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GENOMIC TECHNOLOGIES AND BREEDING APPROACHES FOR EMERGING POULTRY IN AFRICA: A CASE OF GUINEA FOWLS

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Abstract

The origins, evolution and domestication of the helmeted guinea fowl (*Numida meleagris*) in Africa remain elusive. Alongside chicken, guinea fowls are an important dietary source of quality proteins in terms of meat and eggs. They are also a source of livelihoods for small-holder farmers in Sub-Saharan Africa against a backdrop of climate change. Nevertheless, very little research has been done to study the genomes of helmeted guinea fowls in Africa to pave way for their genetic improvement.

Herein, the *de novo* assembly of helmeted guinea fowl genome was performed, based firstly on new whole-genome sequences for 110 domesticated helmeted guinea fowls from Nigeria (24), Kenya (13), Sudan (11), Europe (17 from Italy/Hungary), Iran (23) and China (22). Secondly on whole-genome sequences for 40 wild helmeted guinea fowl species from Nigeria (15), Kenya (13), and Sudan (12). Lastly, whole-genomes for six vulturine guinea fowls and one crested guinea fowl from Kenya. Genome sequences of helmeted guinea fowl in the public databases as published by Vignal *et al* (2019) were also included in the analyses.

The raw data was filtered for quality control and assembled. Comparative genomic analyses with other avian species of economic interest revealed signals of selection on genes related to pathogen resistance

and adaptation to harsh climatic environments as those experienced in Africa. Scanning for selective sweeps also detected a strong candidate gene *GRIK1* for neural change and behavior shift in domestication meaning this gene is a candidate gene for domestication. This study provides valuable genomic resources for researchers and breeders to build on in order to improve breeding, production and productivity of helmeted guinea fowls in Africa using genomic tools against a backdrop of climate change.

Keywords: back yard poultry, climate change, emerging livestock, genomics, candidate genes

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LEADING STUDY ON DETERMINATION OF SINGLE NUCLEOTIDE POLYMORPHISMS IN HEAT SHOCK PROTEIN 70.1 GENE IN FIVE NATIVE BUFFALO BREEDS OF PAKISTAN

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Abstract

Heat and humidity stress is a persistent challenge to farm animals, including buffalo under tropical climatic conditions. The condition is more challenging when environmental thermal stress is escorted with ambient humidity, damaging mainly the animal reproductive performance, alongside the immune system. South-Eastern regions of Pakistan are characterized by hot and humid summers and extended periods of high temperature and relative humidity. In this study, five buffalo breeds (Ravi, Nili, Nili-Ravi, Azakheli and Kundi) were selected to determine the polymorphisms in the promoter and complete coding region of the *HSP70.1*. The complete sequences of *HSP70.1* was composed of the 5'UTR 204 base pair [bp], coding sequence 1926bp encoding 641 amino acids [aa] and 3'UTR 293bp. Among the five buffalo breeds studied, a total of 12 polymorphisms were detected, 6 non-synonymous and 4 synonymous in coding regions, 2 in 3'-UTR and nine polymorphisms were detected in 5'-UTR. Two polymorphic sites were identified among five buffalo breeds in the promoter region. The TATA box was found between -24 and -29 bp and CCAAT box between -64 and -68 bp upstream to start codon of *HSP70.1* gene. Phylogenetic analysis was performed using bovine and bubaline *HSP70.1* gene coding sequences with other species each forming a distinct cluster. We identified four microsatellite markers, one dimer (GT) and three trimmers (GCC, CTG, AAG) in the coding sequences CDS of the bubaline *HSP70.1*. The polymorphisms identified in bubaline *HSP70.1* may act as an imperative molecular marker for thermotolerance.

Keywords: *HSP70.1* gene, polymorphisms, phylogenetic analysis, Buffalo breeds, Pakistan

Introduction

Heat stress has adverse effects on a variety of dairy parameters, including animal health, reproduction, milk production, growth, conception rate and feed intake (West, 2003). Sustainability in livestock production systems is likely to be affected by global warming and climate change. Heat stress is one of the critical factors that impede profitable livestock rearing and has become one of the utmost challenges facing the global dairy industry.

Living organisms respond to a verity of physical stressors and unfavorable conditions at the cellular level by a rapid and transient acceleration of biosynthesis of a highly conserved set of polypeptides termed heat shock proteins (HSPs). They are expressed in many cell types and commonly referred to as stress proteins. On the basis of molecular sizes, the mammalian HSPs are categorized into several families of proteins including HSP10, 30, 60, 70, 90, 100 and 110 subclasses. Environmental heat stress at the cellular level is mediated by various members of HSP70 (HSP70.1 and HSP70.2) is widely studied and considered high temperature sensitive and an indicator of stress in animals (Beckham *et al.*, 2004).

HSPs contain highly conserved stress proteins, expressed in response to stress and playing crucial roles in environmental stress tolerance and adaptation. Due to evolutionary divergence, *Bubalus bubalis* is speculated to have different responses to heat stress. Variation in candidate genes associated with a heat-shock response may provide an insight into the dissimilarity and suggest targets for intervention.

The present work was undertaken to characterize *HSP70.1* promoter and coding regions in different buffaloes (i.e. Ravi, Nili, Nili-Ravi, Azakheli and Kundi) of Pakistan. Buffaloes are economically important livestock species for milk and meat production in developing countries like Pakistan. The breeds haven't been characterized for any immunity genes to ascertain immune response and adaptation to thermal stress. To study the cellular and molecular responses at large is important that may lead to identification of biomarkers for heat stress in animals.

The breeding practices for high genetic merit for increased milk yield have made animals more susceptible to the effects of high heat-load (Al-Katanani *et al.*, 1999). The identification of heat tolerant genes might be effective approaches to mitigate the negative influence of environmental heat stress on dairy animals. In this study, five tropically adapted buffalo breeds Nili, Ravi, Nili-Ravi, Aze-kheli and Kundi from different geographical regions of Pakistan were selected to determine the *HSP70.1* gene structure and existing polymorphisms.

Materials and Methods

To determine genetic variations in the genomic sequences of *HSP70.1*, blood samples were collected from five genetically unrelated buffalo breeds (Nili, Ravi, Nili-Ravi, Aze-Kheli and Kundi) by visiting different government and private farms/tracts of three provinces Punjab, Khyber Pakhtunkhwa and Sindh, Pakistan. Blood samples were collected in EDTA-containing vacutainer tubes and transported to the laboratory in ice and stored at -20ºC till genomic DNA extraction. The experiments were performed at the laboratory of the Virtual University of Pakistan, Lahore. Genomic DNA was extracted using the standard Phenol/chloroform method described by Wajid *et al.* (2014). To determine SNPs in coding sequences of *HSP70.1* as well as 5´ and 3´ flanking regions one set of amplified (*HSP70.1*F and *HSP70.1*R) and three internal sequencing (Int1, Int2 and Int3) primers were used, as previously reported by Sodhi *et al.* (2013). The reaction mixtures of 25µl contained 1µl of 50 ng/µl DNA template, 1.5µl of each forward and reverse primer (10 pmol), 2.5µl of 10µM dNTPs mix, 2.5µl of $MgCl_2$ (2.5mM), 2µl Taq Buffer and 0.5µl of Taq DNA polymerase (5unit/µL). The reaction mixtures were incubated in an ABI thermal-cycler at 95ºC for 10 min followed by 30 cycles (95ºC for 60s, 59ºC for 60s, 72ºC for 2 min) followed by final extension of 10 min at 72ºC. The amplicons were run on a 1.2% agarose gel and the PCR amplicons were purified using the GeneJET gel extraction kit (Thermo Scientific, USA) and sequenced by using an automated sequencer (ABI 3130XL, ABI, CA). The *HSP70.1* sequences obtained in this study were compared with available sequences retrieved from GenBank. MEGA6 software was used for sequence alignment, detecting synonymous and non- synonymous nucleotide substitutions and phylogenetic analysis (Tamura *et al.*, 2013). The domain architecture was predicted using the Simple Modular Architecture Research Tool (SMART), whereas the predicted tertiary protein structure (2Z7X) was generated using the PyMol2.2.8.

Results and Discussion

The complete *HSP70.1* gene was composed of the 204 base pair [bp] 5'UTR, coding sequence 1926bp encoding 641 amino acids [aa] and the 3'UTR (293bp). Among the five buffalo breeds studied, a total of 12 polymorphisms were detected, 6 non-synonymous and 4 synonymous in the coding region, 2 in 3'-UTR and nine polymorphisms were detected in 5'-UTR. Overall, two polymorphic sites were identified among the five buffalo breeds in the promoter region. The TATA box was found between -24 and -29 bp and CCAAT box between -64 and -68 bp upstream to the start codon of *HSP70.1*. Phylogenetic analysis was performed using bovine and bubaline *HSP70.1* coding sequences with other species, with each forming a distinct cluster. We identified 4 microsatellite markers; one dimer (GT) and three trimmers (GCC, CTG, AAG) in the coding sequences of bubaline *HSP70.1* gene. Untranslated regions (UTRs) are involved in RNA stabilization and regulation of gene expression. Polymorphisms in 3´ UTR and 5´ UTR may affect the RNA stability and rate of gene transcription, respectively. The 5´ UTR and 3´ UTR in Bubaline were 204 and 293 bp in length, respectively. The 5´ UTR sequences obtained from the studied buffalo breeds were found to be similar, however, when compared with available bubaline sequences in GenBank, a total of nine polymorphisms were identified. The five

studied buffalo breeds were polymorphic at two positions, Ravi and Nili with C172G and C186G, the other three bubaline were monomorphic. The detection of distinct nucleotide changes in the bubaline *HSP70.1* and its promoter provides context for functional characterization of the variants in order to define cellular components and physiological mechanisms of the species to withstand heat stress.

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SEQUENCE-BASED STRUCTURAL AND EVOLUTION OF POLYMORPHISMS IN BOVINE TOLL-LIKE RECEPTOR 2 GENE IN DHANNI AND JERSEY CATTLE BREEDS

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Abstract

Toll-like receptors (TLRs) are a family of type I transmembrane pattern recognition receptors (PRRs) playing a critical role in the early innate immune response by recognizing and defending against invading pathogens. This study was aimed to characterize the distribution of single nucleotide polymorphisms (SNPs) in bovine *TLR2* gene in Dhani and Jersey cattle breeds from Balochistan province of Pakistan. The complete sequences of *TLR2* comprised the 5'UTR (136bp), coding sequence (2355bp) and 3'UTR (1316bp). Phylogenetic analysis revealed clustering of Dhanni with *Bos indicus* and Jersey with *Bos taurus* as the nearest neighbors. Sequencing of TLR2 in Dhanni cattle showed the occurrence of 35 variable sites within coding region, including 37% non-synonymous and 63% synonymous, while in Jersey cattle, 21 SNPs were identified within the coding region, including 48% non-synonymous and 52% synonymous. The SNPs mostly fell within the leucine-rich repeats (LRR) of the extracellular domain, regions responsible for ligand recognition. The ratio of dS/dN substitutions was <1 at polymorphic sites, indicating purifying selection. The SNPs at position 681 (p.Leu227Phe in Jersey) and 521 (p.Thr174Ile in Dhanni) were presumed to have possible damaging or function altering effects. The predicted bovine TLR2 is a solenoid-like (coil-like) built from 20 LRRs bent into a horseshoe shaped structure. The polymorphisms in *TLR2* can be useful in future research exploring its role in immunity and may be used as markers for disease resistance under selective breeding.

Keywords: Pattern recognition receptor, Toll like receptor 2, SNPs, phylogenetic analysis, Dhanni, Jersey

Introduction

Toll-like receptors (TLRs) are type 1 transmembrane proteins and a significant component of the innate immune response. TLRs are evolutionarily conserved proteins found in both vertebrates and invertebrates functioning in recognition of components of invading pathogens through the conserved microbial patterns. The immune system consists of innate and adaptive immune response, an interaction is needed to eliminate different types of pathogens including viruses, bacteria, fungi and protozoa with maximum proficiency. The TLRs belong to pattern recognition receptor (PRR) molecules (Takeda et al., 2003), activating the innate immune system by detecting pathogen associated molecular patterns (PAMPs). TLRs are distinguished by the presence of Toll-/ Intereukin-1 receptor (TIR) domain and LRRs in the extracellular domain for the recognition of PAMPs (Mukherjee et al 2005). All TRLs recognized so far contain three domains despite their amino acid (aa) length, cytoplasmatic Toll/IL-1 receptor (TIR) domain that assists the downstream signal-transduction, a transmembrane domain (TM) which binds signaling molecules and a large extracellular ligand binding domain (ECD) comprising multiple leucine rich repeat (LRR) motifs of 20-30 amino acids (aa) which are involved in recognition of pathogens' ligands. The ECD shows considerably higher divergence reflecting, their PAMPs

involvement in recognition of multiple pathogens. The TIR and TM domains are highly conserved with functional similarity between species (Werling et al. 2009).
Toll-like receptors (TLRs) are a vastly studied class of PRRs, whose task is rapidly recognizing evolutionary conserved structures on the invading bacteria, viruses, parasites and fungi. Binding to these ligands, TLRs trigger a number of proinflammatory and antimicrobial response, playing a key role in the first line of defense against the pathogens also promoting adaptive immunity responses. The published data suggest that SNPs on various bovine TLRs may reduce the ability to recognize PAMPs and are associated with altered susceptibility to infection.
Several previous studies have demonstrated that the polymorphisms in the TLRs may diminish the capability of the TLR-proteins to recognize the PAMPs and consequently affect the innate immune activation in mammals. The earlier research suggested that disease susceptibility and resistance in animals may be caused by single nucleotide polymorphisms (SNPs) that altered ligand binding by TLRs (Werling et al. 2009).
Dhanni cattle, also known as Pothwari (*Bos indicus*) is a draught purpose breed distributed in various geographical regions (Attock, Jhelum, Rawalpindi and Chakwal) of Punjab province in Pakistan. Jersey (*Bos taurus*) is an imported dairy cattle breed, comparatively smaller in size and considered to be the descendants of cattle from the Norman mainland. These cattle breeds are not yet characterized for any immunity-related genes in Pakistan. The key objective of this study was to determine the variations in the bovine TLR2 gene. To attain this objective, both breeds were selected for nucleotide analysis of *TLR2*. The sequence analysis of *TLR2* of these breeds showed variations that may provide informative genetic markers for future use in association studies of bacterial infection susceptibility or resistance.

Materials and Methods

Samples (n = 60) were collected from both cattle breeds (each breed = 30) from different Government and private farms in various geographical locations of province Punjab. The research work was performed in the Animal Genomics Lab, Virtual University of Pakistan, Lahore. Three ml of blood was collected from the jugular vein in EDTA (Ethylenediamide tetra-acetic acid) containing vacutainer tubes. The blood samples were placed into ice bags and brought nto the laboratory. The samples were stored at -20°C till further use. Genomic DNA (gDNA) was isolated with organic extraction method with the protocol previously described by Wajid et al (2014). The complete (3613 bp) gene was sequenced by six primer pairs used previously by Subhash et al (2018).
The obtained *TLR2* sequences were edited, assembled and analyzed for single nucleotide polymorphisms (SNPs) through BLASTN of NCBI and BioEdit v7 (Hall, 2009). MEGA 6 software was used for phylogenetic analysis, sequence percent identities and (Tamura et al., 2013) for calculation and position of ratio of synonymous and non-synonymous nucleotide substitutions using the Nei-Gojobori method. The Simple Modular Architecture Research Tool (SMART) was used to predict the domain architecture in *TLR2*. The LRR finder tool was used for estimation of the location of LRR. Tertiary protein structure predictions were generated using PyMol 2.2.8, based on the crystal structure 2Z7X structure. The PolyPhen-2 (Polymorphism Phenotyping v2) program used an alignment-based score approach and the SIFT (Sorting Intolerant from Tolerant) program was used to predict if missense variations are likely to affect TLR2 protein function.

Results and Discussion

The complete coding sequence (CDS) of *TLR2* obtained from Dhanni cattle breed by direct sequencing revealed 35 polymorphisms at different position. Twenty newly-discovered polymorphic sites are being reported for the first time in *TLR2*. Thirteen polymorphisms were missense and 22 were synonymous. Out of the 20 novel polymorphic sites, eight SNPs (40%) were identified as missense and 12 SNPs (60%) were synonymous. Ten missense SNPs were distributed in the EC domain (ECD), two SNPs in the TIR domain and one SNP in the TM domain. The ratio of dS/dN substitutions was <1 indicating purifying selection. The novel missense SNPs identified were tested through online tools to predict any effect on the TLR2 protein. Out of 13 non-synonymous SNPs identified in this study, variation at

nucleotide position 521 (p.Thr174Ile) was presumed to have possible damaging or functional altering effects. The CDS of TLR2 gene obtained from Jersey cattle by direct sequencing revealed twenty-one (21) polymorphisms. Three polymorphic sites were novel. Ten polymorphisms were missense and eleven (11) were synonymous. Out of three novel polymorphic sites, two SNPs were missense and one was synonymous. Out of the thirteen missense SNPs identified in this study, variation at nucleotide position 681 (p.Leu227Phe) was presumed to have a possible damaging or functional altering effect. The secondary structure predicted for bovine TRL-2 revealed loops 36%, helices 45.5% helices and β-sheets 18.5%. LRRs are found in both sheets and helices, however LRRs 3 and 13 are formed of purely helical structure. The predicted TLR-2 extracellular domain based on the homology model Q95LA9 / 5d3iA showed the reliability of model with root-mean-square deviation-RMSD value of 0.1A. There were four N-glycosylation sites predicted, at position N114, N199, N248 and N442. The eight predicted active sites, at positions Ser 368, Glu 369, Leu 392, Val 393, Leu 409, Thr 411 and Leu 418, formed a pocket for ligand binding in the concave side. The SNPs observed in the present work may be associated with disease resistance and the functional role of these polymorphisms has to be investigated. These polymorphisms may influence susceptibility or tolerance to specific pathogens.

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COMPETITIVE ALLELE SPECIFIC PCR (KASP™) GENOTYPING OF SINGLE NUCLEOTIDE POLYMORPHIC LOCI ASSOCIATED WITH IMMUNE PATHWAY GENES IN SRI LANKAN INDIGENOUS GOATS AND THEIR JAMNAPARI CROSSBREDS

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Abstract

The study focuses on genetic characterization of Sri Lankan Indigenous (SLI) goats and their Jamnapari crossbreds (JCB) based on the genotypes derived from KASP assays for 141 single nucleotide polymorphic (SNPs) sites located in 72 candidate genes involved in immune pathways. Polymorphism shown by 98% of SNP in both goat types reflected high genetic diversity in immune pathway genes, among which, 57.97% had minor allele frequency (MAF) greater than 0.20. The mean global observed and expected heterozygosities (H_O and H_E) were 0.297±0.009 and 0.309±0.009, respectively. Further, a significant heterozygosity deficit was observed in both SLI (F_{IS}=0.108) and JCB (F_{IS}=0.083) goat types ($P<0.05$). According to global F_{ST}, the total genetic variability explained by population difference was low (0.96%). Morever, principal component analysis revealed the absence of discrete genetic structure between two goat types. The results elaborated the genetic and functional variation in the immune response of both goat types. The information is potentially useful for identifying genetic resistance to diseases in Sri Lankan goats.

Introduction

In the absence of wild relatives in the country, goats in Sri Lanka are introduced animals. Interbreeding of introduced goats with natural pressures of selection for centuries resulted in present-day Sri Lankan Indigenous (SLI) goats with high adaptation to environmental and pathogenic challenges at the field level. However, the attempts in improving SLI goats for economic traits in recent decades have led to unsystematic crossbreeding at field level. As a result, most goats carry crossbred genotypes among which Jamnapari crossbreds (JCB) are predominant. In general, crossbreds are more susceptible to diseases including gastrointestinal (GI) parasitism than are SLI goats. The phenotypic variation within and between breeds for GI strongyle parasitism in goats is mainly under genetic control (Bressani *et al.*, 2014).

To identify parasitic resistance, exploration of the genetic variation in candidate genes involved in innate and adaptive immune response is important. Though many studies have been conducted in this regard, especially in the tropical regions of the world (Bressani *et al.*, 2014) no study has been conducted in goat genotypes in Sri Lanka. Thus, the present study focused on genetic characterization of SLI and JCB goats by using single nucleotide polymorphic (SNP) sites located in candidate genes involved in immune pathways.

Materials and Methods

Blood samples were collected from 521 (SLI-279 and JCB-243) goats. DNA extraction was carried out using the Masterpure® DNA purification kit and quantified using nanodrop spectroscopy. Samples were genotyped using KASP assays for 141 SNP sites located in 72 candidate genes involved in innate and adaptive immune pathways and visualized in the allele discrimination module incorporated in real time PCR system.
Basic genetic diversity indices such as minor allele frequency (MAF), observed heterozygosity (H_O), expected heterozygosity (H_E) and a test for Hardy-Weinberg equilibrium (HWE) were calculated using Genalex version 6.5 (Peakall and Smouse, 2012) and Popgene version 1.3.1 (Yeh *et al*., 1999) software packages. Global F statistics and pairwise F_{ST} among two goat genotypes were computed using FSTAT software version 2.9.3.2 (Goudet, 2002). Subpopulation structure of SLI and JCB goats was investigated through principal component analysis (PCA) using pair-wise F_{ST} values (SPSS version 13.0).

Results and Discussion

Minor allele frequency and genetic diversity of SLI and JCB goats

The KASP assays produced 71,771 identified allele calls (99.03%) and 703 unidentified allele calls. Out of 141 SNP loci, NLRP5_402_CT locus had more than 0.10 unidentified alleles. Hence, this locus was removed from further analysis. Among the 140 selected SNP loci, 138 loci were polymorphic (98.58%) across SLI and JCB. Out of 416 possible SNP genotypes, 410 genotypes were reported in the present study reflecting that both goat types had high genetic diversity. Across 140 SNP loci observed, overall MAF varied from 0.0021 to 0.4896. Observed MAF varied from 0.0019 to 0.4926 in SLI and 0.0023 to 0.4953 MAF in JCB goats. Among 138 SNP polymorphic loci, 30 loci showed MAF<0.10. However, 25.3%, 13.0% and 19.6% SNP polymorphic loci showed 0.20<MAF<0.30, 030<MAF<0.40 and MAF>0.40, respectively suggesting their functional role for the immune response (Table 1).

Table 1: Polymorphic SNP loci with MAF > 0.1

MAF	SNP loci
0.10 to 0.20	ATP2A3_II_92, ATP2A3_II_150, ATP2A3_II_272, NOD2A_352, CXCR4_722, ITGB7_175, MINCLE_492, NOD2B_120, OLA-DRA-I_379, SERPING1_312, FAM62A_Pr40_559, ZBTB39_Pr56_280, NLRC4B_212, NOD2A_1035, SERPING1_615
0.20 to 0.30	LGP2_271, LGP2_874, NLRP12_187, NLRP14_125, NLRP3_636, TLR3B_306, CLEC10A_A_485, CLEC4L_A_151, TLR5B_338, TLR5C_657, TLR8C_260, TLR8C_404, TLR3A_180, TLR4A_751, TLR5A_808, TLR8A_802, FOS_817, SLC11A2_811, COL6A2_834, CLEC4K_A_630, SFTPD_148, CLEC4K_B_1171, CLEC9A_1061, NOD2B_688, OLA-DYB_335, RAVER1_Pr10_845, ITGA5B_194, MARS_628, ANKRD_Pr36_372, TLR5C_326, TLR3_576, TLR3_617, CDKN1A_82, HSPA8_1024, HSPA8_1064, NOD1_146, IL10_Pr133_624
0.30 to 0.40	MBL2_A_546, MBL2_A_658, TLR9A_646, TLR9B_207, ATP2A3_II_210, TLR7C_634, TRAF4_II_264, IL2RA_274, SFTPD_241, SFTPD_701, KLRD1_Pr26_204, ZNF641_Pr8_243, ZNF641_Pr8_421, NOD1_552, VASP_109, VASP_406, NAIP_562, 0LA-DMB_167
> 0.40	NLRP14_481, NLRP3_837, TLR9C_88, TLR9C_732, NLRP9_43, TLR6B_180, TLR4B_422, TLR4A_525, CDKN1A_185, FOS_315, PTPN6_396, STAT5B_197, CLEC4K_A_749, CLEC8A_575, MINCLE_335, CLEC4K_B_552, OLA-DRA-I_760, NLRP8_767, TLR4B_618, MASP2_91, MASP2_141, NOD1_416, PRLR_Pr173_166, PIK3R1_Pr153_716, TLR9C_393, TLR9C_426

The mean global H_O and H_E were 0.297±0.009 and 0.309±0.009, respectively. The mean H_O was 0.284±0.013 and 0.311±0.014 in SLI and JCB goats while the mean H_E was 0.299±0.013 and 0.319±0.013 in SLI and JCB goats, respectively.

Only 24 SNP loci in SLI population and 17 SNP loci in JCB population deviated from HWE ($P<0.05$) due to a heterozygosity deficit, except for four SNP loci in SLI and one SNP locus in JCB populations. The positive F_{IS} values showed a significant heterozygosity deficiency in both SLI ($F_{IS}=0.108$, $P<0.05$) and JCB ($F_{IS}=0.083$, $P<0.05$) which indicated the non-random mating or existence of population substructures with evidence of inbreeding (Hedrick, 2013). The SLI goats for the study were selected from isolated pockets in the eastern region, where there is a possibility of inbreeding. Further, the heterozygosity deficit in both SLI and JCB populations may have occurred through natural selection favoring parasite resistant goats.

Genetic structure of Sri Lankan Indigenous and Jamnapari crossbred goat populations

The global F_{ST} value suggested that only 0.96% of total genetic variation was due to between genotype differences. The global F_{ST} reported in the present study was even less than the observations of Silva *et al*. (2017). This is possible, since JCB goats are mainly from crossing of SLI goats with the Jamnapari male goats. Since the crossing is unsystematic at the field level, goats with different crossbred levels could be observed. However, the presence of high within genotype variability reflects that important genetic characters such as parasitic resistance is still remaining within goat populations, including indigenous and crossbred genotypes. The pair-wise F_{ST} were subjected to PCA and first three principal components were plotted on a three dimensional scattergram to evaluate the genetic structure of two goat types; SLI and JCB (Figure 1). The first three PCs explained 57.67%, 6.19% and 5.10% of the total genetic variation, respectively. The PCA indicated the absence of clear population differentiation between SLI and JCB goats indicating the lack of discrete genetic structure among goat populations, confirming the observation by Silva *et al*. (2017). Further, the absence of separate genetic structures between the goat types in the present study could be due to the high genetic contribution of SLI in JCB cr

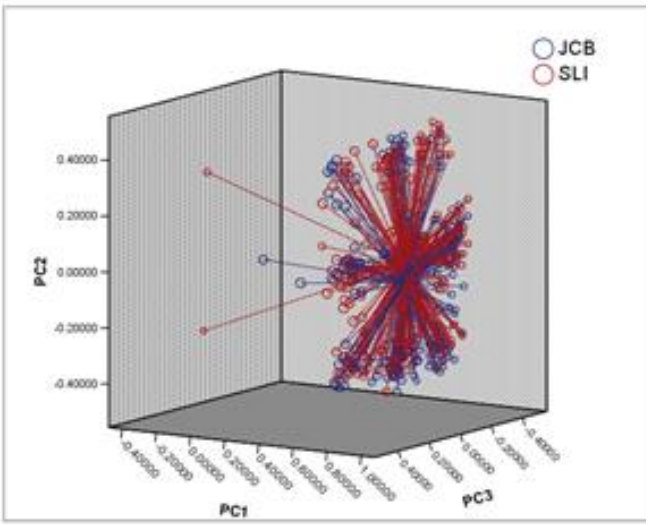

Figure 1. Three dimensional scattergram of first three principal components derived from pair-wise F^{ST} across 140 SNP loci

Conclusion

The SNPs associated in immune pathway genes showed high polymorphism in SLI and JCB goats. High polymorphism of SNP putatively showed their functional role in the immune response. The genetic information generated in the present study provides a directive for identifying genetic resistance to diseases in Sri Lankan goats.

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ALLELIC VARIATION OF BOVINE MAJOR HISTOCOMPATIBILITY COMPLEX - *DRB3.2* IN *BOS INDICUS* CATTLE AND *BOS INDICUS* X *BOS TAURUS* CROSSBRED CATTLE AND THEIR RELATION TO MASTITIS RESISTANCE

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Introduction

The Bovine Major Histocompatibility Complex (*MHC*) class II *DRB3* alleles (*BoLA-DRB3*) are found to have significant association with different indicators of infection. Identifying specific alleles against disease resistance will be a milestone in developing disease resistant crossbred animals. Many workers investigated the genetic association of the *MHC* class *II DRB3* alleles with resistance to mastitis (1, 2, 3). The general consensus from the earlier studies is that these genes are potential genetic markers with higher or lower risk of disease occurrence in cows. The *BoLA* class *II DRB3* allele **16* (*BoLA-DRB3.2*16*) play a role as a risk factor for higher SCC (1). Hence, this study was focused to find out the allelic variations of *BoLA –DRB3* gene present in indigenous as well as in crossbred dairy cattle.

Materials and Methods

Samples used in this study were collected from two different group of cattle *viz.*, Holstein-Friesian crossbred and Jersey crossbred groups as most susceptible to mastitis and group of indigenous cattle (Kangayam, Ongole, Deoni and Bargur cattle) as highly resistance to mastitis. Out of 1073 test samples, screening for *BoLA- DRB3* alleles has been carried out for 894 samples using semi nested PCR (4) followed by Restriction Fragment Length Polymorphism (RFLP) with three different restriction enzymes (*Rsa*I, *Hae*III and *Bst*YI). The PCR RFLP alleles were determined as per BoLA nomenclature committee (5). Direct sequencing of *BoLA-DRB3.2* alleles were custom sequenced (Scigenom, Kerala) to get the confirmation of polymorphism at nucleotide level.

Results and Discussion

The estimated frequencies of *BoLA-DRB3.2* allelic pattern in Deoni, Ongole, Kangayam, Bargur, Jersey and Holstein Friesian crossbred cattle were compared with χ^2 test (Table1) and the frequency distribution showed in figure 1. The most common alleles in Holstein Friesian crossbred and Jersey crossbred cattle were *BoLA-DRB3.2*8* (9.03) and **23* (10.57). Among the major alleles (*BoLA-DRB3.2*8, *10, *13, *15, *16, *22, *23, *24 and *47*), the allele **22* was significantly ($p< 0.05$) higher in crossbred cattle. The *DRB3* alleles of **6, *9, *11, *13, *15, and *23* were very less in frequency (1.08, 1.29, 3.23, 4.95 3.87 and 4.30, respectively) and alleles, **8, *23* and **24* were relatively high in frequency (9.03, 10.57, 7.89, respectively). The common alleles (*BoLA-DRB3.2 *8, *10, *16 *22, *24* and **47*) present in crossbred cattle were less frequent in the native animals.

In crossbred, cows affected with mastitis and the frequency of allele **8* was significantly high ($p<0.05$) when compared to the normal crossbred animals. In Kangayam, Ongole, Deoni and Bargur breeds of cattle, alleles **6* (19.72), **6* (24.00), **15* (22.94) and **6* (19.23) had the highest frequency, respectively. In this study, it was observed that these alleles (**6* and **15*) were significantly ($p<0.05$) higher in low yielding native breeds of cattle.

Table 1. Allele frequencies of *BoLA –DRB3.2* in indigenous and crossbred cattle breeds

Breed	Allelic Frequency			No. of alleles
	< 5%	5 to 10 %	>10 %	
Kangayam N=109	*Bola-DRB3*.2*1, 3,8,12,14,16,20,27,28,36,37,38,41,42,46,N	9, 11, 13,23,31,34	6, 15[a],	27
Bargur N=75	*Bola-DRB3*.2*1, 12,13, 14,16,19, 20, 24,25,27, 31, 34,35, 36,37,38, 41,46,47,51,54	9,15,23	6[a],11[a]	25
Ongole N=110	*Bola-DRB3*.2*1,3,8,9,11,14,16,20,24,27,28,32, 34,36,37,42,47	12,23,31	6[a],13,15[a]	26
Deoni N=91	*Bola-DRB3*.2*1,8,13,14,16,17,19, 20,22,24,25,27,28,31,34,36,37,38, 41,46, 47,54	9,15,23,51	6[a], 11[a]	28
HFx N=230	*Bola-DRB3*.2*1,2,3,6,9,11,12,13,14,15,16,17,18, 20,22,23,26,27,28,31,32,34,36,37,38, 39,41,46, 48,49,51, eaf, fab, gba, xea, N	8,10,24[a],47	-	41
JX N=279	*Bola-DRB3*.2*1,2,3,6,9,11,12,13,16,17,18,19, 20,21,22,25,26,27,28,31,32,34,36,37,39, 41,46, 48,49,50,51,54, gba, N	13, 15, 24[a], 47	23[a]	40

[a] Significantly (P<0.05) higher gene frequency based on Chi-square test

Different sequence patterns were observed between *Bos indicus* groups and *Bos indicus x Bos taurus* crossbred genetic groups. All the sequences from six different genetic groups revealed that the *BoLA-DRB3* exon 2 amplified was 284 bp in length. The nucleotide sequences were analysed with Lasergene DNASTAR and BIO edit software (6). The PCR based sequence (PCR-SBT) analysis of *BoLA-DRB3* exon 2 in all six breeds revealed that, there are numerous variations in exon 2, which led to different restriction patterns. Number of SNP variations varies from three to seven in each sequenced *BoLA-DRB3* alleles.

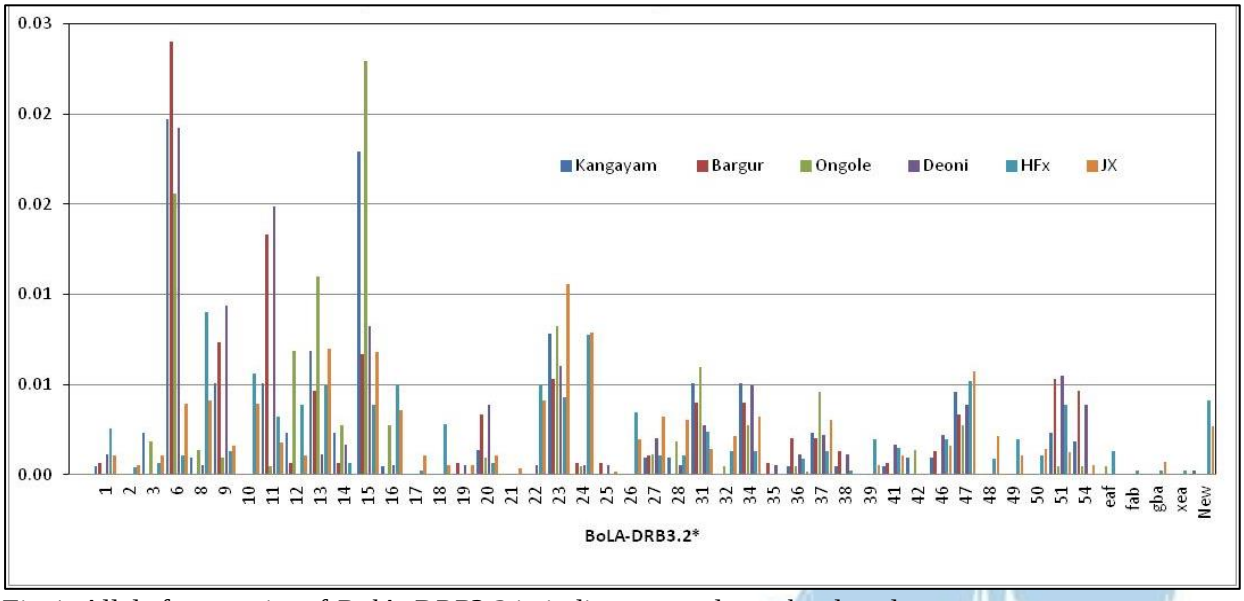

Fig.1. Allele frequencies of *BolA- DRB*3.2 in indigenous and crossbred cattle

The phylogenetic relationship tree involving sequences of *DRB3.2* alleles of different species rooted several clades based on the similarity in the nucleotides present in the selected breed. The sequence pattern that was observed between *Bos indicus* (Kangayam, Bargur, Deoni and Ongole) genetic groups

was different from *Bos indicus x Bos taurus* (Holstein Friesian crossbred and Jersey crossbred) crossbred genetic groups.
Recently, more than 100 different alleles were investigated by PCR-RFLP (4, 7) and PCR-sequence-base typing (5). Various alleles were found to be related with susceptibility and (or) resistance to cattle diseases. Allele *DRB3.2*8* was associated with increased susceptibility, while *DRB3.2*11* and *DRB3.2*23* were associated with increased resistance (8). Forty *BoLA-DRB3* alleles in Holstein x Zebu crossbred dairy cows with frequency ranging from 0.005 to 0.139 (10). Alleles *DRB3*1* and **52* were associated with clinical mastitis, whereas **15, *51*, and **22* were associated with resistance in crossbred populations and the allele *DRB3*10* had the highest effect on increasing milk yield with moderate resistance to clinical mastitis, which could be used as a potential marker for selection in dairy genetic evaluation. The present study revealed that the allele *8 was higher in frequency in crossbred cattle than the indigenous cattle.

Summary

The amplified fragment of *BoLA-DRB3.2* alleles was found to be highly polymorphic as revealed by the PCR-RFLP and PCR-SBT variant data. The number of PCR-RFLP variants and types of DNA sequences indicate the involvement of more than one allele in PCR amplified genomic DNA. Marker assisted selection with exclusion of specific allele *i.e.BoLA-DRB3.2*8* in Holstein Friesian crossbred and Jersey crossbred cattle and inclusion of *BoLA-DRB3.2*6* and *BoLA-DRB3.2*15* alleles in Kangayam, Ongole, Deoni and Bargur breed of native cattle might be the choice in early selection of animals against mastitis infection in dairy herds.

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GENETIC DIVERSITY OF LOCAL PIG IN TOGO USING MICROSATELLITE MARKERS POLYMORPHISM.

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Abstract

Local pig farming in Togo is dominated by the extensive system with a small scale use of inputs. In order to develop the basis for an assessment of the genetic diversity of local pig populations, a survey was carried out in the Maritime and Savanes Regions of Togo. Populations were grouped according to districts. This was the first-ever study of the diversity within the local pig populations. A total of 250 blood samples were collected from 75 farms in the two regions. Molecular analyses were performed on 176 DNA samples at the genotyping laboratories of the Centre International de Recherche Développement en Zone Subhumide (CIRDES) in Burkina Faso. For this study, 15 microsatellite markers were used by referring to the FAO / ISAG list. The allele sizes for these samples ranged from 107 to 290 base pairs, 20% of which were an odd number. The average number of alleles on all markers was 9.53±1.03. The lowest number of alleles was 3 from locus SO227 and the highest was 25, from locus SO005. The smallest genetic distance (GD = 0.067) was observed between pig populations from the Lacs and Yoto districts. It means that these populations are very close genetically, while the pig populations from Tône and Vo districts were the most genetically distant from each other (GD = 0.166). The allelic richness varied from 1.476 ± 0.214 to 1.643 ± 0.228. The degree of morphological differences observed within the two regions followed the trends of the molecular results. Further studies are suggested to identify the true origins of these local populations and the periods during which the crosses occurred.

Keywords: pig, microsatellite markers, genetic, allele, Togo

IAEA-CN-281-197

DECODING OF THE UNIQUE GENETIC COMPOSITION OF TRYPANOTOLERANT AND ENDANGERED EAST AFRICAN SHORTHORN TAURINE-SHEKO

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Trypanosomiasis is one of the economically important diseases impacting livestock production and rural livelihoods in the humid areas of Africa. There are a few African indigenous breeds (6%), mostly from the western Africa region, known to be adapted under high tsetse fly challenges. Of these breeds, Ethiopian Sheko represents the only remnant of East African taurine, which has evolved in the warm humid areas of South-Western Ethiopia where trypanosomiasis is one of the potential selective pressures. On the other hand, Ethiopian indicine cattle and hybrids are trypanosusceptible. To shed light on the unique genetic constitution of Sheko via identifying genomic regions markers differentially selected and linked to trypanotolerance, we compared the genomes of Sheko with Ethiopian zebu, Sanga and Zenga populations genotyped with a high-density SNPs chip and calculation of genetic differentiation. Some of the genes within the highly-differentiated SNPs have roles related innate immune response; the T cell receptor signaling pathway; positive regulation of T-helper 2 cell activation (JAK1, ILRI5, TNFSF13B, MALT1, POLR3H and PRKCQ); DNA repair; response to stress to UV; and response to heat /oxidative stress (RAD18, GPX2, HSPA2, and PRDX1). We further tested a functional enrichment analysis and the candidate genes were enriched in terms related to platelet activation, pathways for cancer and melanoma, and the PI3K-Akt signaling pathway. In light of the immunological functions of the genes detected in this study, it can be inferred that the identified genes and pathways may have been naturally selected in Sheko for adaptation under humid climatic conditions and high tsetse infestation areas.

IAEA-CN-281-215

PHENOTYPIC CHARACTERIZATION AND CONSTITUTION OF A DNA BANK FOR FURTHER GENETIC DIVERSITY STUDY OF BURKINA FASO LOCAL CHICKEN

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The chicken population of Burkina Faso numbered around 44 million head, out of which 90% are local breeds with diverse genetical types about which the genetic knowledge is still basic. The objective of this study was to contribute to a better knowledge of the genetical resources of local chickens in Burkina Faso by performing phenotypic characterization.
A transversal survey was carried out between July 2018 until January 2019 in 6 regions spanning 3 agro-ecological zones in Burkina Faso and involving 251 chicken breeders. A total of 948 individual chicken (692 females and 256 males) were scored for 13 body measurements and 9 qualitative traits. Blood samples were also collected.
The chickens observed displayed 13 different feather colours of which white (19.16%) and light brown (16.75%) were the most common.
The site (agro ecological zone) and the sex of individuals had a significant effect ($P < 0.05$) on all of the 13 body measures analyzed. In fact, the analysis of the main body measurement showed the existence of 2 sub populations, based on body size. One of them (light and small hen: 0.91kg) was observed in all three agro-ecological zones. On the contrary, the second sub population (heavy and large hen: 1.37kg) was almost exclusively seen in Sudan-Sahel and Sudan agro-ecological zones. These two sub populations showed statistically significant differences ($p<0.05$) for all 13 body parameters measured. These data suggested some differences in adaptation linked to body type and environmental factors.
Sexual dimorphism was noted for average body weight (1.64kg for males vs 1.14kg for females), the body length (42.16 cm vs 37.30cm) the thorax perimeter (29.01cm vs 25.96cm).
The information reported in this study will be the basis for the establishment of further characterization, conservation and selection strategies for Burkina Faso chicken populations.
From the blood samples, we were able to extract a stock of 325 DNA samples with an average concentration of 110.83ng/µl. These samples will be used for molecular diversity studies.

Keywords: phenotypic charaterization, local chicken, agro ecological zones, Burkina Faso.

IAEA-CN-281-216

DIVERSITY AND GENETIC STRUCTURE OF 6 POPULATIONS OF LOCAL GUINEA FOWL (*NUMIDA MELEAGRIS*) OF BURKINA FASO.

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Abstract

A total of 190 Guinea fowl individuals with ages ranging between 6 to 7 months were genotyped for 19 microsatellites markers to assess diversity and genetic structure of six local Guinea fowl populations of Burkina Faso. High levels of allelic and gene diversity (7.16 and 0.62 respectively) were found in Burkina Faso local Guinea Fowl populations. The mean inbreeding estimates (F_{IS}) within populations were respectively 0.098; 0.202; 0.114; 0.114; 0.180 and 0.202 for Gaoua, Tenado, Fada, Tenkodogo, Ouagadougou, Dori sub-populations and 0.167 across all the studied populations. The global F_{ST} showed a low genetic differentiation among the studied populations, with 6.9% of total variation being attributed to differences between populations. The qualitative and quantitative test for mutation drift equilibrium revealed no bottleneck events in Burkina Faso Guinea Fowl populations in the recent past. This study revealed a weak genetic structuring indicating a homogeneity within the Burkina Faso local Guinea fowl populations. These results would indicate the existence of a single breed, despite the high genetic diversity within populations observed.

Keywords: genetic diversity, Burkina Faso, Guinea Fowl, microsatellites markers.

IAEA-CN-281-232

GENOME WIDE ASSOCIATION STUDY REVEALS POTENTIAL PLEIOTROPIC VARIANTS FOR CONFORMATION, WEIGHT AND PROLIFICACY IN CAMEROONS' NATIVE GOAT

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Abstract

Goat is one of the most important livestock for African countries with high potential to address sustainably, nutritional and economical major challenges they are facing. In order to improve flock productivity, selection of high potential local breed such as West African dwarf goat (WADG) is one of the key actions. The development of molecular tool appears as a shortcut to identify genetic factor affecting production traits and for a better estimation of the real breeding Value. The present work uses a case control genome wide association study (GWAS) to highlight common chromosomes, genes and SNP variants for height at wither (HW), chest girth (CG), live body weight (BW) and the prolificacy in Cameroon native goat (CNG). The main results show that within the first 20 top p-value, no common variant was found between BL and the other traits studied. On the contrary, variant rs268250889 localised on gene Zinc finger protein 114 (ZNF114) on *Capra hircus* chromosome 18 (CHI18), was the 2nd top p-value for HW ($P < 0.0008$) and was simultaneously present for CG/BW ($P < 0.004$) occupying the 14th top p-value rank. Therefore, ZNF114 may be one of the major gene providing evidence of phenotypic correlation among conformation trait through pleiotropic effect and somehow, confirming the potential usefulness of HW as indicator trait for CG and BW in CNG. Meanwhile, another variant rs268285661 (CHI5) being the 15th top p-value ($P < 0.0038$) for CG/BW was instead the 1st top p-value ($P < 0.0009$) for prolificacy suggesting its pleiotropic effects on conformation, weight and reproduction in CNG. These findings provide potential evidences of the previous phenotypic correlation observed between these traits and might confirm the high potential of CG and BW as indicator trait for prolificacy in CNG. Further investigations are recommended in order to examined these relationships.

Keywords: Small ruminant, genomics, selection, growth, reproduction and indicator traits.

Introduction

WADG is a multiple purposes breed known to be prolific and well adapted to its natural environment. In practice, many goats are slaughtered for ceremonial purposes during their first 3 months (Upton, 1984) suggesting their usefulness even at very young age and the great importance of the numerical

productivity in this breed. However, their small format is considered as a handicap to their meat productivity. Therefore, improving both prolificacy and growth is expected to generate more profit to goat keeper.
When combining many traits in a breeding goal, the degree of the expected response also depends on the relationship between them. Studying those links is of great importance for the selection. Based on the work Kouam *et al.*, (2015) and recent findings (unpublished) in the Western highland (WHAZ) and the bimodal rainforest (BRFAZ) agroecological zone of Cameroon, significant effect between some conformation traits with the life body weight (BW) or prolificacy or life body weight on have been reported. As such, it is hypothesised that those traits are under the control of genes probably acting in a pleiotropic manner or they might be transmitted together. However, the molecular genetic basis of these phenomenon has not yet been studied. GWAS offers an exploratory approach to address such questioning at once in a single study. The present study aims at highlighting knowledge on the genetics determinisms of Cameroon's goat growth and prolificacy in other to improve their productivity. Specifically, to identify common chromosomes, genes, SNP variants between age-type HW, BL, CG and BW and prolificacy in Cameroon native goat.

Materials and Methods

Study area

Data were collected in the WHAZ (5°-8° LN and 9°45 -11°15 LE) and the bimodal rainforest (BRFAZ (2°-4° LN and 11°15 -16 ° LE) of Cameroon (ASEB, 2010).

Animal material and sampling

Up to 383 females, collected in 160 villages, were used in the study. The females have given birth at least once and were pre-characterised for maximum litter size.

Data collection

The records were collected according to the USDA sampling protocol adopted for the African Goat Improvement Network (AGIN) as described by Huson *et al*. (2014). For the DNA-isolation, ear tissues samples were collected on each female using an adapted punch.

DNA extraction and genotyping

The genomic DNA was extracted with the Qiagen PurGene tissue protocol at the BecA-ILRI laboratory. Product from 111 females, whole genome sequenced with the caprine 50K SNP chip panel (Tosser-Klopp *et al.*, 2014) were used. The quality control (QC) of genotypes was performed iteratively as described by Marees *et al.*, (2018).

Data quality control

Loaded SNPs consisted of 51940 variants (autosomes and X chromosome). After quality control and pruning, a total of 1713 variants and 104 does remained for association analysis. Among the remaining phenotypes, 21 are cases and 83 are controls for level of prolificacy.

Identification of molecular genetic marker for HW, BL, CG, BW and prolificacy: Definition of case and control group

For HW, BL, CG and BW **t**he females with a value inferior or equal (≤) to the mean value of the whole females of the same age was considered as control. Otherwise, the individual is considered as case. F**or**

prolificacy**:** The maximum litter size was used to assess the prolificacy. Low prolific does (1-2 kids per kidding) are control while high prolific (3 kids and above) are cases.

Statistical analysis

GWAS was carried by a chi square test using 1 thread (De *et al.*, 2014). Total genotyping rate is 0.990205. The results were then compared to the significant p-value 0.00029 or $3x10^{-3}$ and –log10 (p) =3.53. The analysis was carried in PLINK v1.90b6.15 R-package, run within R (version 3.6.2) (R Development Core Team, 2016).

Identification of SNP

The comparative analysis of the GWAS results output from HW, BL CG/BW and prolificacy has enabled the detection of SNPs. Then, the SNP corresponding to the top 20 variants that reaches the statistical threshold and simultaneously found within at least two of the studied traits was selected.

Results

Common variants between BL and HW, CG or BW

The examination of each top 20 variants for BL, HW, CG and Weight does not reveal any common variant among them. Based on these observations, BL, HW and CG or life body weight are under the control of different set of genes and genomic regions. Then the selection of BL is not expected to impact the other traits.

Common variants between BL and prolificacy

The analysis of both top 20 variants for prolificacy and BL does not show any common marker or genes. Then, the BL and prolificacy may involve specific set of genes suggesting the absence of pleiotropic effect.

Common variants between HW and CG or BW

One single variant, rs268250889, was simultaneously found among the top 20 SNP markers of the GWAS output for HW and CG/BW. Variant rs268250889, localised on gene ZNF114 (CHI18), was the 2nd top p-value for HW ($P < 0.0008$) and was simultaneously present for CG/BW ($P < 0.004$) occupying the 14th top p-value rank. Therefore, ZNF114 may be one of the major gene providing evidence of phenotypic correlation among conformation trait through pleiotropic effect and somehow, confirming the potential usefulness of HW as indicator trait for CG and BW in CNG. The gene ID ENSCHIG00000025224(CHI 18) is among the novel goat gene. It has been labelled as the zinc finger protein 114, 6 (ZNF114 isoform 6). Its molecular function has been inferred from electronic annotation as DNA binding, metal ion binding (UniProtKB-KW) but not Biological data is available. However, the downstream gene variant location of the main SNP (rs268250889) together with 4 others in high LD among which one (rs638991445) having r^2= 0,979 is recorded as a missense variant, while the 3 others (r^2 = 0.815; 0.833; 0.947) are recorded as intron variants), may suggest functional consequence of the gene expression. Interacting proteins for ZNF114 remain unknown and non-available in GeneCards, the human gene Database. Deep studies on this gene is therefore recommended to better assess its potential effect in mammal as a whole and particularly in goat.

Common variants between HW and prolificacy

None of the top variants recorded has been found among the 20 first top variants for prolificacy. Nonetheless, one variant corresponding to gene ATP8A2, been the 21st top variant was among the top

first twelve variants. This observation is highlighting the commitment of many small gene effect in the regulation of polygenic traits. The selection of a specific threshold may therefore lead to missing of some information and the difficulty for few markers to explain the entire genetic variation.

Common variants between CG and BW

It is important to note that the variants for the CG and BW were the same as a result of case and control group designing. The selection cases and controls were based on individual performances in each trait. We finally found that all does have the same level of performance no matter the trait. This result show that CG and BW share the same genes, confirming the high degree of phenotypic correlation between them and their use as predictive tool with barymetric equation. This suggest that both genes for CG and BW have pleiotropic effect.

Common variants between CG and prolificacy

The analysis of both GWAS output for CG/BW and prolificacy show one single marker, rs268285661 (CHI5), simultaneously found among the top twenty ones. The variant rs268285661 (CHI5) was respectively the 11th top p-value ($P < 0.0038$) for CG/BW and the 1st top p-value ($P < 0.0009$) for prolificacy. This finding provides strong evidence of the previous phenotypic correlation observed between these traits, confirming the high potential of chest girth and Weight as indicator trait for prolificacy in CNG.

Conclusion

In general, few SNP variants have been simultaneously identified between different traits, displaying various molecular consequence types depending on the genomic region in which they were located. Simultaneous variant or genes between traits suggest their pleiotropic effect which might highlighted our previous findings of some associations at the phenotypic level. Major recommendations are: Increasing the sample size or using the available sample to access those marker trough collaboration or cooperation with other institutions; exploring quantitative model for analysis workflow; samples resequencing to confirm the existence of the site and assess the polymorphism between animal together with their physiological pathway; settling on station research program to affine and optimize these results.

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AN ANALYSIS OF POPULATION STRUCTURE AND GENETIC DIVERSITY OF SOUTH AFRICAN SMALLHOLDER DAIRY CATTLE HERDS USING SNP MARKERS

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Background

Poor animal productivity, primarily due to a lack of genetic improvement programs, is a major concern for the smallholder dairy sector in South Africa. Most smallholder farmers do not have access to well-adapted germplasm and there are no systems for supporting sound breeding decisions. Consequently, farmers resort to indiscriminate crossbreeding of dairy breeds to improve cow performance for high milk yield and quality (Muntswu *et al.*, 2015). Although crossbred cattle may be the most suitable option for the smallholder sector, there is paucity of information on the current status of breeding practises and the genetic constitution of cattle used in this production system. It was therefore, important to evaluate the population genetic structure of cattle available in South African smallholder dairy (SHD) herds using SNP markers. Such information is a prerequisite for the development of sound genetic improvement programs in this sector.

Aim

To determine the genetic diversity and population structure of cattle on smallholder dairy (SHD) herds in South Africa using SNP markers.

Materials and Methods

A total of 196 animals were sampled from 21 South African smallholder dairy herds, and genotyped using the GeneSeek® Genomic Profiler (GGP) 150K-bead chip. Quality control and data editing were performed using PLINK (Purcell *et al.* 2007). A total of 192 animals with more than 90% SNP genotypes remained for the downstream analysis. Autosomal SNPs that had a median GenCall score more than 0.6 and call rate greater than 95% were kept. SNPs with MAF < 1% and those that deviated from the HWE ($p<0.001$) were removed. Allele frequencies were calculated and used to estimate different genetic diversity parameters including expected heterozygosity (He), observed heterozygosity (Ho), and inbreeding coefficient (F_{is}). Genotypes of animals from four purebred populations, comprising of Ayrshire (n = 200), Holstein(n = 231), Jersey (n = 224) and Nguni (n = 209) were used as the reference populations. Principal Component Analysis (PCA) was used to investigate breed differentiation using the eigenvalues and eigenvectors constructed from the Genome-wide Complex Trait Analysis (GCTA) software (Yang *et al.* 2011). The population structure was evaluated using the model-based Admixture 1.3.0 software (Alexander *et al.* 2009) with the number of clusters defined by the cross-validation (CV) error.

Results and Discussion

The mean MAF values ranged from 0.30 in the Ayshire (AYR), Jersey (JER), and Nguni (NGI) to 0.31 in the Holstein (HOL) and SHD populations (Table 1). There were slight differences in the levels of genetic diversity, ranging between 0.39 (JER and NGI) and 0.40 (AYR, HOL, and SHD). A moderate level of inbreeding (0.02) was observed in the SHD population.

Table 1. Descriptive statistics for the smallholder and reference populations

Population	N	MAF	H_O	H_E	F_{is}
SHD	189	0.31	0.39	0.40	0.02
AYR	200	0.30	0.40	0.40	-0.02
HOL	222	0.31	0.40	0.40	-0.01
JER	222	0.30	0.40	0.39	-0.02
NGI	202	0.30	0.39	0.39	-0.004

The PCA plot separated the NGI from the dairy breeds (Figure 1) with a slight dispersion of the SHD population towards its component. Most of the SHD population dispersed widely between the HOL and JER populations, with a few individuals being closely related to the AYR population.

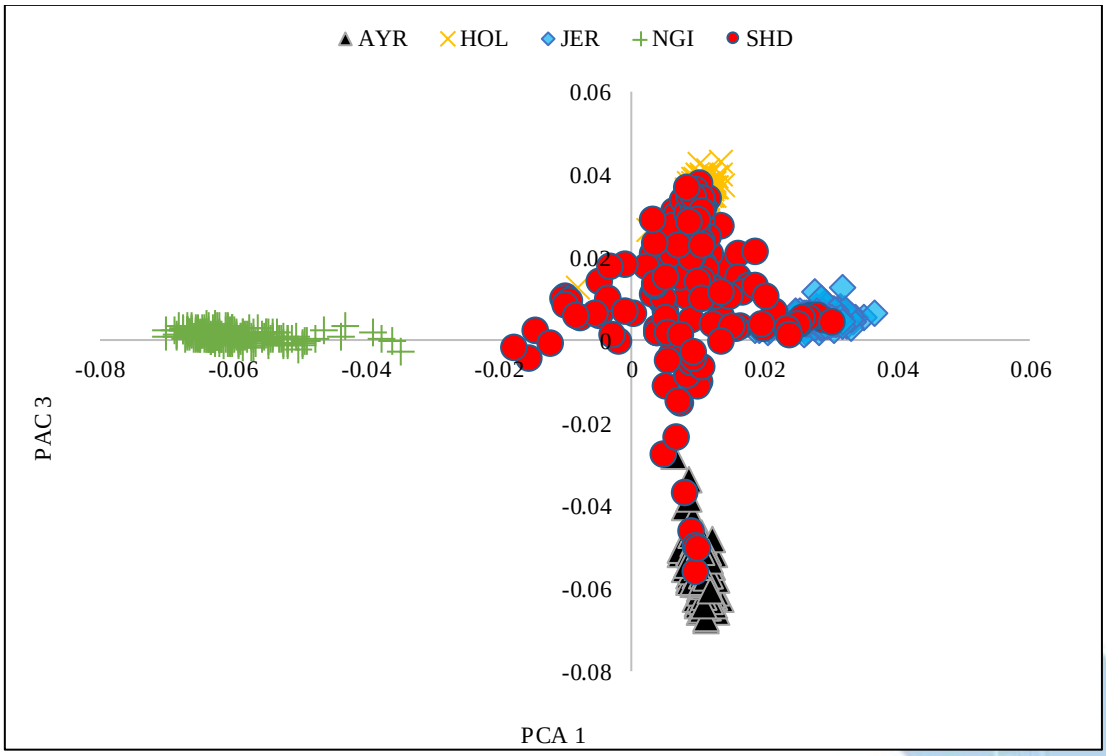

Figure 1. Principal component analysis (PCA) plot constructed for PC1 vs PC3

A low cross-validation error (0.62) was detected at $K = 4$ as shown in Figure 2. The smallholder population displayed a heterogeneous cluster, an indication of a sub-population rather than a distinct population, and confirming the highly crossbred nature of the population. The NGI population separated distinctly from the specialised dairy breeds (AYR, HOL, and JER), and formed a homogeneous cluster closely related to only a few animals from the SHD population. This points to limited use of the indigenous NGI breed in the crossbreeding being practiced on smallholder dairy herds.

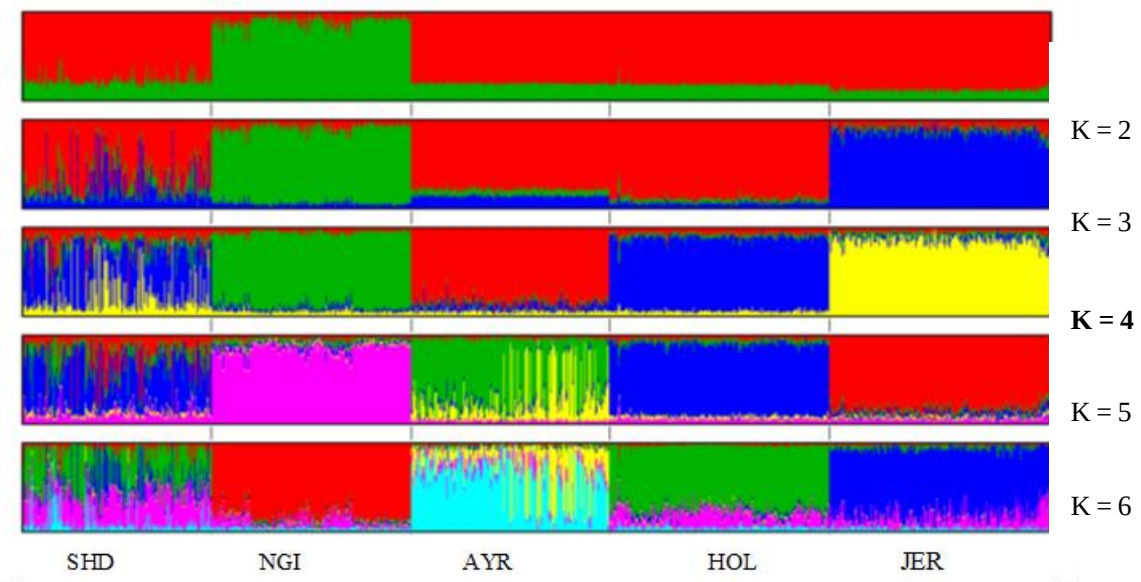

Figure 2. Admixture bar plots of breed compositions (*K* = 2 to *K* = 6), with *K* representing the different clusters per breed

Conclusion

The current study has generated new knowledge of breeding practices in smallholder dairy herds of South Africa. The results have provided a useful insight into the genetic structure and prevailing breeding practices on smallholder dairy herds. Crossbreeding between HOL and JER is presumably meant to complement the high milk production of the former and high solids production of the latter breed, which is an increasingly common practice on commercial herds. There is, however, a need to promote crossbreeding with the indigenous breeds which are adapted to the low input and harsh smallholder environment.

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FIRST MOLECULAR DETECTION OF PSEUDOCOWPOX VIRUS FROM *CAMELUS DROMADARIUS* IN TUNISIA

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Background

Camelus dromadarius is still the preferred livestock species used in extreme dryland areas, in particular the arid zones and the desert of Africa. The economic importance of this multipurpose animal is obvious. African camel population represent 80% of world camels. In Tunisia, the estimated number of camels is 100,000 female units distributed, especially in southern and center of the country (Jemmali B., 2016). Despite this important number, there is a little information on camel pathologies in Tunisia. There are a few field studies on camel diseases; however, most focused on the role of camels as reservoirs of pathogens.

Aim

Skin infections are reportedly frequent in Tunisian camels. Unfortunately, the diagnosis of these is limited to clinical observations. The objective of our research was to study, for the first time, a suspected outbreak of pox diseases in a herd of camel in Tunisia using an HRM-based assay for the differential diagnosis of poxviruses in cattle and camels, and further, characterize the detected virus molecularly.

Materials and Methods

The study was conducted in 2019 in the governorate of Sousse, where six of pox like disease outbreaks occurred. Representative scabs lesions were collected from five camels showing suspected clinical signs of pseudocowpox infection. An HRM assay for the simultaneous detection and differentiation of eight *poxviruses* of veterinary and public health importance was used to detect the genome of pseudocow poxvirus (PCPV). The partial B2L gene encoding the envelop protein of parapoxviruses was sequenced for comparative analyses. A Bayesian phylogenetic analysis was performed with BEAST v1.8.4 the tree was visualized in R using ggtree package.

Results

In the HRM, only one sample was positive, showing the typical melting of PCPV. We sequenced the B2L gene successfully for this sample. In the phylogenetic tree, the sample clustered with Camel Contagious Ecthyma Virus (CCEV), a lineage of PCPV infecting camels.

Keywords: Camelus dromadarius, pseudocowpox virus, B2L, HRM, Phylogenetic analysis, Tunisia

IAEA-CN-281-307

THE IMPORTANCE OF REAL TIME PCR AS A ROUTINE DIAGNOSTIC TOOL FOR THE CONTROL AND ERADICATION OF THE 2019 FOOT-AND-MOUTH DISEASE OUTBREAK IN MOROCCO

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Abstract

Foot and Mouth Disease (FMD) is a major viral disease that severely affect the production of livestock and disrupting regional and international trade in animals and animal products.
FMD is an OIE-listed disease and must be reported to the Organisation, as indicated in the OIE Terrestrial Animal Health Code (1)
Starting from January 2019, an outbreak of FMD has occurred in Morocco, therefore the use of rapid and accurate diagnostic tools was important for the effective control and the prevention of the disease. Reverse-transcription real time polymerase chain reaction (RT-qPCR) played an important role in the routine detection of FMD virus (FMDV) during this outbreak, showing as a the most rapid and effective diagnostic tool.

Keywords: Foot-and-mouth disease, FMD, FMDV, Morocco, Reverse-transcription real time polymerase chain reaction, RT-qPCR

Introduction

In the early 2019, an outbreak of FMD occurred in Morocco, starting from the region of Beni Mellal-Khenifra where the first cases were reported. The Office National de Sécurité Sanitaire des Produits Alimentaires (ONSSA), the national authority of food safety and animal health notified this first case to the Office International des Epizooties (OIE) on 10/01/2019, followed by other reports as the virus started to spread to other regions. ONSSA launched a vaccination campaign among susceptible animals across Morocco (2). The outbreak is officially resolved since 01/07/2019 (3)
FMD virus sequence data generated by international FMD reference laboratories at WRLFMD (Pirbright) and ANSES (France) has characterised the causative virus as belonging to the serotype O/ East Africa 3 (O/EA-3) topotype. Analyses of these sequences shows that these cases are due to a new virus incursion into the region, since the causative FMD virus is distinct to the serotype O (O/ME-SA/Ind-2001d lineage) FMDVs that caused previous outbreaks during 2013-15 in North African countries (4).
Reverse-transcription real time polymerase chain reaction was implemented in the Laboratoire Régional d'Analyses et de Recherches de Meknès, a regional official laboratory covering the regions of Fes-Meknes, Draa-Tafilalet and other nearby provinces, as a routine method for the detection of FMDV. The method selected targeting the 5' UTR region of the FMDV genome (5) (6) was used to detect the presence of the virus in field samples submitted during the period of outbreak.

Materials and methods

Clinical samples

A total number of 558 samples collected in 128 farms in 13 provinces were submitted by ONSSA's veterinary services. Those samples included epithelial lesions, mouth ulcers and/or whole blood. Fig1.

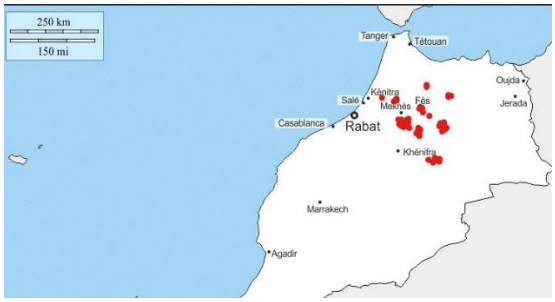

Fig 1. Geographical distribution of FMDV positive cases in the region of FES-MEKNES

Nucleic Acid extraction

Viral RNA was extracted using one of two different kits: MagMAX™ CORE Nucleic Acid Purification Kit (Applied Biosystems) or Maxwell® RSC simplyRNA Blood Kit (Promega). The extraction process was carried out by automated extraction machines: Maxwell® RSC Instrument (Promega) or MagMAX™ Express Magnetic Particle Processor (Applied Biosystems). Fig 2.

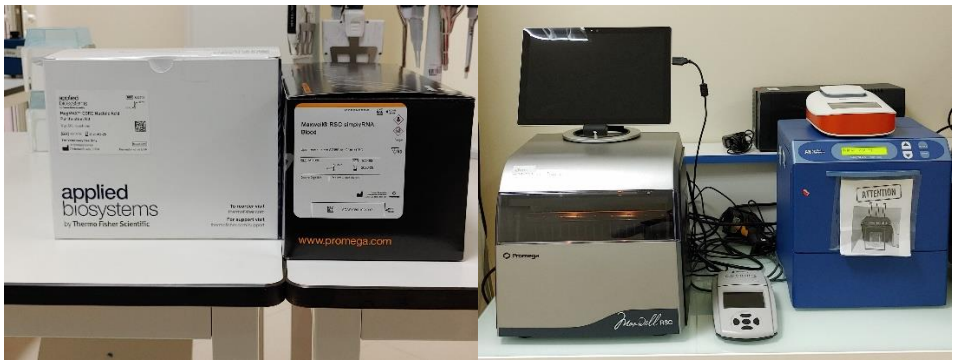

Fig 2. Extraction machines and kits

Primers/Probe set

A couple of primers and an MGB probe targeting the 59 untranslated region (5'UTR) of the FMDV genome were used as described by Reid et al. (5) Table 1.

Table 1. Primers and Probe sequences

Primer/Probe	Oligo name	Sequence (5'-3')	Working concentration
Forward primer	SA-IR-219-246F	CAC YTY AAG RTG ACA YTG RTA CTG GTA C	10 pmol/µl
Reverse primer	SA-IR-315-293R	CAG ATY CCR AGT GWC ICI TGT TA	10 pmol/µl
TaqMan probe	SAmulti2-P-IR-292-269R	FAM-CCT CGG GGT ACC TGA AGG GCA TCC-NFQ-MGB	5 pmol/µl

Reverse transcription real time PCR

For the amplification of RNA, A One-Step RT-PCR Kit (AgPath-ID™ One-Step RT-qPCR by Applied Biosystems) was used according to the manufacturer manual.
The RT-PCR mix is shown in the table below: Table 2.

Table 2. Composition of the fmdv mastermix

Component		Volume (µL)
RT-PCR master mix	2X RT-PCR Buffer	12,5
	Forward and reverse PCR primers	1
	TaqMan® probe	0,5
	25X RT-PCR Enzyme Mix	1
	Nuclease free water	4
RNA sample (or nuclease-free water for Negative Template)		5
Total volume per reaction		25

The Real-Time PCR machine used is QuantStudio™ 5 Real-Time PCR System (Applied Biosystems™) with the thermal protocol shown in the table below: Table 3.
NB: ROX (included in the RT-qPCR Buffer) must be set as Passive reference dye in the machine program.

Table 3. Thermal protocol used for FMDV amplification

Step	Stage	Cycles	Temp	Time
Reverse transcription	1	1	45°C	10 min
RT inactivation/initial denaturation	2		95°C	10 min
		1		
Amplification	3	50	95°C	15 sec
			60°C	60 sec

Results and discussion

From the 558 samples included in this study, 198 were positive for the FMDV, the mean CT values are 36 and 26 for the whole blood samples and epithelial suspensions respectively. The Fig 3. shows the difference in CTs for blood and epithelial samples collected from the same animal. So, when possible, priority should be given to epithelial samples over blood samples.
FMD is an extremely contagious disease, therefore an early detection and warning systems combined with rapid and effective diagnostic tools are important for disease control strategy. In this case, Real-Time PCR tests help monitor the occurrence and prevalence of the disease and provide results in less than 4 hours, giving precious information to the national authorities for decision makings.
Serotyping PCR methods and sequencing are under implementation in the laboratory, also combining the 5'UTR method with the 3D method will be adopted routinely to reduce the risk of having false negatives.

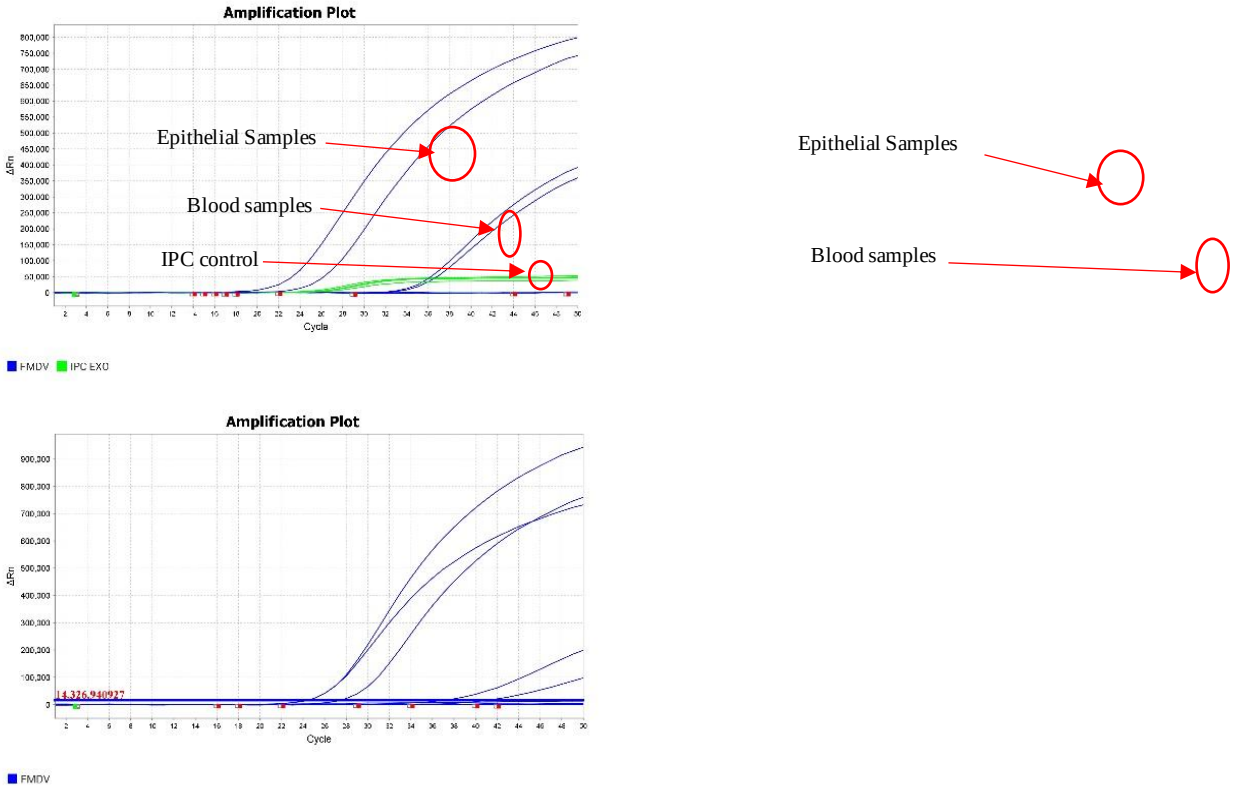

Fig 3. Amplification curves showing the differences of CTs between blood and epithelial samples.

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MOLECULAR DETECTION AND CHARACTERIZATION OF *EHRLICHIA RUMINANTIUM* FROM CATTLE IN MAPUTO PROVINCE, MOZAMBIQUE

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Heartwater (cowdriosis) is a tick-borne disease that affects domestic and wild ruminants throughout sub-Saharan Africa and three Caribbean islands (Guadeloupe, Marie Galante and Antigua) (Jongejan and Uilenberg, 2004). The disease is caused by *E. ruminantium* an obligate intracellular Gram-negative bacterium belonging to the order Rickettsiales and family Anaplasmataceae and transmitted by *Amblyomma* ticks (Allsopp, 2015; Walker et al., 2013) The disease represents a significant obstacle to improvement of livestock production in the tropical and subtropical countries. Control of heartwater through vaccination is not particularly successful because of the high genetic diversity of the pathogen (Vachiéry et al., 2008). Therefore, it is important to evaluate the genetic diversity of *E. ruminantium* in order to design appropriate vaccines in the future (Cangi et al., 2017). In Mozambique the prevalence, spatial distribution and genetic diversity of *E. ruminantium* are still unknown (Matos et al., 2019). The present study aimed to investigate the occurrence and genetic diversity of this agent. For this end 210 EDTA-blood samples were collected, by convenience, from adult cattle apparently healthy in five localities in the province of Maputo, Mozambique (Fig. 1). DNA was extracted from 200 µL of each blood sample using the DNeasy® Blood & Tissue Kit (Qiagen®, Valencia, CA), according to manufacturer's instructions. DNA blood samples were initially submitted to Polymerase Chain Reaction (PCR) assays targeting *E. ruminantium* pCS20 gene fragments (Faburay et al., 2007). Additionally, in order to assess the genetic diversity of *E. ruminantium*, the positive samples were submitted to a PCR assay targeting the *E. ruminantium* map1 gene, according to Faburay et al. (2007). Finally, the amplicons were sequenced in both directions by Sanger's method (Sanger et al., 1977) and the identity values among the nucleotide sequences were assessed by BLASTn tool available in NCBI GenBank database. The phylogenetic analysis was performed by Maximum Likelihood method (ML) by RAxML-HPC BlackBox 7.6.3 (Stamatakis et al., 2008) and models GTRGAMMA (Tamura et al., 2011). PCR results revealed that *E. ruminantium* DNA was detected in all five searched localities with an overall prevalence of infection of 15% of the animals sampled. Nine and 11 nucleotide sequences of the pCS20 and map1 genes were successfully amplified sequenced and deposited in GenBank database. The identity among the *E. ruminantium* pCS20 sequences obtained in the present study ranged from 98 to 100% while the identity among *E. ruminantium*-map1 nucleotide sequences ranged from 85 to 100%. In the phylogenetic analysis, *E. ruminantium* map1 genotypes were positioned into multiple-clades (Fig. 2). Considering that *E. ruminantium* mainly resides in endothelial cells and can only be periodically found in the bloodstream, low prevalence rates based on PCR assays from blood samples are expected (Lorusso et al., 2016). The genetic diversity of map1 observed in the cladogram was further confirmed by low identity values obtained by BLASTn analysis. An *E. ruminantium* prevalence of 15% was reported in this study with all of the positive cattle being apparently healthy. This study also revealed a high genetic diversity of *E. ruminantium* circulating in cattle in Maputo Province, which should be taken into account when delineating future control measures. These data emphasize the importance of conducting extensive studies on genetic diversity of *E. ruminantium* in the country.

Keywords: Heartwater (cowdriosis); PCR; pCS20; map1; Maputo Province; Mozambique

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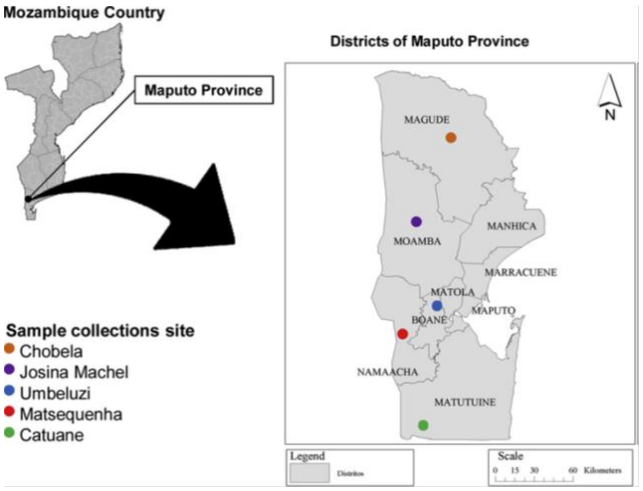

Fig. 1. Sites of cattle blood sample collection in Maputo Province, Mozambique

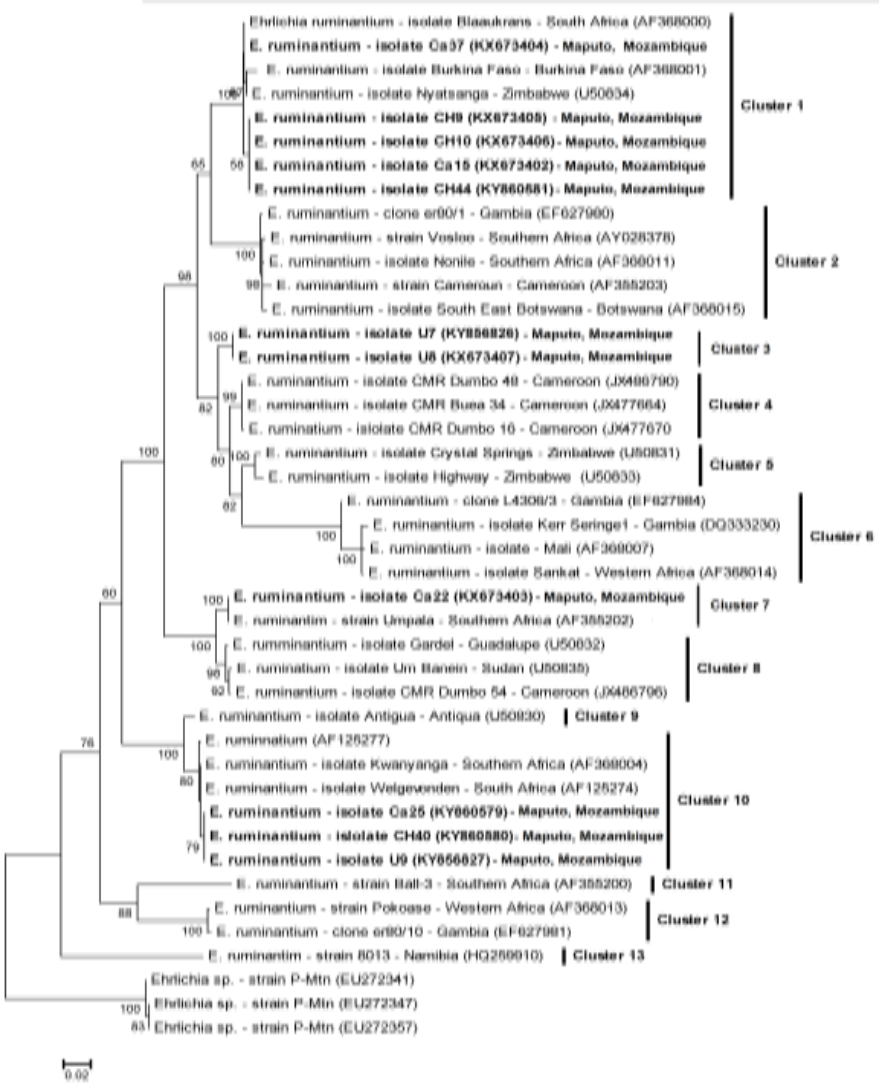

Fig. 2. Phylogenetic positioning of *E. ruminantium map1* sequences obtained from cattle sampled in five localities in Maputo, Mozambique. The sequences determined in the present study are shown in boldface letters. The numbers at the nodes correspond to boostrap values accessed with 1.000 replicates. *Ehrlichia* sp. sequences were used as outgroup. Ca: Catuane, CH: Chobela and U: Umbelúzi locations

IAEA-CN-281-316

CHARACTERIZATION OF THE AFRICAN SWINE FEVER VIRUS IN BURKINA FASO

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Keywords: ASF, Characterization, Burkina Faso

Background

African swine fever (ASF) is a viral disease that affects domestic and wild swine. ASf is endemic in Burkina for since 2003. During October 2018, heavy pig deaths occurred in the Ouagadougou and neighbouring municipalities of Saaba and Koubri in central Burkina Faso (Figure 1). Following these mortalities, the General Direction of Veterinary Services (DGSV) carried out investigations in order to undertake the appropriate control measures. This communication presents the results of the outbreak investigation and the molecular characterization of ASF virus involved in this 2018.

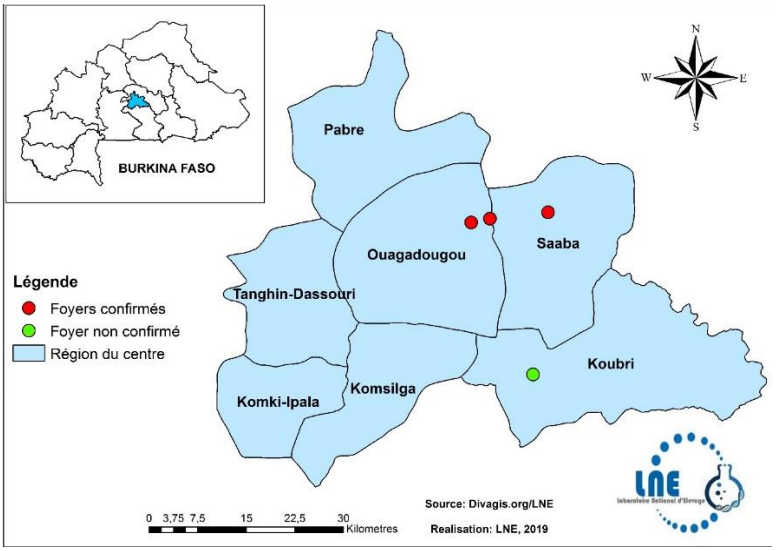

Figure 1: Pig mortality sites in the center region, kadiogo province

Methods

A team of the Animal Health Services and the National Livestock Laboratory visited the sites for outbreaks investigation and epidemiological surveys. Blood samples were collected in dry tubes and EDTA tubes from 69 sick animals for laboratory confirmation by real-time PCR and molecular characterization of the virus. For molecular characterisation, the partial P72 and P54 CD2v genes, B602L were amplified and sequenced.

Results

The field survey revealed that the mortalities had started approximately two weeks before the investigation. In the farms affected, sick animals showed the following symptoms: depression,

inappetence and redness of the skin, especially on the ears (Figure 2). On all affected farms, because of poor biosecurity measures in place, butchers had visit of for the acquisition of new animals introduced without prior quarantine, contributing to the dissemination of the disease.

Figure 2: Skin rash on pigs' ears and feeds

The real-time PCR results confirmed ASFV DNA in twenty-two samples out of sixty-nine blood samples collected in 4 different locations (Figure 1).
The sequencing and phylogenetic analysis showed that viruses responsible belongs to genotype I and serogroup 4 (Figure 2). The analysis of the CVR showed four variants of tetrameric repeats.

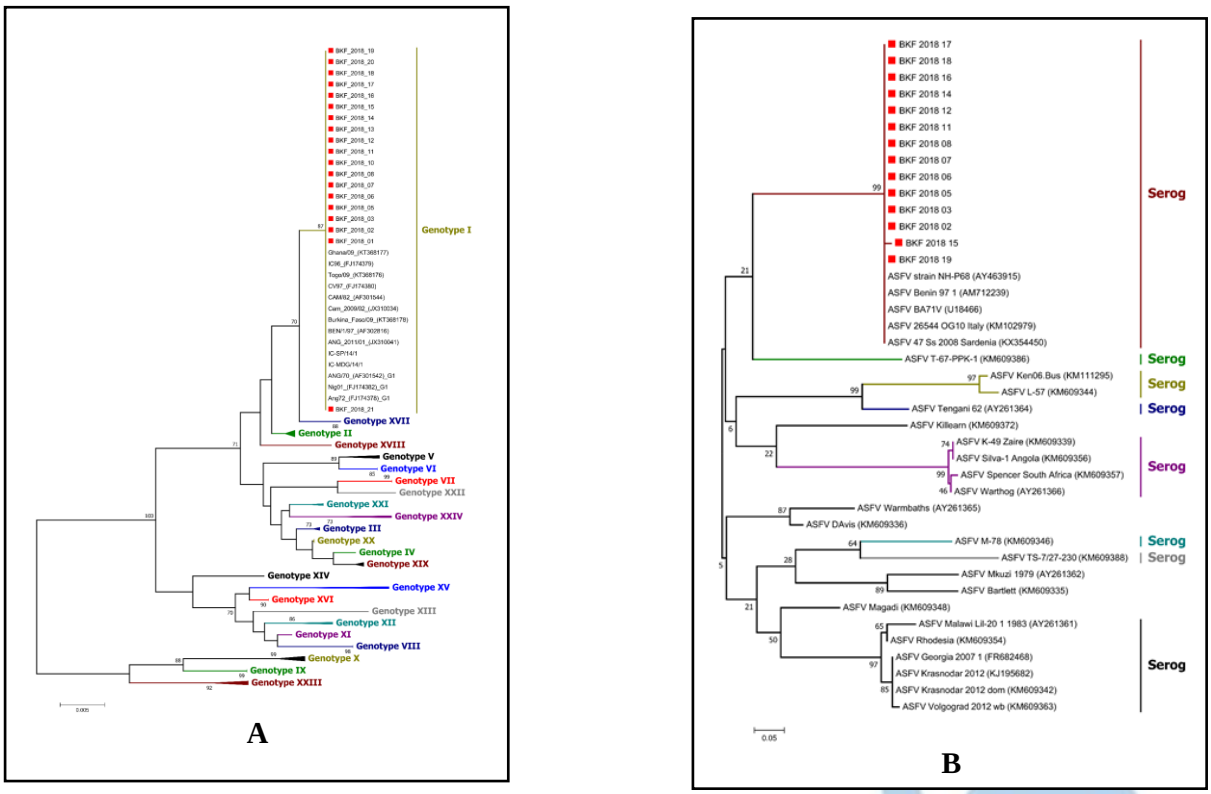

Figure 3. Phylogenetic analysis of ASFVs from Burkina: A) Neighbour-joining tree, of the partial p72 gene; B) Maximum Likelihood tree based on the partial amino acid sequence of the CD2v protein.

Our findings that ASF genotype I caused these outbreaks is consistent with previous reports showing that only ASFV genotype 1 is present in West Africa (**Couacy et al., 2018**).
Nevertheless, the four CVR variants in the 2018 samples were different from previously described variants in the in Burkina Faso (**Alkamis et al., 2018).**

Further studies are needed to understand how these variants have emerged. The presence of multiple CVR variants involved in these outbreaks shows the importance of molecular characterization to understand in real-time the evolution of ASFV isolates and the link between epidemics.

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IAEA-CN-281-322

FIRST MOLECULAR CHARACTERIZATION OF AFRICAN SWINE FEVER VIRUS IN MONGOLIA

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ASF virus was first isolated in Africa in 1907 and it became widespread in the eastern and northern countries of Europe in 2007. Last August 2018, the first case of ASF in Asia was reported in China specifically in Liaoning Province where in several farms were infected by the disease. African Swine Fever (ASF) occurred in 7 provinces as part of the ASF incursion into Mongolia during 2019. Infection of pig was confirmed by PCR and qRT-PCR for each affected country but not necessarily for every outbreak cluster involving more than one herder. In January, the samples were sent to and subsequently confirmed for ASF by the Animal Production and Health Laboratory, Joint FAO and IAEA, and the Pirbright Institute, World Organization for Animal Health (OIE) ASF reference laboratory. On 15 January, following confirmation by the SCVL, the Government of Mongolia (GoM) notified the OIE about the outbreak. Based on the result of the gene sequencing, it was found out that the P72 gene which is under the Genotype II is 100% the same as the Chinese strain isolated in 2018.

IAEA-CN-281-324

MOLECULAR DETECTION AND CHARACTERIZATION OF PAPILLOMAVIRUS IN CATTLE IN GAZA, MOZAMBIQUE

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Introduction

Bovine papillomatosis is a cosmopolitan, chronic infectious and contagious disease that occurs mainly in cattle and is caused by viruses belonging to the papillomaviridae family, which presents tropism for squamous and mucous epithelial tissue. The viruses infect the basal cells inducing them to form tumor lesions, known as papillomas, in the skin and mucosal epithelia (Claus et al, 2007; Carvalho, 2008). The lesions are generally benign and tend to regress, but under special conditions, they can turn into malignant tumors (Araldi, 2014;). Cutaneous papillomatosis is widespread worldwide and occurs in several species of mammals and birds (Daudt et al, 2018).

Figure.1: Animals infected by Papillomavirus

The papillomavirus genome consists of a double circular DNA strand containing 10 open reading frames (ORF), distributed in two main regions (E and L), according to the transcription phase (Claus et al, 2007: Torre et al, 2017).

Objective

The present study aimed to detect and to identify the type of virus involved in a case of papillomatosis in cattle in Chidenguele, Gaza province, Mozambique.

Methodology

Biopsies of skin lesions were taken in 8 cattle, in a production unit in the family sector, located in Chidenguele, Manjacaze District, in Gaza Province, south of Mozambique. The samples were preserved in glycerin for viral DNA detection and phylogenetic analysis. Virus DNA extraction was performed at the Central Veterinary Laboratory using DNeasy Blood and Tissue kit (Qiagen) according to the

manufacturer's instructions. The DNA samples were sent to APHL, IAEA Laboratories Seibersdorf, Austria, for diagnostic confirmation and further characterization.
The partial L1 open reading frame (ORF) of the Delta papillomaviruses was amplified and sequenced using primers Aup-CCAGAYTAYYTMAAAATGGC and Adw-ATAAMKGCTAGCTTATATTC (Araldi, et al., 2014). The sequences were edited and assembled using Vector NTI 11.5. Multiple sequence alignments were performed with BioEdit and MEGA 7. A Neighbour Joining tree was constructed using the Maximum Composite Likelihood method in MEGA7.

Results

Papillomatous lesions were observed in young and adult animals, and were located on the head, neck and back.
A 430 bp product corresponding to PBV was detected in 7 out of the 8 samples by PCR (Figure 2).

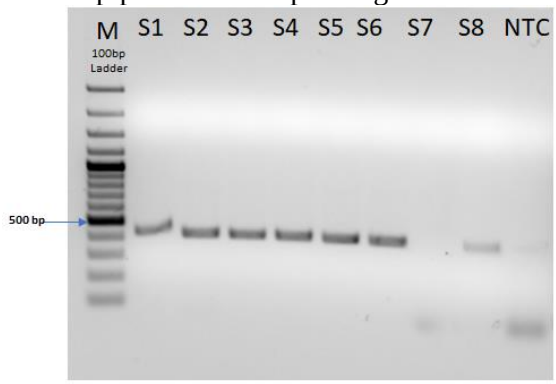

Figure 2: PCR detection of papillomavirus. Note the presence of the 430 bp product in the Mozambican samples

The multiple sequence alignments showed that all 7 Mozambican BPV sequences were identical.
According to phylogenetic analysis (Figure 3) the virus involved in this outbreak belongs to PBV1 (Delta papillomavirus).

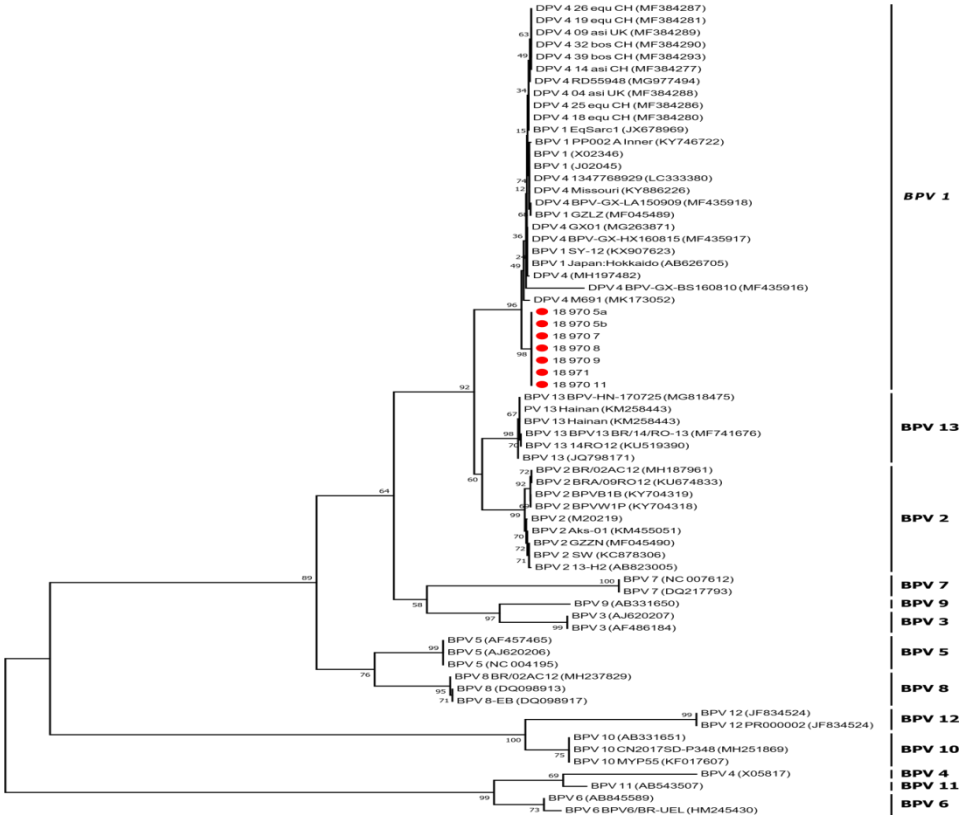

Figure 3: Phylogenetic tree of the identified virus

Discussion

According to phylogenetic analysis, the virus was identified as PBV-1. To our knowledge, this is the first study of PBV genetic characterization in Mozambique.

Studies carried out in South Africa revealed the circulation of PBV-1 in sables, buffaloes, giraffes and zebras (Van Dyk E, et al, 2009; Van Dyk E, et al, 2011; Williams JH, et al, 2011). In our study the viruses was isolated in animals that are located and grazing areas somehow close to Limpopo National Park, so there is a high probability of wildlife animals being the source of the BPV.

The multiple sequence alignments also revealed that the sequences are 100% identical, therefore the viruses are homogeneous. The isolates from our study are closely related to BPV1 identified in Asian, European and USA countries like Japan, China, UK and Switzerland, however, they formed a separated cluster.

Future epidemiological studies are needed for better understanding the genetic differences between the Mozambican BPV1 and those found elsewhere and assess the real impact of BPV in Mozambique to enable the implementation of suitable control measures.

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ADVANCES IN VACCINOLOGY

IAEA-CN-281-103

EFFECTS OF ENCAPSULATED GAMMA-IRRADIATED *ICHTHYOPHTHIRIUS MULTIFILIIS* TROPHONTS IN CALCIUM PHOSPHATE AND ALGINATE NANOPAPRTICLES ON OXIDATIVE STRESS BIOMARKERS IN RAINBOW TROUT LIVER TISSUE

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Abstract

The effects of encapsulated gamma-irradiated *Ichthyophthirius multifiliis* trophonts in calcium phosphate and alginate nanopaprticles (as delivery and protective systems) on the oxidative stress biomarkers of the liver tissue in rainbow trout were studied. The level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), cholinesterase (CholE), Homocysteine (Hcy), adenosine deaminase (ADA) and heat shock protein (HSP) activity in the liver tissues of rainbow trout treated with gamma-irradiated trophonts and calcium phosphate and alginate nanoparticles were significantly higher than in control groups. In fish treated with calcium phosphate nanoparticles, there was a significant increase in lipid peroxidase, as evident from the increased MDA content in the liver tissue. Therefore, gamma-irradiated trophonts, alginate and calcium phosphate nanoparticles, encapsulated gamma-irradiatied trophonts with alginate and calcium phosphate nanoparticles have the potential to improve the immunity responses against *I. multifiliis* in rainbow trout. Furthermore, these results suggest that alginate nanoparticles could be more useful for development of gamma irradiated trophonts compared to calcium phosphate nanoparticles, which increases the lipid peroxidase activity in liver tissue.

Introduction

Ichthyophthirius multifiliis is a large ciliated protozoan parasite with distribution of throughout the world. The life cycle of *I. multifiliis* involves three stages: an infective theront, a parasitic trophont, and a reproductive tomont (Tucker and Robinson, 1990; Schäperclaus, 1991; Fu *et al*., 2014). The chemical agents currently used to control *I. multifiliis* have potential environmental and host toxicities (Rintamäki-Kinnunen and Valtonen, 1997). These compounds are restricted from use in food fish due to their carcinogenic and teratogenic properties (Shinn *et al.*, 2012). Thus, there is a crucial need to investigate effective routes to control *I. multifiliis*. Fish treatment against *I. multifiliis* and immunotherapies can be considered as options for chemotherapy in fish (Maki and Dickerson, 2003). This study aims at investigating the effects of encapsulated gamma-irradiated *I. multifiliis* trophonts in calcium phosphate and alginate nanoparticles on the oxidative stress biomarkers in rainbow trout liver tissue.

Materials and Methods

Gamma irradiated *I. multifiliis* trophonts were prepared as described previously. Irradiation of parasite trophonts was performed at 170 Gray gamma-ray doses (Osman *et al.*, 2009; Heidarieh *et al.*, 2014a). Alginate nanoparticles were synthesized by the irradiation method (irradiated at 30 KGy gamma-ray doses) (Heidarieh *et al.*, 2014b). Calcium phosphate nanoparticles were prepared of calcium chloride dibasic sodium phosphate and sodium citrate (Sigma-Aldrich Chime GmbH, Germany). The components of a formulation of 12.5 mM calcium chloride, 12.5 mM dibasic sodium phosphate, and 15.6 mM sodium citrate were mixed together and stirred for 48 h and then subject to 30-min of sonication (Sheikhzadeh *et al.*, 2017). The formation of the encapsulated irradiated trophonts with alginate and calcium phosphate nanoparticles was achieved by addition of 4 mL of the alginate/calcium phosphate nanoparticles solution to irradiated *I. multifiliis* trophonts (37˚C, pH 7.5) under magnetic stirring. Subsequently, 1 ml of PBS (pH 7.5) solution was added dropwise to the above solution under magnetic stirring at room temperature. The alginate/calcium phosphate irradiated *I. multifiliis* vaccines nanoparticles were centrifuged (30 min, 8000 × g at 4 °C) and the supernatant was discarded (Mohamed *et al.*, 2018). Finally, the sediment was dried and frozen in liquid nitrogen and freeze-dried overnight. The dry powder was kept frozen until further use. A total of 210 parasite free rainbow trout (mean weight = 50 - 60 g) were randomly assigned to 7 groups in triplicate, 30 fish per each aquarium. Dose rate of vaccine was 100 gamma-irradiated trophonts (170 Gray) per 150 g fish body weight (Heidarieh *et al.*, 2015).

Group 1 served as a healthy control (negative control). Group 2 was administered with gamma-irradiated trophonts. Groups 3 and 4 were administered with alginate and calcium phosphate nanoparticles, respectively. Groups 5 and 6 were administered with encapsulated gamma-irradiatied trophonts with alginate and calcium phosphate nanoparticles, respectively. Group 7 was non-immunized but infected with 10000 live trophonts as positive control one. Except for the negative control group, a second treatment with the same amount of irradiated parasite and nanoparticles was given after 10 days. In the ten days after the second treatment, fish were challenged with 1000 live trophonts (except negative and positive control groups) by the immersion method. Liver was removed from treated, healthy and infected fish under MS222 anesthesia (200 mg L^{-1}) after 30 days following immunization and washed with cold saline buffer. To obtain the enzymatic extract, tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) using an appropriate manual pestle. The homogenates were centrifuged (30000 g for 10 minutes), and the supernatant obtained after centrifugation was transferred into another tube and frozen at –20˚C. AST, ALT, LDH, ALP, SOD, CAT, GST, CholE, MDA, Hcy, ADA and HSP activities in the tissues were assayed by Olympus AU 600 autoanalyser by using commercial kits.

Results and Discussion

The level of AST, ALT, LDH, ALP, SOD, CAT, GST and MDA activity in the liver tissue of rainbow trout treated with vaccines and nanoparticles significantly differed from that in the control groups (Table 1). Treated fish caused a significant increase in the level of AST, ALT, LDH, ALP, SOD, CAT and GST activity, especially in the group treated via encapsulated gamma-irradiatied trophonts with alginate nanoparticles ($p<0.05$). The hepatic CholE, Hcy, ADA and HSP contents were significantly increased in all treated groups as compared to controls ($p<0.05$) (Table 1). The highest levels of hepatic CholE, Hcy, ADA and HSP are found in fish that treated with calcium phosphate nanoparticles ($p<0.05$) (Table 1). The MDA level was also observed to be significantly high in liver tissues of fish treated with calcium phosphate nanopartilces ($p<0.05$). We studied the effects of encapsulated gamma-irradiated *I. multifiliis* trophonts in calcium phosphate and alginate nanoaprticles (as delivery and protective systems) on oxidative stress biomarkers in rainbow trout liver tissue. Based on these results, it was observed that gamma-irradiated trophonts, alginate and calcium phosphate nanoparticles, and encapsulated gamma-irradiatied trophonts with alginate and calcium phosphate nanoparticles had the potential to improve the immunity response against *I. multifiliis* in rainbow trout. Furthermore, these results suggest that alginate nanoparticles could be more useful for development of encapsulated gamma irradiated

trophonts compared to calcium phosphate nanoparticles, due to the latter's tendency to increase lipid peroxidase activity in liver tissue.

Table. 1. Bioarker enzymes levels in the liver tissue of immunization rainbow trout against *I. multifiliis*

Biomarker Enzymes	G1	G2	G3	G4	G5	G6	G7
ALT	0.85 ± 0.01^{e}	1.39 ± 0.035^{d}	1.86 ± 0.01^{c}	1.98 ± 0.015^{b}	2.19 ± 0.015^{a}	1.95 ± 0.025^{b}	0.59 ± 0.01^{f}
AST	0.95 ± 0.01^{f}	1.17 ± 0.04^{e}	1.55 ± 0.01^{d}	1.74 ± 0.005^{b}	1.98 ± 0.015^{a}	1.61 ± 0.05^{c}	0.876 ± 0.011^{g}
LDH	1.17 ± 0.026^{e}	1.38 ± 0.03^{d}	1.17 ± 0.02^{e}	3.06 ± 0.04^{b}	3.35 ± 0.04^{a}	2.36 ± 0.06^{c}	0.87 ± 0.026^{f}
ALP	1.31 ± 0.12^{e}	2.42 ± 0.04^{d}	1.13 ± 0.04^{f}	3.04 ± 0.04^{b}	3.19 ± 0.02^{a}	2.85 ± 0.06^{c}	0.94 ± 0.03^{g}
SOD	0.94 ± 0.02^{f}	1.44 ± 0.07^{d}	1.08 ± 0.01^{e}	2.14 ± 0.04^{b}	2.9 ± 0.12^{a}	1.95 ± 0.02^{c}	0.82 ± 0.02^{g}
CAT	0.573 ± 0.015^{e}	0.823 ± 0.03^{d}	0.97 ± 0.005^{c}	1.07 ± 0.011^{b}	1.22 ± 0.06^{a}	0.975 ± 0.021^{c}	0.336 ± 0.025^{f}
GST	0.77 ± 0.01^{c}	0.96 ± 0.01^{c}	1.98 ± 0.01^{a}	2.17 ± 0.01^{a}	1.95 ± 0.05^{a}	1.37 ± 0.59^{b}	0.36 ± 0.02^{d}
MDA	1.1 ± 0.02^{e}	1.51 ± 0.02^{d}	2.77 ± 0.06^{b}	3.13 ± 0.11^{a}	2.33 ± 0.1^{c}	1.61 ± 0.03^{d}	0.75 ± 0.02^{f}
CholE	1.58 ± 0.03^{e}	2 ± 0.005^{d}	3.95 ± 0.1^{b}	4.39 ± 0.22^{a}	3.18 ± 0.15^{c}	2.1 ± 0.04^{d}	1.04 ± 0.04^{f}
Hcy	0.5 ± 0.01^{f}	0.72 ± 0.03^{e}	1.1 ± 0.005^{b}	1.32 ± 0.01^{a}	1.04 ± 0.03^{c}	0.8 ± 0.005^{d}	0.31 ± 0.005^{g}
ADA	0.39 ± 0.005^{e}	0.48 ± 0.005^{d}	1 ± 0.03^{b}	1.1 ± 0.06^{a}	0.78 ± 0.03^{c}	0.5 ± 0.01^{d}	0.26 ± 0.01^{f}
HSP	1.11 ± 0.02^{e}	1.63 ± 0.07^{d}	2.4 ± 0.01^{b}	2.9 ± 0.02^{a}	2.23 ± 0.07^{c}	1.81 ± 0.01^{d}	0.7 ± 0.01^{f}

Values are Mean ± S.E (n= 30); significant ($p<0.05$). Unlike letters in a row show significant differences.

G1: Healthy fish (negative control); G2: Rainbow trout immunized with gamma-irradiated trophonts of *Ichthyophthirius multifiliis*; G3: Rainbow trout immunized with alginate nanoparticles G4: Rainbow trout immunized with calcium phosphate nanoparticles; G5: Rainbow trout immunized with encapsulated gamma-irradiatied trophonts of *Ichthyophthirius multifiliis* with alginate nanoparticles; G6: Rainbow trout immunized with encapsulated encapsulated gamma-irradiatied trophonts of *Ichthyophthirius multifiliis* with calcium phosphate nanoparticles; G7: Infected with 10000 live trophonts of *Ichthyophthirius multifiliis* as positive control group.

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POST- VACCINATION MONITORING TO ASSESS IMMUNE RESPONSES OF GAMMA IRRADIATED FMD TYPE O/IRN/2010 VACCINE AND DNA VACCINE ON GUINEA-PIG MODEL BY PRIME BOOST STRATEGY

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Abstract

Vaccination of cattle against foot-and-mouth disease (FMD) is a well-established strategy for control of the disease. In this research, gamma irradiated FMD virus type O/IRN/2010 was used as an inactivated vaccine and a DNA vaccine was constructed based on the VP1 gene. The immune responses of vaccinated guinea pigs identified significant differences for gamma irradiated vaccine (GIV) and conventional vaccine groups relative to a negative control group ($P<0.05$), but there were no significant differences between these two groups ($P>0.05$). Also, the MTT test showed that proliferation of spleen T lymphocyte was increased significantly for the two vaccinated groups versus the negative control. PD50 of GIV was more than conventional vaccine. A heterologous prime boost strategy with DNA vaccine + GMCSF and inactivated vaccine is the best method for inducing humoral and cellular immunity and increasing Th1 cytokines.

Introduction

Prevention and control of disease is the main aim of livestock vaccines. Foot and Mouth Disease (FMD) is a severe contagious disease in cloven-hoofed animals (1). There are 7 serotypes for this virus, and three of them are in Iran. Nowadays, an inactivated vaccine is used for the prevention of this disease. Some disadvantages of the inactivated vaccine are short shelf life, chemical residues in the final product, viral escape and a long inactivation time (2). The aims of this research are preparation of inactivated FMD vaccine using gamma irradiation and evaluation of humoral and cellular immune responses in vaccinated guinea pigs.

Materials and Methods

Virus and Vaccines: FMD virus type O/IRN/2010 was multiplied and irradiated by different doses of gamma rays. Dose/responses curve were drawn by Origin software and D_{10} Value and the optimum dose of gamma irradiation for complement inactivation were calculated. Antigenic characteristics of irradiated and un-irradiated viral samples were compared using the complement fixation method, and a safety test for the inactivated virus was confirmed by four blind cultures. The inactivated virus was formulated as an gamma irradiated vaccine (GIV) with $Al(OH)_3$ as an adjuvant and saponin (3 and 4). The VP1 gene was also sub-cloned into the unique *Kpn* I and *Bam*H I cloning sites of the pcDNA3.1+ vector to construct the VP1 gene cassette as a DNA vaccine. A conventional vaccine was prepared by binary ethylene imine (BEI) inactivation (5 and 6).

Vaccination schedule: Eight guinea-pig groups were respectively immunized with 1) the sub-cloned pcDNA3.1+-VP1 gene cassette as the DNA vaccine (100 µg), 2) DNA vaccine (50 µg) and PCMV-

SPORT-GMCSF vector (50 µg, as molecular adjuvant), 3) GIV (200 µl), 4) conventional vaccine (200 µl), 5) prime boost (PB) 1 (DNA vaccine and PCMV-SPORT-GMCSF vector as the prime vaccine and conventional vaccine as boost vaccine), 6) PB2 (DNA vaccine and PCMV-SPORT-GMCSF vector as a prime vaccine and GIV as a boost vaccine), 7) PB3 (Gamma Irradiated vaccine as prime and DNA vaccine and PCMV-SPORT-GMCSF vector as boost vaccine) and 8) PBS (as negative control), each group contained 5 animals. Vaccination was done in two doses, separated by a 21 day interval. Blood samples and spleens of vaccinated animals were collected 2 weeks after the second vaccination.

Post-vaccination monitoring to assess immunity: Antiserum titration was detected by the sero-neutralization test (SNT), and proliferation of splenic T lymphocyte was measured by the MTT test (Cell Proliferation Kit 1 Roche, Cat No: 11-465-007-001). Cytokine assays were done by using ELISA kits for IFN-γ, Il2, Il4 and Il 10.

Challenge and PD50 assay: Seventy eight guinea pigs (weighing about 250 g) were divided into 26 groups (each group included 3 animals) for the viral challenge test. One group each was vaccinated by one of five serial dilutions (1, 1/2, 1/4, 1/8 and 1/16) for one of five kinds of vaccines including; GIV vaccine, Conventional vaccine, DNA vaccine + GMCSF, PB1 and PB2 groups and one group served as a negative control. The vaccination was given subcutaneously and the two booster doses followed 3-week intervals. On the fourteenth day after the last immunization each guinea pig was subcutaneously challenged with 0.2 ml of 100 ID 50 of guinea pig-adapted live virus (seventh passage) injection into the footpad and housed separately for a 7-day period of examination in a BSL-2 laboratory. In the vaccinated animals total protection was defined as absence of lesions on the footpad, partial protection was defined as lesions occurring on the injected foot only and without protection was defined when lesions were found on two or more feet and the tongue after challenge. The protective dose 50 (PD 50) was calculated for the five kinds of vaccines [16, 17].

Results

According to the Dose/Response curves for FMD virus type O/IRN/2010, with the first titration at about $10^{6.32}$ $TCID_{50}$/ml, the D_{10} Value and optimum dose of gamma irradiation for complement inactivation were calculated as 8.33 and 52.5 kGy, respectively. The safety test after four blind cultures for irradiated virus samples on the BHK_{21} cell line showed that the best dose of gamma radiation for complete inactivation is 50 kGy. The principal results are in Table 1. The antibody titration showed the highest humoral immunity in GIV and conventional vaccine groups and confirmed protection for PB1, PB2 and PB3 groups. Cellular immunity was the best in PB1, PB2, PB3 and DNA vaccine+GMCSF as the Stimulation Index (SI) for splenic lymphocyte proliferation. IFN-γ concentration in the GIV group increased significant compared to the negative control, but it was significantly less than with the DNA vaccine+GMCSF ($P<0.05$). IL2 and IFN-γ are the cytokines which were secreted from T helper cell class I (Th1), in the PB1 and PB2 groups there was a significant increase for Th1 cytokine concentration. There were no significant differences for IL10 concentration in DNA vaccine, DNA vaccine+GMCSF, GIV, PB1,PB2 and negative control groups, but a significant increase was observed for the PB3 group ($P<0.05$).
ID_{50} for guinea pig-adapted live virus (after seventh passages) was calculated as 297 ID_{50}/ml and it was used for challenge of the vaccinated groups and the PD50 of the five vaccine groups (GIV, Conventional vaccine, DNA vaccine + GMCSF, PB1 and PB2) were 7.07, 6.25, 4.44, 7.14 and 7.20, respectively.

Table 1: The results of neutralizing antibody titration, splenic lymphocyte proliferation (SI), IFN-γ, IL2, IL4 and IL10 two weeks after second vaccination

No.	Vaccine groups	Antibody titration ±SD	SI ±SD	Mean of IFN-γ concentration (pg/ml) ±S.D	Mean of IL2 concentration (pg/ml)	Mean of IL4 concentration (pg/ml) ±S.D	Mean of IL10 concentration (pg/ml) ±S.D

				±S.D			
1	DNA vaccine	0.9±0	1.23±0.123	619.76±144.28	22.39±8.51	247.19±72.17	73.04±52.12
2	DNA vaccine+GMCSF	1.2±0.17	1.44±0.131	1098±233.61	96.39±25.79	1339.38±1317	75.46±67.22
3	Gamma Irradiated vaccine	2.1±0	1.23±0.046	386.29±168.45	74.44±18.34	1641.80±841.64	818.78±276.10
4	Conventional vaccine	2.1±0	1.26±0.095	464.27±355	82.01±32.57	1278.26±1010.56	1543.07±1171.62
5	PB1	1.5±0	1.52±0.166	885.76±189.46	118.66±26.11	1759.427±1153.19	16.82±9.48
6	PB2	1.5±0	1.55±0.153	925.76±389	118.81±42.88	1988.46±1969	83.98±31.64
7	PB3	1.7±0.17	1.43±0.100	368.92±82.434	23.84±5.96	936.84±329.63	1698.69±1225.94
8	PBS (Negative Control)	0.9±0	0.98±0.051	3.844±0.58	1.25±0.39	79.11±10.18	32.17±6.87

Conclusion

Gamma Irradiated FMD virus type O/IRN/2010 can be used for inactivated vaccine preparation. Antibody titration showed humoral immunity was the same for GIV and conventional vaccine, but PD50 of GIV was greater. A heterologous prime boost strategy with DNA vaccine + GMCSF and inactivated vaccine is the best method for inducing humoral and cellular immunity and increasing Th1 cytokines.

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MODELING SENECA VIRUS A REPLICATION IN IMMORTALIZED PORCINE ALVEOLAR MACROPHAGES TRIGGERS A ROBUST IMMUNITY

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Seneca virus a (SVA), a causative agent of vesicular diseases in swine, has caused great economic loss in pig industry. Due to its genetic diversity and rapid evolution, SVA infection has spread pandemically in different regions and countries. The adaptive immunity against SVA has been elaborately studied, characterized by early neutralizing antibody response. However, research on cellular immunity triggered by SVA has largely been hampered by the lack of permissive immune cell lines, which support the life cycle of SVA. In the present study, we demonstrated that an immortalized porcine alveolar macrophage (iPAM) could robustly and productively support the replication of prototype SVV 001 and its field isolate strain SVA FJ. Meanwhile, a plasmid-based reverse genetics system for SVA could produce infectious SVA particles in iPAM. Meanwhile, SVA possessed sensitivity to ribavirin, a broad-spectrum antiviral agent and melanoma differentiation-associated protein 5 (MDA5), a key member of the RIG-I-like receptor (RLR) family in iPAM, indicating the availability and reproducibility of iPAM in supporting SVA infection.
Subsequent analysis showed that SVA could activate the IRF3/NFkB pathways, resulting in the expression of IFNs and a subset of cytokines. Meanwhile, the JAK-STAT pathway was also activated, which was coincident with the fact that SVA induced IFNs expression. Extra works will be done, including RNA sequence and proteomics analysis to deeply understand the innate immunity towards SVA infection in iPAM.

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EVALUATION OF IMMUNOGENICITY AND PROTECTIVE EFFICACY OF IRRADIATED *SALMONELLA GALLINARUM* AGAINST HOMOLOGOUS CHALLENGE INFECTION IN CHICKENS

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Background

Fowl typhoid is a systemic poultry disease caused by *Salmonella Gallinarum* (SG). It is responsible for significant economic losses, particularly in developing countries. Irradiated vaccine is one of the possible alternatives to prevent and control fowl typhoid. This study aimed to evaluate safety, immunogenicity and protective efficacy of irradiated SG. A field strain of SG was exposed to different doses of gamma irradiation to determine the effect of irradiation on the viability of SG. Safety and immunogenicity were assessed by administering irradiated SG orally at 2400, 2500 and 2600 Gy to 5 week old Bovans brown chickens. The protective efficacy of SG (10^8 CFU) irradiated at 2400 Gy administered orally and subcutaneously was then evaluated using homologous challenge infection and compared with SG 9R commercial vaccine.

Results

Irradiation at 2600 Gy caused complete inactivation of SG, whereas SG exposed to 2400Gy showed better immunogenicity with 2500 Gy and was safe for chickens. Antibody response in a group of chickens vaccinated with irradiated *SG* administered subcutaneously (SC) was significantly higher than those vaccinated with SG 9R vaccine at first ($p=0.003$) and second ($p=0.002$) week post immunization. Comparative evaluation of the protective efficacybased on mortality rate of chickens after challenge showed that 2400Gy irradiated SG vaccine administered SC and SG 9R vaccine induced equal protection of 50% while the irradiated vaccine administered orally protected only 10% of chickens against homologues challenge infection. SG was not isolated from liver, spleen and feces of chickens that survived challenge infection at the end of the experiment.

Conclusion

Irradiated SG administered SC is a promising vaccine against fowl typhoid. Furtherstudies using larger sample sizes and involving tuning of the irradiation dose to improve immunogenicity, use of booster vaccination and assessment of efficacy of vaccine preserved under different conditions are recommended.

Keywords: Chicken, Fowl typhoid, Gamma irradiation, *Salmonella Gallinarum*, Vaccine

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DEVELOPMENT OF A MUCOSAL VACCINE AGAINST PEST-DES-PETITS RUMINANT VIRUS FOR SMALL RUMINANTS

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Abstract

Peste des petits ruminants (PPR) is a highly contagious viral disease affecting mainly small ruminants and is associated with high mortality and morbidity causing severe economic losses. The principal method for the control of PPR is per subcutaneous (s.c) vaccination with a live attenuated vaccine like the PPRV/Nigeria/75/1 strain. Needle vaccination is costly as it requires professional staff to apply. Therefore we developed an ocular vaccine formulation based on the PPRV/Nigeria/75/1 strain to allow the application by trained village vaccinators. Different formulations of cryo-protectants and viral concentrations were tested in goats and the immune response determined by ELISA and Virus neutralization test (VNT). It revealed that a dose of 100 ul ocular vaccine having viral concentration of 10^5 $TCID_{50}$/ animal was optimum for a consistent immune response in tested animals. The immune response of the ocular vaccine was compared with conventional subcutaneous vaccine having same viral concentration per animal in 1 ml dose. All vaccinated animals produced neutralizing antibodies with very similar patterns of antibody titers for subcutaneous route or through ocular route over the 6 months follow up. These promising results for the ocular application of a vaccine against PPR virus in goats warrants further studies on the minimal protective dose of the vaccine virus and its formulation for heat stability to confirm if the ocular application could be used easily under field conditions for PPR control programs in small ruminants.

Keywords: Mucosal vaccine, Immunity, Peste des petits ruminants, PPRV, Cell Culture, VNT

Table 1: Experimental design of the animal trial-1

No.	Experimental group	No. of animals	Treatment	Dose of vaccine	Composition of vaccine	$TCID_{50}$ of vaccine
1	Group -1	5	Subcutaneous with booster	1 ml	Sucrose and Lact Albumin	10^4 $TCID_{50}$/ Animal
2	Group -2	5	Ocular with booster	100ul	Trehalose	10^4 $TCID_{50}$/ Animal
3	Group -3	5	Subcutaneous single dose	1 ml	Sucrose and Lact Albumin	10^4 $TCID_{50}$/ Animal
4	Group -4	5	Ocular single dose	100ul	Trehalose	10^4 $TCID_{50}$/ Animal
5	Group -5	5	Negative Control	1 ml	PBS	No virus

Table 2: Experimental design of the animal trial-2

No.	Experimental group	No. of animals	Treatment	Dose/ animal	Composition of vaccine	$TCID_{50}$ of vaccine

1	Group -1	5	Ocular vaccine	100 ul	Sucrose and Lact-Albumin	10^6 $TCID_{50}$/ Animal
2	Group -2	5	Ocular vaccine	100 ul	Sucrose and Lact-Albumin	10^5 $TCID_{50}$/ Animal
3	Group -3	5	Ocular vaccine	100 ul	Sucrose and Lact-Albumin	10^4 $TCID_{50}$/ Animal
4	Group -4	5	Negative Control	100 ul	Sucrose and Lact-Albumin	No virus

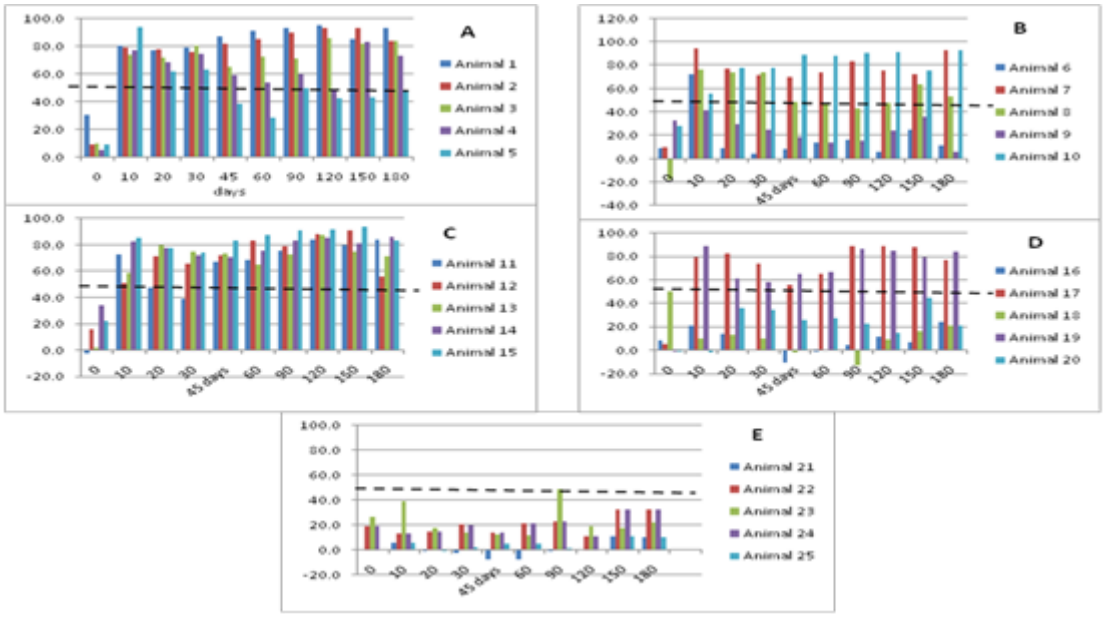

Figure 1: ELISA serum antibody levels (as PI) of experimental goats vaccinated with NIG 75/1 log 1x 10^4 TCID50/ dose during animal trial-1. Percent inhibition values (P.I) ≥ 50 is considered as positive (Dotted line indicates cutoff value). Group 1: Animals got S/C immunization on day 0 and day22, Group 2: Animals got ocular immunization on day 0 and day 22, Group 3: Animals got single S/C immunization on day 0, Group 4: Animals got single ocular immunization on day 0, Group 5: Animals without immunization served as negative control.

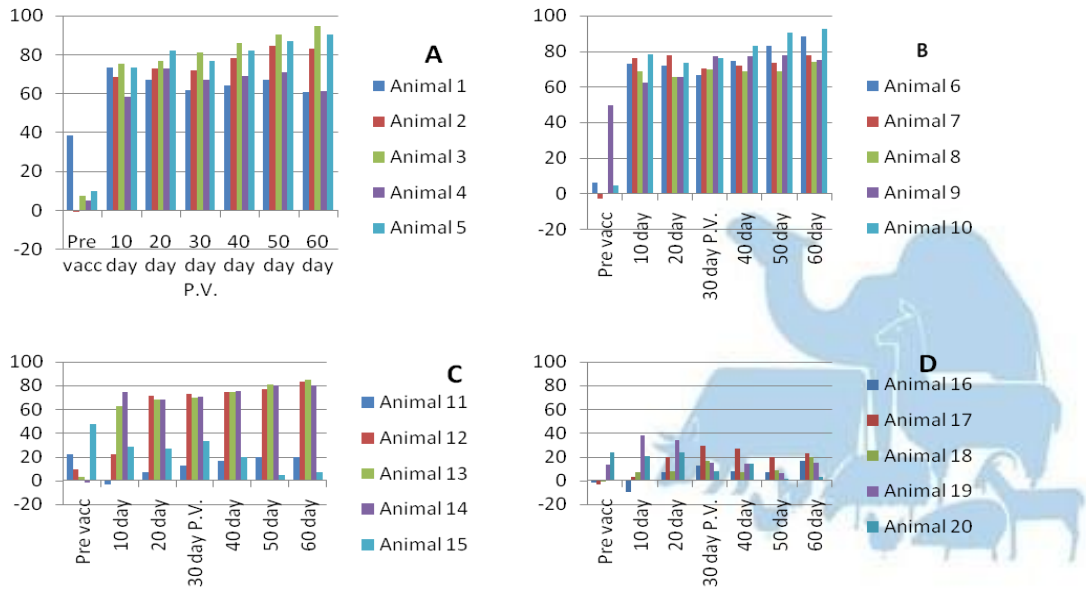

Figure 2: ELISA serum antibody levels (as PI) of experimental goats vaccinated with NIG 75/1 ocular vaccine having different viral titres (1 x log 10^6/animal, 1 x log 10^5/animal and 1 x log 10^4/animal) during animal trial-3. Percent inhibition values (P.I) ≥ 50 is considered as positive. Group 1: Animals got single ocular immunization with log 10^6 TCID50/ dose having sucrose, lact-albumin as cryo-protectant. Group 2: Animals got single ocular immunization with log 10^5 TCID50/ dose having sucrose, lact-albumin as cryo-protectant, Group 3: Animals got single ocular immunization with log 10^4 TCID50/ dose having sucrose, lact-albumin as cryo-protectant, Group 4: Animals without immunization served as negative control.

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LOW-ENERGY ELECTRON IRRADIATION FOR THE GENERATION OF INACTIVATED VACCINES

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Inactivated vaccines are commonly produced by incubating pathogens with chemicals such as formaldehyde or beta-propiolactone. This is a time consuming process, the efficiency is highly variable and extensive downstream procedures are often required. Moreover, application of chemicals alters the antigenic components of the pathogens, resulting in reduced vaccine specificity and therefore stimulation of a less effective immune response. An alternative method for inactivation of pathogens is ionizing radiation. Thereby mainly the pathogen´s genome is damaged, while structural components remain largely intact. However, high radiation doses are required to inactivate viruses or bacteria, and common technologies such as gamma- or X-rays require massive shielding constructions due to emission of substantial amounts of radiation into the environment. This has so far precluded the integration of ionizing radiation in vaccine production processes including good manufacturing practice (GMP) environment. As a consequence, irradiated viral or bacterial vaccines are not yet available. Low-energy electron irradiation (LEEI) delivers the same dose range as gamma- or X-rays, however, it has two critical advantages: firstly, it has a much higher dose rate, shortening the time required for applications in the multiple kiloGray (kGy) range to seconds; secondly, it generates only almost no secondary radiation (Bremsstrahlung) and does not require significant shielding. LEEI can, therefore, be applied in any laboratory or production facility. However, LEEI has a very limited penetration depth and was therefore until now only used for surface treatment. In a research consortium lead by the Fraunhofer Institute IZI and also containing the Fraunhofer Institutes FEP and IPA, LEEI was adapted to treat pathogen-containing liquids. In a variety of studies we have shown that LEEI inactivated pathogens are well suited as vaccines against several human and veterinary infections. Moreover, we have developed principles and research-prototypes for the industry-scale application of LEEI. In the talk I will present examples of vaccine projects and will give an overview of the current developments and future perspectives to transform LEEI into a valid alternative to chemical treatment in vaccine production.

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INDUCTION OF PROTECTIVE IMMUNE RESPONSE IN EXPERIMENTAL BOVINE CALVES BY Γ-RADIATION ATTENUATED *TRYPANOSOMA EVANSI*

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Synopsis

'Surra,' caused by *Trypanosoma evansi*, is an economically significant disease of livestock. The low host specificity and mechanical mode of transmission by hematophagous flies have facilitated the spread of the parasite beyond the tsetse belt of Africa to many tropical countries of Asia and South America. Cattle and buffaloes usually suffer from a chronic form of the disease. The clinical signs of surra include fever, anaemia, inappetence, nervous symptoms, abortion and cachexia that eventually cause the death of the host. The extensive prevalence of surra in almost all agro-climatic zones of India has adversely impacted the farm-based economy through reduced productivity, morbidity and mortality of livestock. The estimated annual economic loss is to the tune of US $ 671.1 million. Currently, surra is managed by chemotherapy with a limited number of commercially available drugs. It has not been possible yet to develop a protective vaccine against the disease. The systematic antigenic variation by the parasite during infection has limited the value of the surface glycoproteins as vaccine candidates. The present investigation reports the induction of protective immune response in cattle by γ-radiation attenuated *T. evansi*.

A cryopreserved horse isolate of *T. evansi*, used in the study, was propagated *in vivo* in experimental mice. The parasites were purified by DEAE cellulose chromatography and were exposed to γ-ray (500Gy) from a cobalt-60 source. Sixteen male bovine calves of Vrindavani breed, aged 2-3 months, were randomly divided into four equal groups. The trypanosome-free status of the calves was established before their inclusion in the experiment. The calves in Group I and II were immunized with 5×10^6 and 1×10^7 attenuated *T. evansi*, respectively, on days 0 and boosted on days 15 and 35 via the subcutaneous route. The animals in Group III and IV served as non-immunised infected and healthy controls, respectively. The calves in the first three groups (Group I to III) were challenged with 1×10^3 virulent *T. evansi*, homologous strain, through subcutaneous route on day 50. The clinical, parasitological, haematological, and immunological parameters were compared between the treated and the non-immunised control groups.

No parasite detected in the immunized calves post-challenge and the animals were free from parasitemia during the observation period of 150 days. In the non-immunised group, parasitaemia developed on day 14 post-challenge (PC). The calves were clinically ill with elevated rectal temperature and remained PCR positive for *T. evansi* ITS1 till weeks 4 to 6 PC. Progressive anaemia and weakness recorded in these calves. Further, a gradual decrease in Hb, PCV and TEC counts recorded in Group III.

The humoral IgG, IgM, IgG1 and IgG2 responses were monitored post-immunization (PI) and challenge (PC) by ELISA using sera samples collected at weekly intervals. The radiation-attenuated *T. evansi* induced a robust IgG and IgM antibody responses. Although, both IgG1 and IgG2 responses were noted in the immunized calves, the IgG2 response was predominant, suggesting a Th1 polarisation. This observation was consolidated by the data on upregulation of Th1 cytokines. The non-immunised animals in the control group (GroupIII) showed exclusive IgG1 response which implied an anti-inflammatory Th2 polarization of the immune response. The expression profile of the IFN-γ, TNF-α, IL-1β, IL-2, IL-4, IL-10, and IL-17 genes was studied by real-time PCR, keeping GAPDH as the endogenous control. Upregulation of the pro-inflammatory cytokine transcripts, viz. IFN-γ, TNF-α, IL-

1β, IL-2, and IL-17 recorded on day one and three post-immunization, whereas, the IL-10 and IL-17 transcripts were down-regulated throughout, except on days one and 3-PI in Group II. Increased expression of IL-1β and IL-2 recorded after the first immunization whereas, the same decreased after the second and third immunizations. A significant increase ($p<0.001$) in the post-challenge upregulation of the cytokine transcripts recorded in Group I. Conversely, calves in Group II showed decreased expression of TNF-α, IL-1β, IL-2, IL-10 and IL-17 transcripts on days one and three post-challenge. However, significant ($p<0.001$) upregulation recorded on days 14 and 28-PC when compared to the mon-immunized calves (Group III). The immunized calves showed higher expression of Th1 cytokine transcripts, vis-a-vis the IL-10 mRNA transcripts. This balance might have played a crucial role in protection. On the contrary, expression of IL-10 transcripts was maximum on day 28 PC with significant downregulation of the pro-inflammatory cytokine genes in the non-immunised calves.
The data indicate the pivotal role of IgM and IgG2 antibodies, supported by Th1 cytokines, in the protection of the calves immunized with radiation-attenuated *T. evansi*.

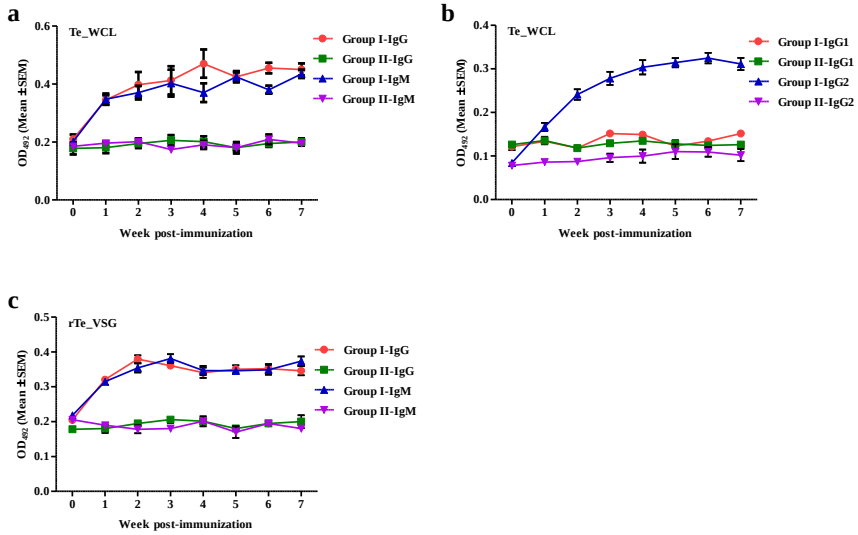

Fig.1 Post-immunisation *T. evansi*-specific IgG and IgM (a); IgG1 and IgG2 (b) and VSG-specific IgG and IgM (c) response in bovine calves. The antibody response was measured by ELISA and compared with pre-challenge humoral response data from unimmunized challenge controls by linear mixed-effects model analysis. The data presented as Mean±SEM of OD_{492} values recorded on different weeks, derived from four biological replicates. Error bars denote natural variation within the group.

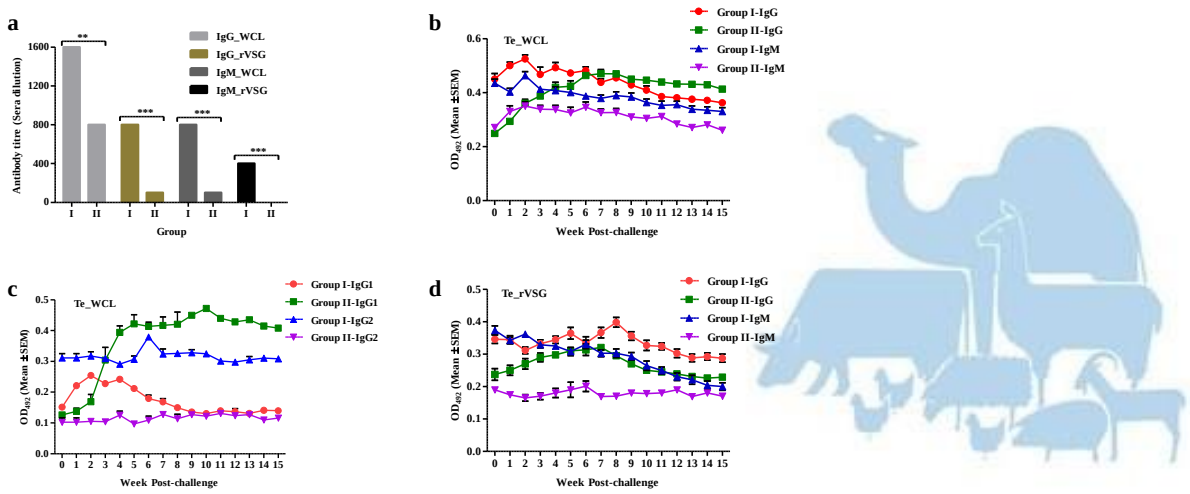

Fig. 2. *T. evansi* specific post-challenge humoral response. Bar diagram represent peak antibody titre recorded (a); *T. evansi*-specific IgG and IgM (b); *T. evansi*-specific IgG1 and IgG2 (c) and VSG-specific IgG & IgM (d). The data were analysed by linear mixed-effects model. Data presented as Mean±SEM of OD_{492} values recorded on different weeks post-challenge, derived from at least four biological replicates. Error bars denote biological variation within the group.

Antibody titre was determined by ELISA with a serial dilution of sera samples. Data presented as the dilution of sera scored above the cut-off as the antibody titre. Asterisk indicates the significant differences between groups ($p<0.001$). The cut-off value (Mean+2SD) calculated using data from healthy controls was 0.26 for WCL_IgG and WCL_IgM; 0.25 and 0.26 for rTeVSG_IgG and rTeVSG_IgM, respectively.

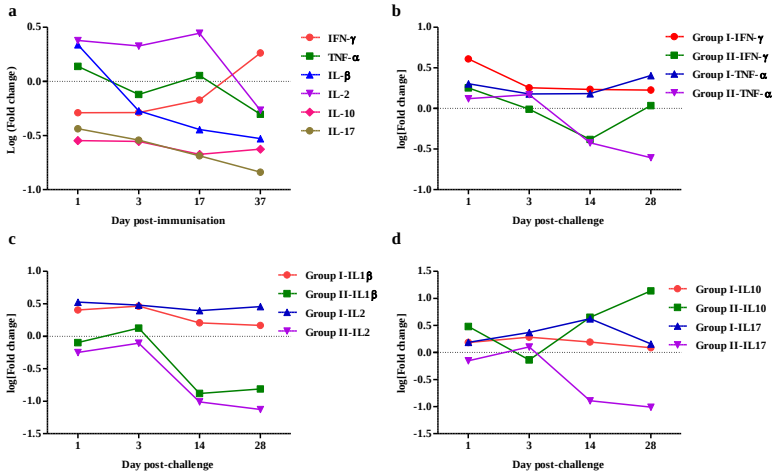

Fig.3 Dynamics of cytokine expression in calves post- immunization and virulent *T. evansi* challenge. The immunization induced changes in cytokine dynamics (a), Profile of IFN-□ and TNF-□ following *T. evansi* challenge (b), IL-1□ and IL-2 (c) and IL-10 and IL-17 (d).

The cytokine expression in the treated calves was monitored by qPCR. The data presented as mean relative fold changes of cytokine gene transcription in log values (y=log[y]), derived from four biological replicates. Data were analysed by the linear mixed-effects model to determine the effect of time and treatment. Between Group I and II, there was a significant difference ($p<0.05$) in the expression of IFN-□, TNF-□, IL-1□, IL-2, IL-10 and IL-17.

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PRODUCTION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST A 35 KDA OUTER MEMBRANE PROTEIN OF OVINE *MANNHEIMIA HAEMOLYTICA*

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Mannheimia haemolytica is a commensal and opportunistic bacterial pathogen that is the causative agent of respiratory tract infection in sheep (Ackermann and Brogden, 2000). Previous studies have suggested that a 35 kDa outer membrane protein (OMP) of *M. haemolytica* is playing an important role in immunogenicity and pathogenicity of *M. haemolytica*. It was shown that high antibody responses to several specific OMPs correlated with resistance to challenge with virulent *M. haemolytica* (Confer et al., 1995; Pandher et al., 1999) and a 35 kDa heat-modifiable OMP of *M. haemolytica* was involved in adherence to bovine bronchial epithelium *in vitro* (Kisiela and Czuprynski, 2009). Therefore, and because most of reported studies are focusing on bovine but not ovine originated isolates of *M. haemolytica* the present study was undertaken to characterize a 35 kDa protein of ovine *M. haemolytica* biotype A strains, to determine the role of the protein in adhesion of *M. haemolytica* to lamb kidney (LK) cells by using monoclonal antibodies (Mabs).

Nine ovine field isolates including 8 isolates of *M. haemolytica* (Biotype A) and 1 isolate of *Pasteurella multocida* (serogroup A) were used in the present study. The field isolates were obtained from Syrian Awassi sheep suffering of bronchopneumonia (6 isolates) and infected sheep lungs (3 isolates) (Al-haj Ali and Al Balaa, 2019; 2020). In addition, 6 reference strains were also used (Fig. 3). Two types of antigens were prepared: 1) Killed whole-cell bacterial lysates (Wbl) were prepared from all strains of the bacteria (Castro et al., 2017). 2) Detergent insoluble fraction of outer membrane proteins DIF-OMPs of *M. haemolytica* were prepared by the method of Bötcher et al. (1991). Protein profiles of Wbl and DIF-OMPs antigens from 8 ovine field isolates of *M. haemolytica* are shown in fig. 1a and 1b, respectively. A 35 kDa protein appears as a common protein with various degrees of thickness in all isolates. Mabs were produced by immunization of BALB/c mice with DIF-OMPs extracted from ovine field isolate Q11 of *M. haemolytica*. Hybridomas were produced by fusing of plasma cells from immunized BALB/c mouse with non-Ig-secreting Sp2/0-Agl4 (SP2/0) myeloma cell line (Zola and Brooks, 1982). Positive hybridomas were screened by indirect ELISA (iELISA) and the targeted epitope was identified by immunoblot. Five hybridomas belonged to IgG_1 and IgG_{2b} were obtained and stably produced desired Mabs. Reactivity of cloned Mabs with DIF-OMPs or Wbl antigens of ovine isolate Q11 was examined by immunoblot. All Mabs gave a unique reaction with DIF-OMP at a 35 kDa molecular weight position only (Fig. 2). These results indicated that the 35 kDa protein is a major immunogenic antigen for ovine *M. haemolytica*. Furthermore, Indirect ELISA revealed that Mabs reacted strongly with all Biotype A isolates of *M. haemolytica* but weakly with all types of bacteria not belonging to *M. haemolytica* (Fig. 3) that were used in the present study suggested that the major 35 kDa OMP is may be a common and high conserved immunogenic protein for ovine isolates of *M. haemolytica*. The epitope recognized by Mabs was subjected for proteinase K treatment (Khosraviani et al. 1990) and periodate oxidation (Woodward et al., 1985) and tested by iELISA. The results showed that proteinase K treatment of DIF-OMPs reduced ELISA values (Fig. 4) inferring that the Mab binding site(s) had been destroyed. However the treatment with periodic acid had no effect (Data not shown), indicating that the epitope recognized by Mabs was proteinaceous. Mab Gmh1 was conjugated with

fluorescein isothiocyanate (FITC) and Immunofluorescence microscopy on ovine isolate Q11 reacting directly with Mab Gmh1 conjugated with FITC or indirectly with unconjugated Mab Gmh1 and goat anti-mouse IgG conjugated with FITC is shown in Fig. 5b, c and d. Mab Gmh1 appeared to react strongly with the bacteria, which was represented by intense aggregation of FITC-Mab on bacterial surface (Fig. 5b and 5c) especially on capsular material (Fig. 5d). These results indicate that the 35 kDa OMP is a capsule associated protein.
Adhesion (Fig. 6a) and adhesion inhibition assays (Fig. 6b) of 7 isolates of *M. haemolytica* to LK cells were designated *In vitro*. The results showed that Mab Gmh1 significantly inhibited the adherence of *M. haemolytica* to LK cells suggested that a 35 OMP is may be an adherence factor.

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Figures

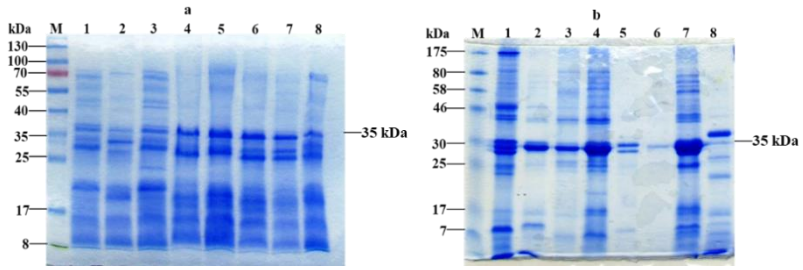

Fig. 1. Protein profiles of Wbl (a) and DIF-OMPs (b) antigens prepared from 8 field isolates of ovine *M. haemolytica* as shown by SDS-PAGE analysis. M, Low molecular weight marker; Lanes 1-8 field isolates Ha2, Ha6, Ho8, Q11, Ho10, Dz14, Rq17 and Rq3 respectively. 6 µg/lane.

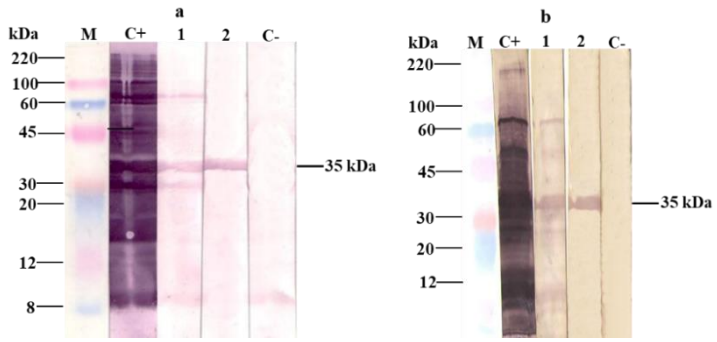

Fig. 2. Immunoblot analysis of Mab Gmh1 reacting with DIF-OMPs (a) and Wbl (b) of *M. haemolytica* ovine field isolate Q11 before (a1 and b1) and after (a2 and b2) 3 times of cloning. C+, reaction of diluted antiserum from immunized BALB/c mouse with Wbl (aC+) and DIF-OMPs (bC+) of the isolate Q11 (positive control); C-, reaction of diluted serum from unimmunized BALB/c mouse with Wbl (aC-) and DIF-OMPs (bC-) of the isolate Q11 (negative control).

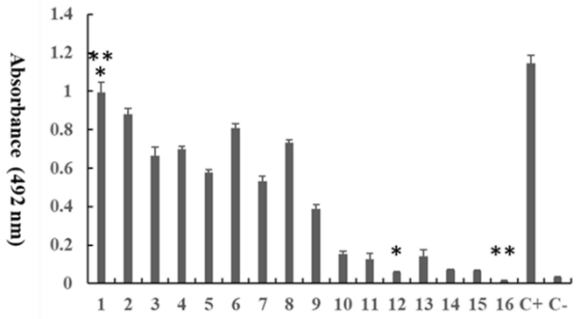

Fig. 3. Reactivity of Mab Gmh1 with OMPs and Wbl antigens extracted from all strains used in the present study as revealed by iELISA. Diluted Mab Gmh1 was reacted with: 1 and 2 Wbl and DIF-OMP of the isolate Q11; 3-9, *M. haemolytica* field isolates: Ha2, Ha6, Ho8, Ho10, Dz14, Rq17 and Rq3 respectively; 10, *P. multocida* field isolate Ho13; 11-16, reference strains: *C. perferingens* NCTC 8798, *S. aureus* NCTC 10788, *S. typhimurium* NCTC 12023, *B. cereus* NCTC 7464, *B. abortus* S19 and *B. melitensis* Rev-1 respectively; C+, reaction of diluted antiserum from immunized BALB/c mouse with Wbl of the isolate Q11 (positive control); C-, reaction of diluted serum from unimmunized BALB/c mouse with Wbl of the isolate Q11 (negative control). *, $P<0.05$; **, $P<0.005$.

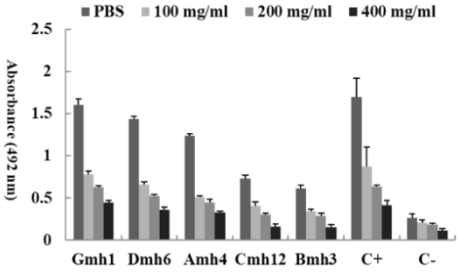

Fig. 4. The effect of treatment of DIF-OMPs antigen from the isolate Q11 with increasing concentrations of proteinase K on the binding of produced Mabs.

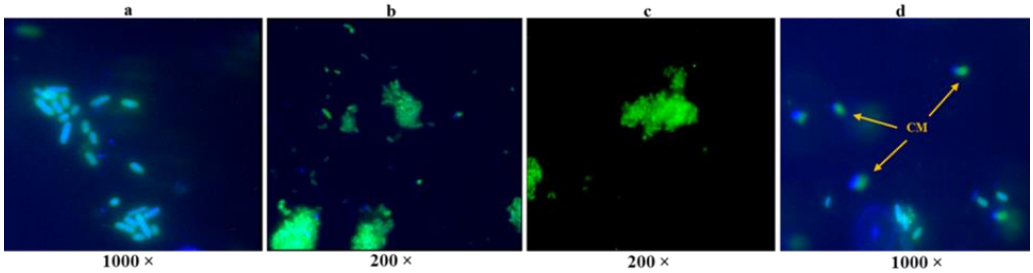

Fig. 5. Immunofluorescence microscopy of whole bacterial cells of ovine field isolate Q11. a, bacteria stained with DAPI; b, bacteria were reacted with Mab Gmh1 and labeled with FITC-conjugated goat anti-mouse IgG; c and d, Bacteria were labeled with Mab-FITC; CM, capsular material.

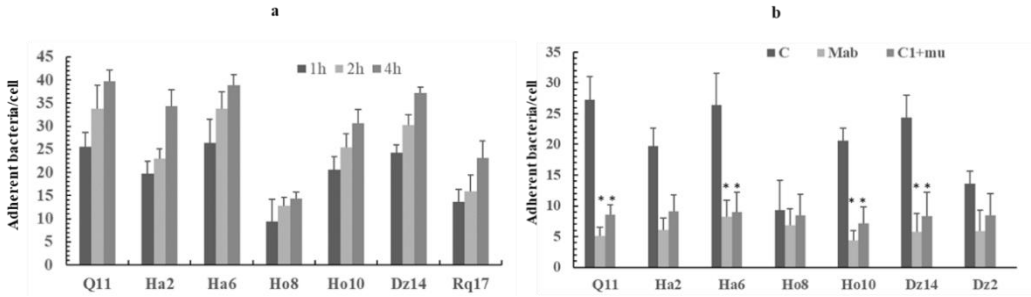

Fig. 6. Adhesion assay of 7 strains of *M. haemlytica* to LK cells before (a) and after treatment with Mab Gmh1. C, bacteria treated with RPMI without antibiotics; C+mu, bacteria treated with antiserum from immunized BALB/c mouse. ** $P < 0.01$.

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INACTIVATION OF THE TICK-BORNE ENCEPHALITIS VIRUS WITH A NOVEL AUTOMATED IRRADIATION PROCESS BASED ON LOW ENERGY ELECTRON IRRADIATION

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Inactivated vaccines are commonly produced by incubating pathogens with chemicals like formaldehyde or beta-propiolactone. But despite a successful history in inducing protection against many diseases there is a need for alternative inactivation methods. The procedures for chemical pathogen inactivation are time consuming and require long incubation periods as well as the removal of chemicals. The substances used are potentially toxic and can damage or alter necessary antigens. This can reduce the desired immune responses caused by the vaccine or lead to an atypical course of the disease. An alternative technology is the use of ionizing radiation. Without any addition of harmful chemicals, pathogens are inactivated by gamma or electron radiation. Since nucleic acids are mainly targeted and damaged, protein structures stay intact, which is a good prerequisite for vaccine production. Major drawbacks are the need for complex shielding constructs to safely use this technology and difficulties in applying exact doses. Low energy electron irradiation (LEEI) produces minimal amounts of secondary radiation, thus compact shielding structures suffice. A very high dose rate ensures a quick application and it is possible to set exact, reproducible doses. One disadvantage is the low penetration depth limiting the amount of material that can be irradiated. For a high throughput-use of LEEI a unique proof-of-concept prototype was designed and constructed. The prototype, termed ELLI300 (**el**ectron Irradiation of **li**quids, Fig. 1A), enables the automatized irradiation of liquids (milliliters to several liters) with low energy electron irradiation in short spans of time. Thin liquid films necessary for complete penetration with LEEI are generated with different exchangeable modules, one of them being termed the Bag-Module (Fig. 1B). For this module liquid is sealed inside a special PE-bag to be irradiated. With the filled bag clamped between conveyor belts it is pulled through the electron beam by a motor-driven transport roller. One bag can hold a volume of 10 to 20 mL and needs about 5 minutes to be processed.

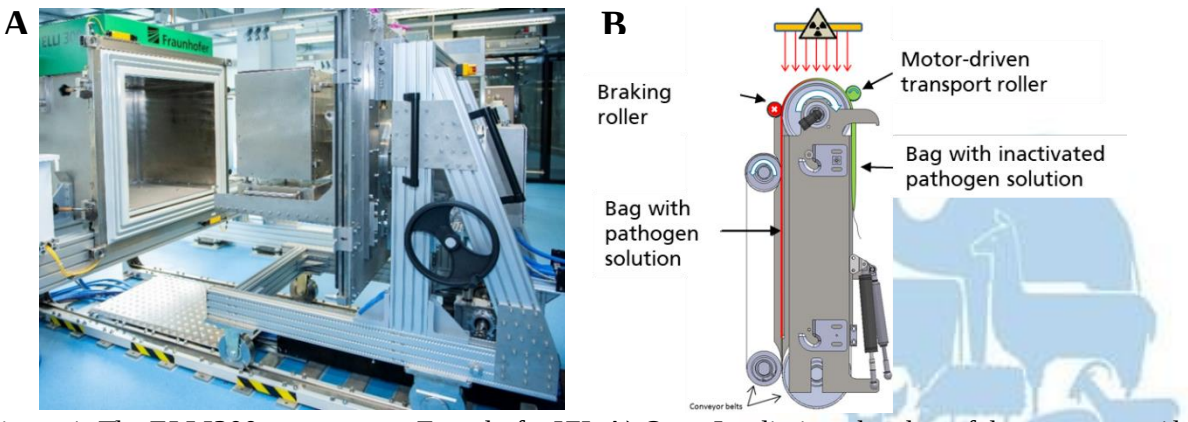

Figure 1: The ELLI300-prototype at Fraunhofer IZI. A) Open Irradiation chamber of the prototype with the closed module box containing the module for liquid handling. B) Schematic drawing of the working principle of the exchangeable Bag Module for handling of liquid pathogen solutions. A PE-bag filled with pathogen solution is inserted between conveyor belts and transported through the electron beam.

One candidate for the development of an automated LEEI-based inactivation process is the tick-borne encephalitis virus (TBEV). The currently available TBEV vaccines are based on formalin-inactivated virus and while inducing protection against the disease after three initial shots, require booster injections every 3 to 5 years for lasting protection. The LEEI-based inactivation of TBEV holds the potential to improve the quality of TBEV vaccines and the duration of immunity. To generate the automated process for TBEV-inactivation, virus solution was irradiated in the ELLI300 prototype and subsequently analyzed. As a control for every run a sample of the TBEV solution was processed without irradiation. A dose killing curve was generated in the Bag-Module by applying doses between 5 and 30 kGy to TBEV-containing cell culture supernatant from BHK-21 cells (10 mL per bag, $2{,}3x10^{8}TCID_{50}$/mL). Titers of active TBEV in the samples were measured in infectivity assays on BHK-21 cells with serially diluted virus. A dose dependent reduction of the titer was observed after irradiation. Depending on the starting titer and composition of the solution a reproducible complete inactivation of TBEV was observed at 30 kGy.

The effect of LEEI-treatment on antigenic structures of TBEV was analyzed in ELISA experiments and compared to formalin-inactivated TBEV. Before treatment TBEV was purified and diluted in PBS + 12% (w/v) trehalose. Samples were coated on ELISA-plates and probed with TBEV-positive human blood sera to detect TBEV-binding antibodies. Compared to untreated TBEV a slight decrease in antibody signal was observed in the non-irradiated control, suggesting that this is caused by the process itself. In the inactivated samples an dose dependent decrease was observed.compared to the active samples. There is no significant difference in the signals of LEEI-inactivated and the formalin-inactivated TBEV samples.

Immunization studies in BALB/c mice with LEEI-inactivated or formalin-inactivated TBEV are currently performed. The analysis of binding and neutralizing antibodies in the serum will provide details about induced immune responses. Finally, in order to reveal the protective potential of LEEI treated TBEV, the vaccinated animals will be challenged with active virus.

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HAEMONCHUS CONTORTUS IRRADIATED LARVAL VACCINE: STORAGE CONDITIONS AND LARVAL ACTIVITY FOLLOWING IRRADIATION

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Background

Haemonchus contortus is an abomasal blood sucking parasite of goats causing significant economic losses in the goat industry in Sri Lanka. The infection is most prevalent in the dry zone regions of the country where small and medium holder semi-intensive farming system is practiced. An abattoir study conducted to evaluate the prevalence of gastro-intestinal nematodes in goats in the dry zone of the country revealed 81% of the animals had *H.contortus* infection. *H. contortus* has shown a great ability to develop resistance against anthelmintic drugs in a particularly short period of time. Anthelmintic drugs are commonly used to control the disease and to prevent associated deaths in the field, but the frequency of medication has been increased exponentially over the recent years suggesting development of anthelmintic resistance. Cheap and sustainable means of novel disease control strategies such as development of vaccines should be thoroughly investigated in order to minimize the losses associated with the industry.

Methodology

H.contortus irradiated larval vaccine was developed by exposing L3 infective stage larvae at 200 Gy gamma irradiation. In order to achieve the maximum larval vaccine efficacy, the storage conditions, larval mortality following irradiation is of prime importance. The current study was designed to evaluate the larval mortality following irradiation and to determine the optimum storage conditions for the vaccine. Once irradiated, the vaccine was stored in 5mL aliquots, with 10 IU/mL Penicillin and 10 µg/mL Streptomycin in cell culture flasks at 4, 15 and 37 ^{0}C along with non-irradiated controls. Larval viability and motility were determined with direct observation through the inverted light microscope, and metabolic activity was evaluated by metabolic reduction assay using resazurin (Alamar blue) assay.

Research Questions

1. What is the most suitable storage temperature to store irradiated larval vaccine?
2. How long the larval activity is maintained following irradiation?

Hypothesis

The hypothesis of the study is that the shelf life of irradiated larval vaccine is at least more than a month with continuous maintenance of larval activity.

Results

The percentage of live irradiated larvae at 4 ^{0}C remained at 70% by 7 dpi (days post irradiation) and 57% by 14 dpi. The percentage of dead irradiated larvae raised above 90% at 15 ^{0}C and 37 ^{0}C by 21 dpi and remained at 78% at 4 ^{0}C. This suggests the maximum livability of irradiated larvae could be achieved for at 14 days, but the best is to administer the vaccine within 7 days of production. The metabolic activity of irradiated larvae stored at 4^{0}C compared to controls on 0 dpi showed maximum larval activity by 14 dpi (417.86%) and declined thereon. At 15 ^{0}C, metabolic activity declined from 0 dpi. At 37 ^{0}C, it reached a maximum 1 dpi (103.47%) and declined thereon. So, in order to achieve the vaccine efficacy, it is best to use the vaccine within 14 days of production given that highest efficacy will be achieved when it is administered at day7 of production.

Literature Review

H.contortus is a highly pathogenic abomasal parasite causing significant damages to the host including anaemia and hypoproteinemia. Dynamic approaches has been taken in order to control the infection in sheep and goats. Usage of anthelmintic drugs, bioactive forages, behavioral management and vaccines are among the effective control strategies. With the prompt development of anthelmintic resistance by *H.contortus*, much attention has been given towards development of vaccines. *H.contortus* irradiated larval vaccine has been developed to control the infection as shown by Jarett et al. 1959. The study confirmed the development of protective immunity in sheep and maintenance of the immunity over 29 days post vaccination when challenged with 50,000 infective larvae. However, usage of the above vaccine has not been in practice to control the infection in the field. This vaccine was developed by exposing infective *H.contortus* larve to 350.8 Gy irradiation. Later the researchers focused more on developing recombinant subunit vaccines locally in many countries and being effectively used to control the infection. The need of cheap, reliable and sustainable effective vaccine is of paramount importance to be used in developing countries like Sri Lanka where the *H.contortus* infection is a big economic burden to the goat industry. Revisiting the irradiation attenuated vaccine development, current study focuses to develop irradiation attenuated *H.contortus* larval vaccine for the development of protective immunity in goats. The determination of proper storage conditions in order to achieve maximum larval activity fulfils the suitability of the vaccine to be used effectively in the field to control the infection.

Limitations

The irradiated *H.contortus* larval vaccine has a shelf life of 14 days with more than 50% larval activity maintained when stored at 4 ^{0}C temperature. In some replicates, fungal growths were detected upon storage, thus addition of an antifungal agent may increase the shelf life while maintaining the larval activity for a longer time period.

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VACCINE ANTIGEN EXPRESSION AND BOVINE IMMUNISATION USING A FLEXIBLE AND MODULAR TRYPANOSOMATID VACCINE DELIVERY PLATFORM

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Summary

We have developed a novel non-pathogenic protozoan parasite that is found is almost all cattle worldwide as a flexible and safe subunit vaccine delivery vehicle that is well suited to deployment in low and middle-income countries, as well as managed farms globally. Specifically, the single-celled organism, *Trypanosoma theileri*, can be easily and rapidly engineered to express pathogen antigens using modularised expression constructs and these can be delivered systemically into recipient cattle over a sustained period. This results in low-level, prolonged immune stimulation that can be differentiated immunologically from infection and which has the potential to generate effective immune responses to target antigens at protective levels.

Introduction

Some trypanosomatid species are significant eukaryotic pathogens of humans and animals in the tropics. However, non-pathogenic trypanosomatid species are also globally ubiquitous, including among livestock. For example, *Trypanosoma theileri* (spread by horse flies) is ubiquitous in cattle worldwide (incidence levels are above 80%) but causes no ill effects in healthy animals[1]. Recently, we have developed *T. theileri* as a vaccine delivery vehicle to combat cattle pathogens, with the benefit of sustained antigen expression and immune stimulation by the live parasite, which is maintained systemically at low level long-term. We have successfully developed culture systems and transfection methods for protein expression and delivery in *T. theileri* and have derived complete genome sequence and expression data for the vehicle[2], aiding its manipulation and expression optimisation. Moreover, expression of a test antigen derived from *Babesia divergens* in cattle has successfully achieved effective immune responses at protective levels[3]. Expression of Rabies G antigen and *Clostridium* antigens has also been achieved, demonstrating flexibility for the expression of pathogen antigens from viruses, bacteria and eukaryotic parasites. Antigen expression is driven from high efficiency expression cassettes (Figure 1) that can either be integrated into the genome of the delivery vehicle, or maintained episomally, these being progressively lost over 12 weeks restoring the wild-type status of the vehicle.

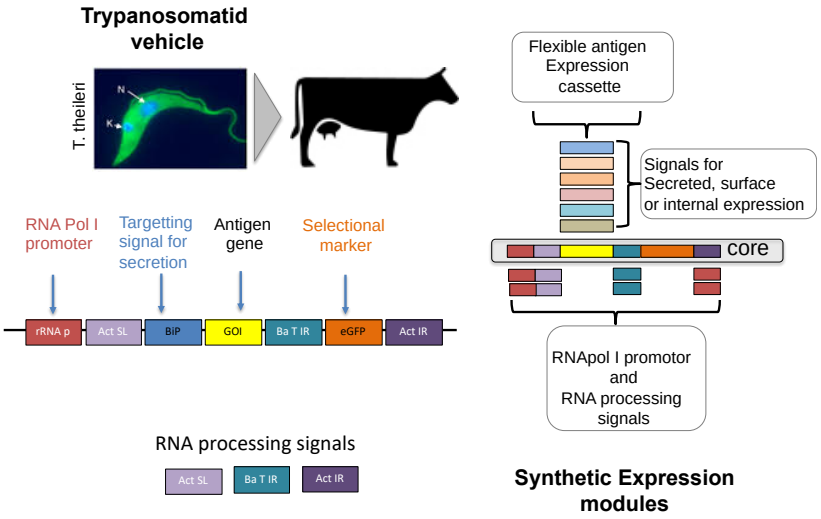

Figure 1. Use of *Trypanosoma theileri* to deliver antigens to cattle. Expression cassettes drive expression of the antigen (GOI) using appropriate RNA processing signals from *T. theileri*. The expressed antigen can be trafficked for secretion, surface or cytosolic expression using modular expression cassettes which can be integrated or maintained episomally.

Results

As a further proof-of-concept for the vehicle, we have evaluated the antigen expression and immunogenicity of an antigen from the agent of East coast fever, *Theileria parva*. The target antigen, p67, can generate effective immune responses and variable protection in cattle after delivery by conventional vaccination using viral delivery vehicles[4]. Using existing data mapping epitopes conferring effective immune responses, the p67 antigen was expressed either intact, or as distinct fragments comprising the N-terminus, C-terminus, or internal domains of the p67 protein[5]. In each case, expression was attempted from an episomal expression vector, with expressed antigen subunits being trafficked for secretion, surface presentation, or cytosolic presentation by the delivery vehicle using appropriate *T. theileri* protein trafficking signals[3]. All but one of the antigen fragments were successfully expressed with strongest expression observed with a C terminal fragment displayed on the surface of the transgenic *T. theileri* vehicle (Figure 2). In vitro, the maintenance of expression without selection was sustained for 9-21 weeks depending on the expressed antigen. Using the unusual multi-gene (polycistronic) transcription mechanism of trypanosomatids parasites, we could also demonstrate effective expression of multiple antigens from the same episome, transcribed from a single strong upstream promoter. This highlighted the ability of the *T. theileri* expression vehicle to simultaneously express multiple distinct subunit antigens or immunological variants of the same antigens from a single transgenic delivery vehicle.

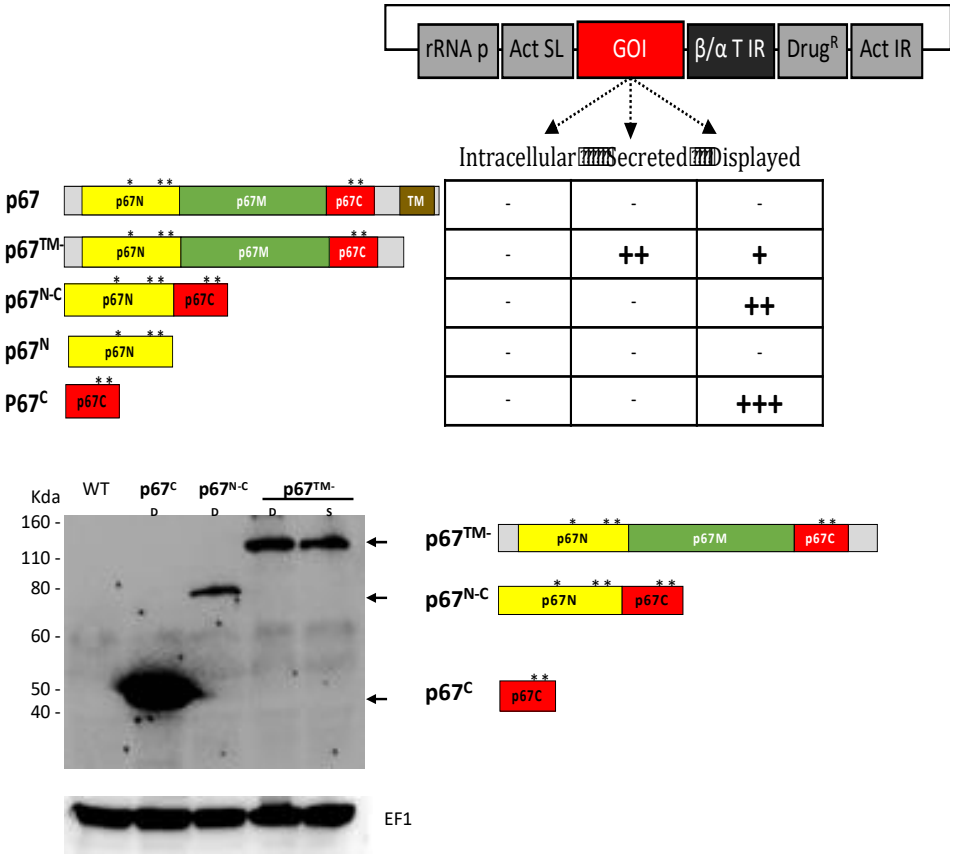

Figure 2. Expression efficiency of different fragments of the p67 antigen when trafficked to different sites. Optimal expression was achieved using the p67-C terminal fragment.

Having confirmed effective expression of different components of the p67 antigen, the highly expressed p67-C antigen was tested for immunogenicity in vivo. Six calves were inoculated twice with live delivery vehicle at week 0 and week 2 and thereafter the prevalence of the transgenic *T. theileri* monitored weekly by PCR assay. Two control animals were also evaluated, these being inoculated with *T. theileri* not expressing any antigen. Although detection of the delivery vehicle was variable due to its natural maintenance at low level in host animals, all inoculated calves successfully established detectable populations of the *T. theileri* delivery vehicle. Currently, serum from the experimental and control animals is being evaluated for the detection of immune responses generated to the expressed p67 fragment via ELISA assay and these are being compared to serum from animals inculcated against the p67 antigen by conventional prime boost vaccination. The molecular and immunological data from the study will be presented.

Conclusion

The trypanosomatid vehicle offers an innovative and alternative route to prevent bovine infections, which is sustained, systemic and can be delivered to target multiple antigens of the same pathogen, or antigens from distinct pathogens. The expressed antigens can be flexible, with a focus on existing priority targets or promising new candidates, identified by reverse vaccinology approaches.

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EMERGENCY PREPAREDNESS AND RESPONSE

IAEA-CN-281-104

DIAGNOSIS OF *CRYPTOSPORİDİUM* SPP. İN NEONATAL CALVES BY ELISA, NESTED PCR AND CARBOL FUCHSIN STAINING METHODS

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Abstract

Cryptosporidiosis is a common diarrhea-inducing coccidian parasite infection in animals throughout the world, with importance especially for immunosuppressive adults and children. Many techniques have been developed for detection of *Cryptosporidium* oocysts. The objective of this study was to diagnose cryptosporidiosis in neonatal calves by the carbol fuchsin staining method, ELISA and nested PCR and to compare these methods. For this aim, a total of 150 fecal samples were collected from calves (≤2 mo.) with diarrhea. Fifty-two (34.6%) fecal samples were found positive by the carbol fuchsin staining method and 56 (37.3%) gave a positive reaction for *Cryptosporidium* spp. with ELISA. The PCR of 150 fecal samples showed a total of 56 (37.3%) positive amplications. This study indicated that the carbol fuchsin staining method can be used for diagnosis of clinical cases, however PCR, and ELISA are more sensitive than conventional staining methods.

Keywords: cryptosporidiosis, carbol fuchsin, ELISA, nested PCR, calves

Introduction

Cryptosporidium is a widely distributed coccidian parasite that cause enteric disease in humans and animals. It has been reported as a common seriouse primary cause of outbreaks of diarrhea in newborne and young calves (Fayer, 2004; Ebrahimzade *et al.*, 2009). *Cryptosporidium* has gained much attention in the last 20 years as a clinically important human pathogen. Presently, the increasing population of immunocompromised persons and the various outbreaks of cryptosporidiosis through infection by water-borne Cryptosporidium oocysts have placed an even greater emphasis on this pathgens (Hannahs y College, 2005).

Cryptosporidiosis is mainly a problem in neonatal calves. The parasite frequently acts alone, but the losses are pronounced when concurrent enteropathogens are present. Economic losses associated with cryptosporidisis are retarded growth and mortyality, and a number of hard to estimate costs resulting from interventions necessitatedby diarhoeic problems (de Graaf *et al.*, 1999).

Early diagnosis of *Cryptosporidium* spp. is central to the control of this disease. There are a variety of methods, including microscopy, immunulogical and molecular techniques for the detection of *Cryptosporidium* oocyst in different laboratories. Microscopic methods include concentration techniques and staining of fecal samples (e.g. with carbol fuchsin). It has been reported that the *Cryptosporidium* ELISA can detect *Cryptosporidium* in more speciments than microscobic examination, and is sufficiently sensetive and specific to detect clinical cases of Cryptosporidiosis (Sevinç *et al.*, 2003). In epidemiological screenings and genetical examinations PCR with its high sensitivity and specificity is highly benefical (Carey *et al.*, 2004; Sakarya *et al.*, 2010).

The purpose of this study was to compare fuchsin staining method, ELISA and nested PCR used in routine examination of samples for *Cryptosporidium* spp.

Material and Methods

A total of 150 fecal samples were collected from up to calves (≤2 mo) with diarrhea. Samples were kept at +4°C until laboratory analyses. To reveal the presence of *Cryptosporidium* in the feces, microscopy via the carbol fuchsin staining method, ELISA and nested PCR were employed. The carbol fuchsin staining method was performed according to the methods described by Heine (1982). Detection of *Cryptosporidium* Coproantigens used a commercial ELISA kit (Diagnostic Automation, Inc California, USA). It is a double antibody (sandwich) ELISA using an anti-Cryptosporidium antibody to capture the antigen from the stool supernatant. Samples were processed according to the manufacturer's recommendations and results were assessed at a wavelength of 450 nm using an ELISA reader.

At this stage, 200 µl, was taken from each homogenized fecal samples and DNA extraction was performed by using the QIAamp DNA stool kit (Qiagen, Maryland, USA). This DNA was stored at -20 °C. Nested PCR was carried out by applying the protocol and primers described by Xiao *et al*. (2001). The samples which had been proven to be *Cryptosporidium* positive in previous studies carried out in Parasitology Laboratory of Faculty of Veterinary Medicine inAdnan Menderes University were used as positive control.

At the first stage of PCR, the primers CryptoF 5'-TTCTAGAGCTAATACATGCG-3' and CryptoR 5'-CCCATTTCCTTCGAAACAGGA-3' were used, which amplify DNA fragment encoding SSU rRNA as 1.325 bp in length. For each reacti on, master mix was prepared as 25 µl including 200 nM from each primer, 0.2 mM from each dNTP, 0.025 U Taq DNA polymerase, 6 mM MgCl2, 1x PCR buff er and the sample DNA of 1.5 µl, 1 µl of the reacti on products were later subjected to nested PCR. During Nested PCR, a base set of CryptoNF 5'- GGAAGGGTTGTATTTATTAGATAAAG-3' and CryptoNR 5'- AAGGAGTAAGGAACAACCTCCA-3' were used, which amplify a region at about 826-864 bp in length in the former product. The reacti on was carried out at the same way as the the previous process; however, the quanti ty of the sample DNA was taken as 1 µl. Reaction products of 15 µl were used for gel electrophoresis.

Results and Discussion

In this study 52 (34.6%) of 150 samples examined by the carbol fuchsin staining method were found positive regarding to be *Cryptosporidium* spp. Oocysts (Table 1). Fifty-six (37.3%) of these samples gave positive reaction for *Cryptosporidium* spp. with ELISA (Table 2. The nested PCR also showed a total of 56 (37.3%) positive amplification in 150 fecal samples (Table 3).

Table 1. Detectin of *Cryptosporidium* spp. in neonatal calves by carbol fuchsin staining method.

Method	No of fecal samples	No of positives	Positive rate %
Carbol fuchsin staining	150	52	34.6%

Table 2. Detectin of *Cryptosporidium* spp. in neonatal calves by ELISA

Method	No of fecal samples	No of positives	Positive rate %
ELISA	150	56	37.3%

All of the carbol fuchsin positive specimens were detected positive by ELISA and nested PCR methods. 4 (2.6%) negative carbol fuchsin specimens were moderately positive by ELISA and nested PCR methods. Fifty- six samples that were positive by ELISA were also detected as positive by nested PCR method (Table 4).

Table 3. Detection of *Cryptosporidium* spp. in neonatal calves by Nested PCR

Method	No of fecal samples	No of positives	Positive rate %
Nested PCR	150	56	37.3%

Table 4. Positve results of carbol fuchsin staining, ELISA and Nested PCR methods

Method	No of positives	Positive rate %
Carbol fuchsin staining	52	34.6%
ELISA	56	37.3%
Nested PCR	56	37.3%

There are a variety of methods, including microscopy, immunological and molecular techniques for the detection of *Cryptosporidium* oocysts. Carbol fuchsin staining technic is regarded by laboratories to be advantageous due to its simplicity and practicability. The cost of this method is lower than the other methods and gives quick results. Staining techniques, however, is time consuming and require an experienced microscopist, and the sensetivity and specifity vary when the shedding of oocysts is intermittently or in low numbers. Our study indicated that the carbol fuchsin staining method can be used for diagnosis of clinical cases, however in diagnosis of subclinical cases, PCR and ELISA are more sensitive and specific than conventional staining methods.

In conclusion, new and rapid diagnostic techniques have played an important role in animal health management and control of cryptpsporidiosis. These techniques are easy to use, rapid, sensitive, specific and and can offer significant advantages over other methods. Fast action can not only limit damage to the affected herds, but can also prevent the diseases from spreading into neighbouring villages or even other countries.

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LABORATORY CONFIRMATION OF AFRICAN SWINE FEVER AT THE NATIONAL REFERENCE LABORATORY FOR ASF, IDAH, ROMANIA

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Abstract

At present, African swine fever is considered to be a real challenge for the pig breeding industry, due to the high rates of mortality and morbidity recorded in the affected herds. The economic impact on the pig breeding industry is a major one. Tests used to confirm the onset of the disease have shown positive results.
In Romania, first case of African swine fever has occurred in July 2017, in Satu Mare county, near the border with Ukraine and Hungary, in the North part of the country. The second county affected by this disease was Tulcea county, in June 2018, near the border with Ukraine, in the South-East part of Romania. In all cases, the primary laboratory diagnostics were made in the county laboratories, and the confirmation of the disease was performed at the National Reference Laboratory for African Swine Fever within the Institute for Diagnosis and Animal Health (IDAH), Bucharest, using Real Time PCR and direct immunofluorescence tests. The disease has spread and is now present also in other counties of Romania.
Since the first outbreak, the National Reference Laboratory for African Swine Fever of IDAH has performed several thousand confirmatory tests.

Keywords: diagnosis, African swine fever, Real Time PCR, direct immunofluorescence tests

IAEA-CN-281-158

ASSESSMENT OF BIOSECURITY LEVEL IN PIG AND POULTRY PRODUCTION SYSTEMS USING INNOVATIVE TECHNOLOGY TO PREVENT AFRICAN SWINE FEVER OUTBREAKS IN VIETNAM

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Abstract

Biosecurity is one of the main factors affecting disease occurrence and antimicrobial use. However, the importance of specific measures could vary depending on the national context. The objective of this study was to describe the biosecurity status in the Pig and poultry production systems in Vietnam by using Biocheck technology (Gent, Belgium). The project conducted surveys to collect information from 30 pig farms and 30 poultry farms in the Hanoi and Dong Nai provinces of Vietnam, using the Biocheck.Ugent tool from 2018 to 2019. The Biocheck.UGent™ system for pig production and poultry production consists of 109 and 79, mainly di- or trichotomous questions, subdivided into six subcategories for internal biosecurity and six for external biosecurity. Every subcategory consists of 2-13 questions, where the answer to each question results in a score between 0 (when the measure in question is not implemented) and 1 (when the measure is implemented). The score per question is then multiplied by a weight factor that depends on the importance of the measure. Also, the subcategories have a specific weight factor to account for their assessed relative importance for disease prevention. The average over internal and external biosecurity categories results in a score for the total biosecurity. The result showed that internal and external biosecurity scores in pig farms were similar (55.05% and 53.68%, respectively). By contrast, in chickens farms the internal biosecurity score was higher than external biosecurity score (64.05% and 58.85%, respectively). For the external biosecurity of pig farms, the purchasing of animals factor in the external biosecurity had a high score, while some other factors had low scores such as vermin and bird control, personnel and visitors (people), environment and water resources. For the internal biosecurity of pig farms, some biosecurity factors had high fluctuations such as measure of animal and disease management. For chicken farms, the factors of one-day-old chick purchase, material supply and disease management had high biosecurity scores. Meanwhile, other factors such as the entrance of visitors and personnel, removal of manure and dead animals, cleaning and disinfection were generally low, showing a need to improve biosecurity management. The factors including supply and management of materials and measures between compartments have a big variability in scores. In conclusion, the analysis and assessment of biosecurity levels of surveyed pig and chicken farms in Hanoi and Dong Nai via Biocheck.ugent tools show that all farms need to improve many factors in both internal and external biosecurity.

Keywords: Biosecurity, internal biosecurity, external biosecurity, pig production, poultry production, Biocheck

IAEA-CN-281-162

EVOLUTIONARY ANALYSIS OF *PESTE DES PETITS RUMINANTS* VIRUS IN BANGLADESH DURING 2008-2017

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Synopsis

Peste des petitsruminants virus (PPRV) infection is one of the major constraints of sustainable goat farming and is the number one killer of Black Bengal goats in Bangladesh. The first outbreak of PPR was reported in 1993. Since then, outbreaks of PPR have been continually reported across the country (Begum et al., 2018; Chowdhury et al., 2014). The genome of PPRV codes for six structural and two non-structural proteins in the order of 3´-N-P(C/V)-M-F-H-L-5´. The nucleoprotein (N) and fusion (F) protein genes have been used extensively for genetic evolutionary analysis. The virus is circulating in Africa, the Middle East and Central to South-East Asia as four genetically distinct lineages, among which lineage IV is currently circulating worldwide. Our recent molecular phylogenetic analysis using partial F and N gene sequences of a limited number of samples showed that Bangladeshi PPRV isolates belonged to lineage IV but formed a separate sub-lineage along with recent isolates from Nepal, Bhutan and China (Rahman et al., 2016). However, full length sequence analysis of Bangladeshi PPRV isolates is required to confirm the sub-lineage and to further assess the evolutionary dynamics of the virus. This paper describes the genetic diversity of three PPRV isolates from Bangladesh collected in 2008, 2015 and 2017 based on their complete genome sequence analysis.

Two PPRV isolates from 2015 (BD10/2015) and 2017 (BD11/2017) were obtained from local field outbreaks and RNA was extracted from lymph node homogenates of infected goats. The amplification of the full genome of PPRV was performed by 26 overlapping RT-PCR using custom designed primer sets (Dundon et al., 2014). The amplified RT-PCR products were gel cleaned and sequenced by a commercial company. Sequence data were edited and assembled using Bioedit and MEGA 7 software to obtain the full genome sequences. In addition, the full genome sequence of a previous Bangladeshi PPRV strain (BD2/2008, GenBank Accession No. MG581412) was downloaded from GenBank. For comprehensive phylogenetic analysis, full genome sequences of an additional 71 PPRV strains were retrieved from GenBank. Phylogenetic relationships were inferred by the maximum likelihood method based on the General Time Reversible model with the MEGA 7 software.

The genomes of all three field isolates of PPRV were 15948 nucleotide (nt) in length and encoded six structural genes in the order of 3′-N-P-M-F-H-L-5′with a 52 nt long leader at 3′ untranslated region (UTR) and a 73 nt long trailer at the 5′ UTR. All genes were separated by similarly conserved noncoding intergenic trinucleotides (CTT). Phylogenetic analysis using complete genomes (Fig. 1) and individual gene segments showed that all three PPRV isolates of Bangladesh belonged to lineage IV and were closely related to the viruses from Bangladesh, India, China, Tibet and Pakistan. Analysis of nucleotide divergence between the three Bangladeshi strains and the four lineages of PPRV based on their complete genome sequences is shown in Table 1. The mean divergence between the four lineages of PPRV varied from 9.7% to 14.8%. The highest divergence (14.8%) was found between lineage II and lineage IV. Bangladeshi PPRV isolates from 2015 (BD10/2015) and 2017 (BD11/2017) showed 1.7% and 2.3% divergence from isolate of 2008 (BD2/2008), respectively, indicating that Bangladeshi PPRV are slowly

evolving. Individual gene-based comparative analysis of nucleotide identity among strains of the four lineages showed that the M gene was the most conserved (divergence: 7.1%-10.9%) whereas the H gene was the most divergent (divergence: 9.6%-13.6%) one. These findings suggest that the higher divergence of the H gene compared to the other genes of the PPRV could allow for the selection of the H gene for genetic evolutionary analysis of PPRV.

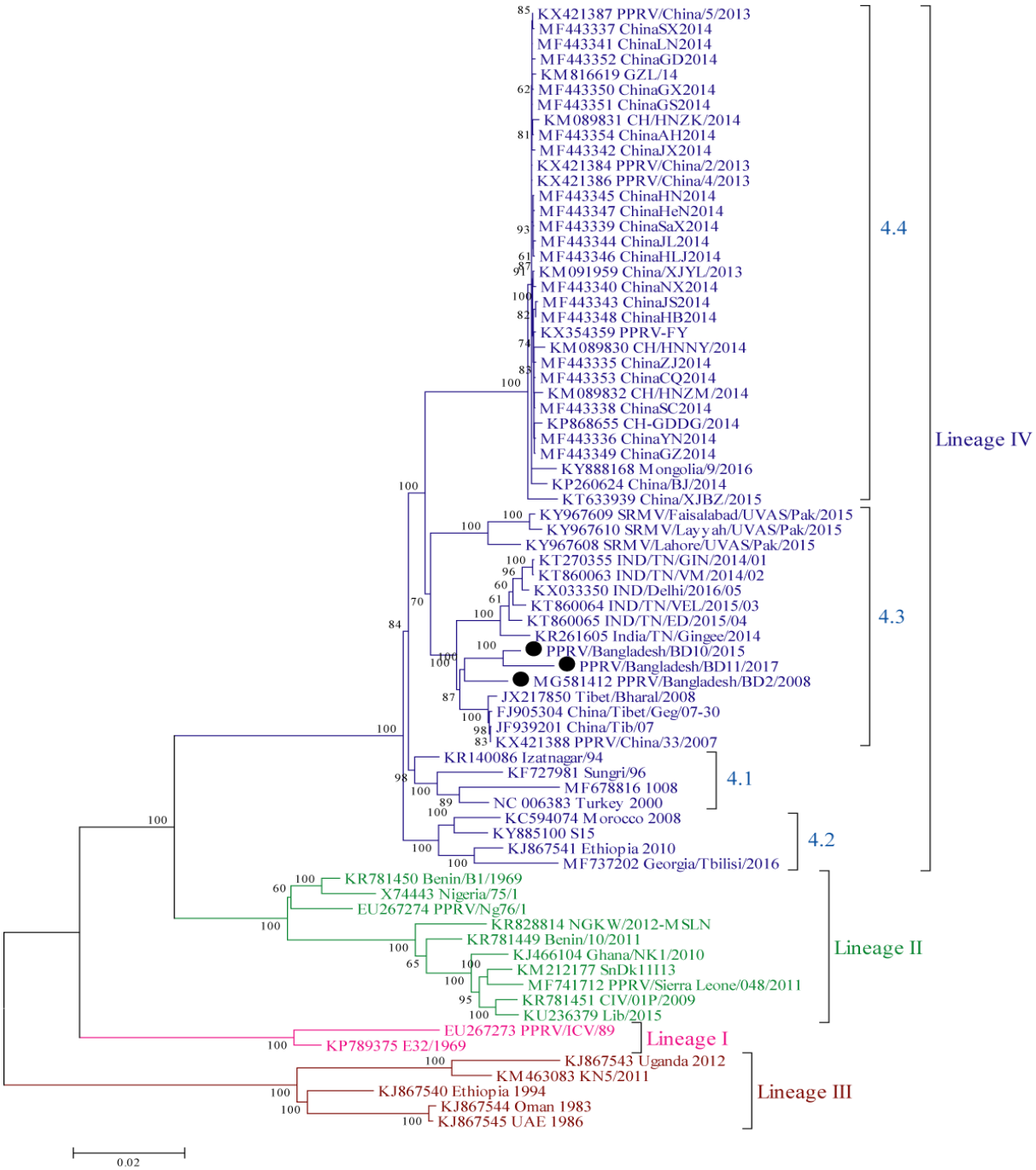

Fig. 1: Maximum likelihood phylogenetic tree of PPRV strains based on complete genome sequence analysis. Bangladeshi isolates are marked with filled dots (•).

Table 1: Analysis of evolutionary distances of PPRV strains

Nucleotide distances between lineages of PPRV				
	I	II	III	IV
I	-			
II	0.106	-		
III	0.130	0.135	-	
IV	0.122	0.097	0.148	-

Nucleotide distances between Bangladeshi strains of PPRV			
	BD2/2008	BD10/2015	BD11/2017
BD2/2008	-		
BD10/2015	0.017	-	
BD11/2017	0.023	0.012	-

Further phylogenetic analysis of global lineage IV PPRV strains showed that the three Bangladeshi PPRV sequences clustered under clade 4.3 according to the genotypic classification described by Bao and colleagues (Bao et al., 2017). This clade 4.3 contains PPRV strains from several Asian countries including India, Pakistan and China. Other 3 clades include; clade 4.1 contains PPRV strains from Eurasian countries such as India, Israel and Turkey, clade 4.2 contains PPRV strains from African countries such as Morocco, Algeria, Ethiopia and Georgia, and clade 4.4 contains PPRV strains from China and Mongolia (Bao et al., 2017). Nucleotide divergence analysis between four clades showed a mean divergence of 3.2% to 4.1%.

Comparative residues analysis of the complete genome showed several conserved motifs in our three field isolates. These include three motifs in the N protein: a nuclear export signal motif (^{4}LLKSLALF^{11}), a nuclear localization signal motif (^{70}TGVMISML^{77}) and the RNA binding motif (^{324}FSAGAYPLLWSYAMG^{338}) involved in the interaction of N with N monomers of RNA during genomic RNA binding and thought to be required for N-N self-interaction (Yu et al., 1998). The Soyuz1 motif (^{4}EQAYHVNKGLECIKSL^{20}) in the P protein, which may bind the nucleoprotein and prevent its self-assembly (Karlin and Belshaw, 2012), was also conserved. Two conserved motifs were also noticed in the F protein: a cleavage site motif (^{103}GRRTRR^{108}) responsible for virulence and adaptation in the environment and a leucine zipper domain (^{459}LGNAVTRLENAKELLDASDQIL^{480}) involved in the maintenance of protein tertiary structure (Lamb and Parks, 2007). The binding of the PPRV to SLAM is suggested to be mediated by an asparagine residue at position 481 of the H protein (Vongpunsawad et al., 2004) was also conserved. Finally, the ^{771}QGDNQ^{775} and the ^{1464}GDDD^{1467} motifs in the L protein associated with RNA polymerase activity for negative single-stranded viruses were also conserved in three PPRV strains of Bangladesh (Dundon et al., 2014).

In conclusion, phylogenetic analysis based on complete genome sequences clustered all three Bangladeshi strains under lineage IV and indicate that the viruses are under continuous evolution. This is the first report of complete genome sequencing of PPRV from Bangladesh.

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FASCIOLIASIS AS IT AFFECTS LIVESTOCK PRODUCTIVITY THROUGH METABOLOMICS IN MÉXICO

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Abstract

Parasitic diseases are one of the most frequent problems in livestock in Mexico, the metabolic changes produced by each parasite is unknown. Fascioliasis produced by *Fasciola hepatica* is widely distributed in the country, however, the efforts to detect other methods of diagnosis and control of it are very few. The objective of this study was to determine the pathophysiological changes (metabolic, enzymatic profile, differential blood cell count and histological analysis of liver parenchyma) caused by F. hepatica in cattle in different zoogeographic areas in Mexico. Blood samples were taken from 7892 cattle. Obstruction and increase in the diameter and thickening of the bile ducts, decrease in the thickness of the left and ventral lobe were observed and chronic cases showed cirrhosis; at cellular levels, loss of cell morphology and eggs of F. hepatica were observed in the hepatic parenchyma. Liver damage was directly proportional to the number of parasites that also caused alteration in metabolic components. The most significant indicators of liver damage due to *F. hepatica* at the metabolic level were Urea / BUN, γ-GT, ALT / GPT, AST / GOT and alkaline phosphatase

Keywords: Fascioliasis, Physiopathology, liver damage, parenchyma

Introduction

Mexico is one of the Latin American countries with the highest number of ruminants (bovines and sheep), however the population of ruminants is affected by a parasitic disease called fasciolosis and that affects herbivorous animals and less frequently to man, this parasitosis is caused by the Fasciola hepatica parasite (Dalton, 1999). This disease is emerging or resurfacing in many countries, and its prevalence, intensity and geographic distribution are increasing (Mas-Coma, 2004). Today, fascioliasis is the vector-borne disease that has the widest known latitudinal, longitudinal and altitudinal distribution (Mas-Coma et al., 2003). This parasitosis affects Mexico, 35% of cattle in very humid areas (tropical and sub-tropical) and 13.3% in sheep, nowadays this parasitosis after having decreased its prevalence, for approximately 10 years due to causes The use of beta-agonists adrenergic (β_2-AA) is now resurfacing in animals with high doses of β_2-AA (Clenbuterol-Clb) blood serum concentrations of 1253.5±87.5 ng / ml (Caicedo *et al*., 2009; Paz-Calderón *et al*., 2011; Caicedo *et al*., 2011); in sheep its prevalence is lower, because there are no definite studies of this disease. Recently, diagnostic and control techniques for this parasitosis are implemented. The purpose of this study was to evaluate the pathophysiological effects caused by F. hepatica in cattle

Materials and Methods

***Blood samples and livers*:** Clinically healthy cattle and animals with fascioliasis slaughtered in different municipal slaughterhouses and private farms in Mexico were collected, for two years the population of sampled animals was 7892 cattle. The number of trematodes in the liver parenchyma was determined by examining the affected livers. To observe the damage of liver parenchyma, liver tissue samples were taken and subjected to a histological process and stained with H&E.

Blood samples were taken to count white blood cells. The metabolic profile analysis was performed on blood serum samples, and various enzymes and metabolites were measured with different diagnostic kits (Bio-System/Randox).
An analysis of variance (ANOVA) with the statistical program Stat2 was used (Olivares, 1994) and to determinate the significance between averages was used *Duncan's new multiple range test*.

Results and Discussion

A) *Number of worms in liver parenchyma*: In the liver ducts of the confiscated livers, flukes were found in a variable number, with a minimum of 1 individual and a maximum of 273 individuals for liver, the largest number of flukes found in the liver of females was of 54, whereas for males was 273. This variation is due to the number of metacercariae ingested by each animal, which was impossible to detect in this study because the analyzed animals were naturally infected by *F. hepatica*.
According to Dalton, the majority of the confiscated livers showed an increase of about 3 cm in diameter of the liver ducts; besides their color was whitish-yellowish, due to the obstruction of these ducts by the parasite (Figure 1a), as well decrease in the thickness of the affected lobes (left and ventral) as the disease progresses and loss of liver consistency, caused by fibrosis and cirrhosis in chronic cases (Figure 1b). As a result, the liver loses its metabolic faculties and allows the accumulation of substances rich in salt, which crystallizes causing obstruction of ducts (Figure 1c) bringing favorable conditions for the reproduction of bacteria as *Clostridium spp* (Robles, 1998), *Escherichia coli*, *Enterococcus faecalis*, y *Klebsiella pneumoniae* (Valero *et al*., 2006). All the above characteristics and based on literature (Mas-Coma, 2005; Guy *et al.*, 2001) indicates that most animals with *F. hepatica* that were sampled were in the chronic phase of the disease.

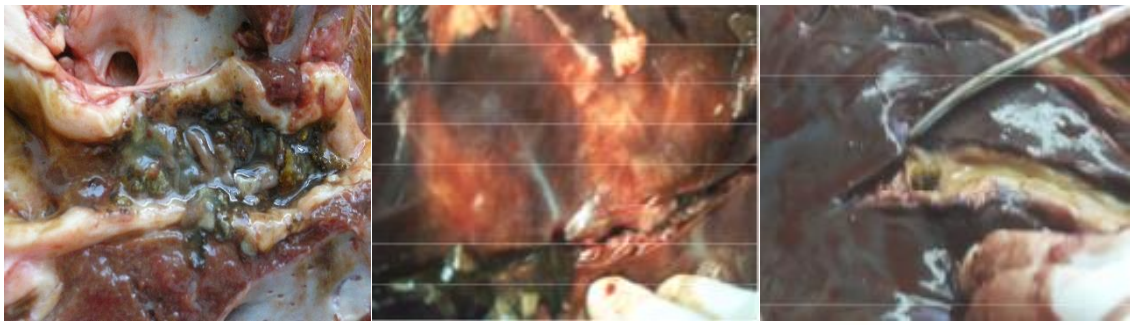

Figure 1. a) Opening of a liver duct where we could observe crystallized salts and individuals of *F. hepatica*, b) change of the liver coloration (fibrosis-cirrhosis), and c) crystallization of biliary liquid inside a duct liver.

At cellular level, comparing with clinically healthy liver tissue (Figure 2a), we could observe loss of the morphology, chronic hepatitis, inflammation forming micro-abscesses, proliferation of bile ducts, intrancytoplasmic cholestasis, chronic inflammatory infiltrate based of lymphocytes, dilation of the sinusoids (Figure 2b), thickening of the capsule of Glisson by a chronic process, presence of fibroblasts, fibrosis, cirrhosis, and eggs of *F. hepatica* in the ducts and in the hepatic parenchyma (Figure 2c).

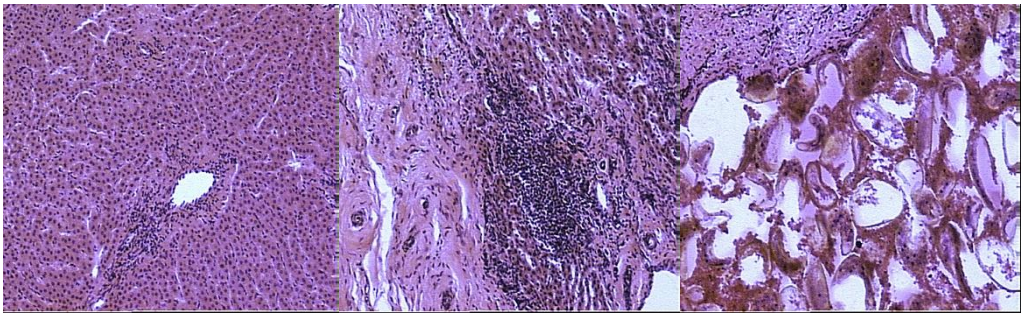

Figure 2. a) Show the arrangement of hepatocytes (H), sinusoids (S), central vein liver (CVL), terminal liver vein (TLV), and Kupffer cells (KC). b) Presences of inflammatory infiltrate predominantly of lymphocytes, proliferation of capillaries and fibrosis, hepatocytes cords with degenerative changes. c) Accumulation of *F. hepatica* eggs (*Fh*) surrounded by necrosis (NT) and cellular detritus, delimited by fibrous tissue liver (FTL) with inflammatory infiltrates.

B) *Differential recount:* About the counting of white blood cells, we found significant differences (*P<0.01*) in eosinophils values in the analyzed groups, also in monocytes in males (Table 1). According to the number of flukes, there were significant differences in males who presented a decrease of neutrophils, an increase of lymphocytes; and in both groups (males and females) there was a decrease of monocytes and an increase of eosinophils, all of this according to the number of flukes increase (Table 2).

Table 1. Values of the counting of white blood cells of clinically healthy bovines and bovines with *F. hepatica*.

		n	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Males	**Healthy**	**992**	54.9 ± 1.6	38.7 ± 1.5	2.0 ± 0.3^{a}	3.8 ± 0.4^{a}	0.6 ± 0.1
	F. hepatica	**996**	47.8 ± 3.0	45.7 ± 2.9	4.3 ± 1.1^{a}	1.8 ± 0.4^{b}	0.3 ± 0.1
Females	**Healthy**	**956**	50.7 ± 2.2	43.8 ± 2.0	2.9 ± 0.4^{b}	2.4 ± 0.4	0.2 ± 0.1
	F. hepatica	**914**	46.5 ± 4.1	42.1 ± 3.8	8.4 ± 2.3^{a}	2.3 ± 0.4	0.8 ± 0.4

Different letters show significant differences (*P<0.01*).

Table 2. Values of the counting of white blood cells of bovines with *F. hepatica* according to the number of flukes into the liver.

		n	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Monocytes (%)	Basophils (%)
Males	**Healthy**	**992**	54.9 ± 1.6^{a}	38.7 ± 1.5^{b}	2.0 ± 0.3	3.8 ± 0.4	0.6 ± 0.1
	1 – 10	**249**	50.4 ± 5.3^{ab}	42.2 ± 4.0^{b}	4.8 ± 0.26	2.6 ± 0.7	0 ± 0
	11 – 25	**301**	49.8 ± 2.8^{ab}	43.0 ± 3.7^{ab}	5.5 ± 0.17	1.3 ± 0.6	0.5 ± 0.3
	26 – 50	**154**	54.5 ± 0.5^{ab}	41.5 ± 1.5^{b}	2.5 ± 0.5	1.5 ± 1.5	0 ± 0
	>51	**292**	31.0 ± 1.0^{b}	64.0 ± 1.0^{a}	2.5 ± 0.5	1.5 ± 0.5	1 ± 0
Females	**Healthy**	**956**	50.7 ± 2.2	43.8 ± 2.0	2.9 ± 0.4^{b}	2.4 ± 0.4	0.2 ± 0.1
	1 – 10	**436**	50.5 ± 6.9	44.4 ± 6.0	3.0 ± 0.7^{b}	1.9 ± 0.6	0.3 ± 0.2
	11 – 25	**478**	45.0 ± 2.0	45.5 ± 5.5	8.0 ± 0.8^{a}	1.5 ± 0.5	0 ± 0

Different letters show significant differences (*P<0.01*).

The increase in the number of eosinophils in animals with *F. hepatica* concord with the diagnostic characteristics of the disease (Mas-Coma, 2005; Guy *et al.*, 2001); however, this increase is more evident during parenchymal phase and this cells increase when the parasite enter into the bile ducts (Poitou *et al.*, 1992, 1993; Jemli *et al.*, 1993).

C) *Metabolic profile*: In relation to metabolic profile, there were significant differences (*P<0.05* y *0.01*) in the values of Urea/BUN and phosphorus between the two groups of males, whereas females only in Urea/BUN (Table 3).

Table 3. Values of the metabolic profile of clinically healthy bovines and bovines with *F. hepatica*.

	n	Calcium mg/dL	Phosphorus mg/dL	Urea/BUN mg/dL	Bilirubin mg/dL	Cholesterol mg/dL	Total Proteins g/L	γGT (U/I)	GPT (U/I)	GOT (U/I)	LHD (U/I)	Alkaline Phosphatase (U/I)
Healthy	**992**	8.4 ± 0.5	5.2 ± 0.01[b]	26.4 ± 1.5[a]	0.2 ± 0.01	182.9 ± 19.4	49.8 ± 1.9	28.5 ± 4.7	258.1 ± 66.6	131.8 ± 39.5	1838 ± 88.4	375.8 ± 39.3
F. hepatica	**996**	8.8 ± 0.4	5.5 ± 0.1[a]	13.9 ± 1.9[b]	0.3 ± 0.02	163.8 ± 22.6	47.1 ± 1.7	29.3 ± 5.1	259.7 ± 51.6	51.9 ± 20.4	1338 ± 227.4	431.3 ± 41.5
Healthy	**956**	8.2 ± 0.5	5.3 ± 0.1	22.8 ± 2.6[a]	0.4 ± 0.08	161 ± 28.5	52.5 ± 1.8	26.4 ± 6.3	258.3 ± 44.3	68.2 ± 28.9	1980 ± 155.1	250.9 ± 48.1
F. hepatica	**914**	8 ± 0.3	5.3 ± 0.1	14.9 ± 1.6[b]	0.3 ± 0.02	162.7 ± 16	47.4 ± 2.9	22.9 ± 2.3	245.5 ± 44.9	67.7 ± 17.4	1500 ± 192.9	404.8 ± 59.9

Different letters show significant differences (*P<0.01*)..

Based on metabolic profile of animals with fasciolosis grouped according to the number of flukes, we observed in males that the values of urea/BUN decrease when the number of flukes in the liver parenchyma increases. The values of FA, ALT, AST and Cholesterol in males and AST in females showed a declining trend line when the number of flukes in the liver increases (Table 4).

Table 4. Values of the metabolic profile of bovines with *F. hepatica* according to the number of flukes into the liver.

	n	Calcium mg/dL	Phosphorus mg/dL	Urea/BUN mg/dL	Bilirubin mg/dL	Cholesterol mg/dL	Total Proteins g/L	γGT (U/I)	GPT (U/I)	GOT (U/I)	LHD (U/I)	Alkaline Phosphatase (U/I)
Healthy	**992**	8.4 ± 0.5	5.2 ± 0	26.4 ± 1.5[a]	0.25 ± 0.01	182.9 ± 19.4	49.8 ± 1.9	28.5 ± 4.7	258.1 ± 66.6	131.8 ± 39.5	1838 ± 88.4	375.8 ± 39.3
1 – 10	**249**	7.8 ± 0.6	5.4 ± 0.2	17.4 ± 3.2[ab]	0.29 ± 0.02	193.6 ± 38.5	42.8 ± 1.2	17.4 ± 2.9	239.1 ± 41.2	77.5 ± 56.1	1048.3 ± 498.8	490.9 ± 69.5

11 – 25	**30 1**	9.2 ± 0.7	5.4 ± 0.1	18.7 ± 1.2[ab]	0.28 ± 0.01	199.2 ± 21.6	50.7 ± 0.5	23.2 ± 2.9	151.7 ± 11.7	59.4 ± 13.1	1740.4 ± 129.5	490.6 ± 20
26 – 50	**15 4**	10.9	5.8	13.5[ab]	0.28	143.2	45.4	29	155	60.2	2064.2	371.2
>51	**29 2**	8.1	5.4	9.2[b]	0.24	97.8	39.6	29	60	11.3	1440.9	151.8
Healthy	**95 6**	8.2 ± 0.5	5.3 ± 0.1	22.8 ± 8.2	0.37 ± 0.08	161 ± 28.5	52.5 ± 1.8	26.4 ± 6.3	258.3 ± 44.3	68.2 ± 28.9	1980.4 ± 155.1	250.9 ± 48.1[b]
1 – 10	**43 6**	7.6 ± 0.4	5.2 ± 0.1	16.9 ± 2.4	0.27 ± 0.03	171.7 ± 31.3	49.2 ± 3.3	22.4 ± 2.6	230.6 ± 77.1	73.9 ± 36	1455.5 ± 152.6	505.4 ± 88.8[a]
11 – 25	**47 8**	8.2 ± 0.6	5.4 ± 0.2	18.2 ± 3	0.31 ± 0.02	163.5 ± 7.9	58.9 ± 4.9	29.7 ± 8.4	300 ± 114.8	47.8 ± 16	1413.6 ± 458.4	357.4 ± 26.1[ab]

Different letters show significant differences (*P<0.01*).

As a result of damage, this parasitic disease, caused heavy economic losses in livestock, by causing a negative effect on production, causing a decrease of weight gain (Loyacano *et al*., 2002), reproductive efficiency and milk production (Chirinos y De Chirinos, 1993) and the confiscation of liver (Ortega *et al*., 2007).

Conclusion

The study demonstrates the significant damage caused by this parasitic disease (distomatosis) in ruminants of economic importance (more than 182 million dollars are lost in Mexico for this disease annually, in treatments and deaths of animals) for human consumption, currently its prevalence It is rising from 1.7% in 2009 to 23.4% in 2019 in trails, although for a period of 10 years it remained almost lost, this rise is probably due to climate change or resistance to food additives, such as is Clenbuterol, administered indiscriminately to animals intended for fattening. This study aims to demonstrate that fascioliasis is a parasitic condition that increases productive and reproductive deficiencies in the country, so far there is no effective control or diagnosis of it.

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DEVELOPING MOBILE APPLICATIONS BY APPLYING SPATIAL MODELLING FOR PREDICTING DISEASE SPREADS AND FARM PREVENTION

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Introduction

Under the situation that the novel diseases have emerged globally, for instance, African swine fever (ASF) caused damage to pig production and Bat-borne pathogens such as SARS, MERS, Ebola and Nipah virus infection harmed human and animal life. Attempts to control these emerging diseases have been conducted over the past decade. Under the Emerging Pandemic Threat Program Phase II (EPT2), FAO/USAID provided funds for Department of Livestock Development (DLD) to conduct an operational research in Thailand. Two mobile applications were developed by applying spatial models in order to evaluate pig farms on disease preventive levels and to predict disease spreading areas.

Methods and results

Multi-criteria Decision Analysis (MCDA), a knowledge-based model, was applied to evaluate pig farm on 6 important diseases in pig including ASF, CSF, PRRS, FMD, PED, and Nipah. The equations were coded under mobile application which the risk level of pig farm for each disease was calculated automatically. Improvement of farm biosecurity will be suggested after evaluation. Moreover, the location and risk level of farm will be shown on Google map. Simultaneously, the spatial risk of important diseases were determined and the risk maps were generated which this allows the outputs of farm evaluation to show together on the map. The other mobile application was developed by modifying the infectious models for predicting disease outbreaks in animals. Pig movement networks were analyzed and used to build a SIR model. The output destination areas as well as the magnitude of outbreaks were predicted and shown on the map and spread sheet.

Conclusion

These two novel technologies apply complicated algorithms to predict the disease outbreaks as well as to evaluate farm from diseases and visualize the outputs with simply transformed interpretation. This allows the responsible officers to receive information with simple way and better understanding resulting in controlling the diseases effectively. Furthermore, the improvement of farm biosecurity has been conducted after risk assessment to support sustainable livestock production systems.

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EXPERIMENTAL PATHOGENESIS OF *PESTE DES PETITS RUMINANTS* IN BLACK BENGAL GOATS

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Abstract

Peste des petits ruminants (PPR), also known as Goat Plague, is a disease of goats, sheep, and other taxonomically related species. It is caused by a *morbillivirus* in the family *Paramyxoviridae.* In Bangladesh, the first outbreak of PPR was recorded in the year 1993 and now PPR is endemic in Black Bengal and causes huge economical losses (Chowdhury et al., 2014). Knowledge of pathology and pathogenesis helps controlling diseases and formulation of supportive therapy when required. Information on pathogenesis of PPR disease is available elsewhere for different goat and sheep breeds but is not available in Black Bengal goats. Bangladesh has joined the PPR Global Eradication Program 2017 – 2030. Since pathology and localization of antigen of infectious agents varies across animal species and type, information on pathogenesis of PPR is required for the national control and eradication plans. This paper describes experimental pathogenesis of the disease and its consequences in Black Bengal goats. Eight (8) Black Bengal goats were experimentally infected with 2 ml of goat kidney cell culture fluids containing a virus titer of $\log_{10}$ $TCID_{50}$ 5.6/ml. Goats were sacrificed at different post infections (dpi). Signs of infectivity were developed in 100% experimentally infected goats. Based on the clinical signs produced, we divided the PPR disease into four overlapping phases: incubation period (first 4- 8 dpi), phase of primary viremia (5^{th} to 13^{th} dpi), phase of secondary viremia (8^{th} to $\geq 13^{th}$ dpi) and phase of convalescence or fatality ($\geq 13^{th}$ dpi). During the incubation period, the infected goats showed no visible clinical signs except very mild dullness (in a few cases) and scanty nasal secretion only at the morning. Fever started within 5 to 8 dpi when the virus moved to the nearby lymphoid organs, multiplied extensively, entered the circulation and produced the primary viremia. This resulted in onset of clinical signs *viz.* dullness, clear watery nasal and ocular discharges and rise of temperature (104-105^0F). During secondary viremia, similar but more extensive clinical signs were noticed with further rise of temperature (>106^0F); the discharges were more purulent, some ulcerative lesions developed in the mouth and nostrils, eyelids were severely inflamed, which sometimes closed the eyes. At the end of the secondary viremia, severe diarrhea was noticed. At the 4^{th} stage, 3 of 8 goats died, the rest of the goats had been sacrificed at different time points.

The gross pathological findings were consistent with clinical disease in goats. Those dying at 13, 15 and 18 dpi after showing characteristics clinical signs. Hemorrhages and congestion were common in the respiratory, digestive and lymphoid organs. Oral mucosa, tongue and nostrils showed erosive necrosis after 10 dpi and severity increased with time. There were cases of severely congested trachea after 7dpi, filled with white froth. Lungs showed gradual consolidation and at the later stages most of the lungs consolidated. At this stage the goat showed labored open mouth forceful breathing. White to grayish necrotic spots on the outer surface of the PPR affected liver was found after 7 dpi in our experimental infection. Kidneys in a few of the infected goats were severely hemorrhagic and inflamed. Similar lesions in liver and kidneys have been described in Black Bengal goats at natural outbreaks in Bangladesh recently, but not reported earlier elsewhere (Begum et al., 2019). The major histopathological findings included hemorrhages, congestion, mononuclear infiltration and extensive necrosis in respiratory, digestive and lymphatic organs. Ulcerative pyogranulomatous lesions in mouth and its associated structures were also common in goats that survived for a longer period. Viral inclusion

bodies were visible in epithelial cells, lymphocytes and antigen presenting cells in various organs and in exudates. Salivary glands of the epiglottis and associated structures were found filled with mucus after 5 dpi and also consistent with inclusion bodies in the acinar cells. Mononuclear infiltrations, sloughing off tracheal epithelium with hemorrhages in the lamina propria were seen on the 7 dpi, 14 dpi and 18 dpi and in dead goats. In PPR affected goats, the first lesions in lungs were started with slight congestion, and focal accumulation of some mononuclear inflammatory cells at 5 dpi. Over time, the alveoli ruptured and collapseddue tomassive infiltration of large mononuclear cells in the interstitial space and luminal sites was observed at 7 dpi. In addition, pyogranulomatous pneumonia, severe hemorrhage and congestion, mono nuclear infiltration, syncytia formation and degeneration of syncytial cells, presence of giant macrophages and accumulation of pneumocyte II cells were observed after 10 dpi. In our experiment, PPR infected livers and kidneys were showed remarkable lesions after 7 day of post infection. In the liver, at an early stage the fatty change and single hepatocellular necrosis were noticed. At the later stage single hepato-cellular necrosis converted to multi-focal necrosis. The most striking lesions were found in the kidney. These were necrotic tubules, fusions of tubular epithelia and haemorrhages and necrotic materials accumulated in some of the tubular lumen. Lesions in the intestine, spleen and lymph nodes were similar to the previous research findings.

On haemato-biochemical analyses, leukocytosis was found at the first febrile phase of the disease, however, immediately soon after the initial febrile phase, leukopenia was developed. On differential counts, lymphopenia and neutrophilia were noticed at 10 dpi. Other routine parameters were within the normal ranges. Total protein (Fig. 1a) and albumin decreased significantly after 10 dpi and urea B and blood urea nitrogen (Fig. 1b) also found increased at the 18 dpi. Likewise, the level of aspartate transaminase (AST) (Fig. 1c), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and creatine kinase (CK) (Fig. 1d) increased significantly after 7 dpi when compared to uninfected control goats. These indicate liver and kidney dysfunction in the infected goats. Our pathological investigation also revealed necrotizing hepatitis and nephritis. Furthermore, sodium and chloride ions (Fig. 1e-f) were seen with elevated values at the 14 dpi due to severe diarrhea that leads to severe dehydration.

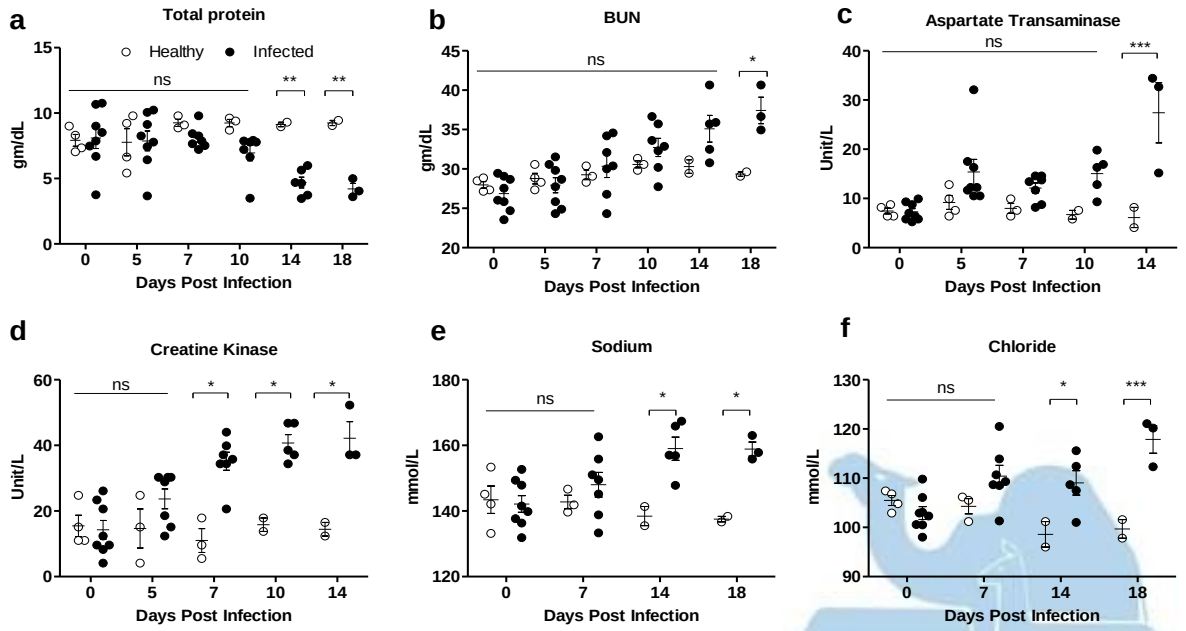

Fig. 1: Experimental *peste des petits ruminants virus*-infected Black Bengal goats showed altered haemato-biochemical and electrolyte profiles.

Real Time RT PCR revealed very high virus loads in pre-scapular lymph nodes at 5 dpi (Fig. 2a). Tracheal mucosa and intestinal mucosa also revealed comparatively higher virus titer (Fig. 2b). The titer further increased many fold at 7 dpi in lungs, blood and spleen whereas virus load decreased in other organs (Fig. 2c). The virus travelled from the site of entry to the lung and spleen and then

replicated further and entered into circulation (primary viremia). At day 14 dpi, almost all visceral organs revealed high titer of viruses (Fig. 2d-e), released into blood and thus produced secondary viremia (blood also revealed high titer of virus). After day 14 the titer decreased sharply in all organs. Details can be seen in Fig. 2. 100% sero-conversion of experimental goats occurred against PPR virus at 14 dpi. Urine, feces and all other secretions revealed very high titers of virus after secondary viremia. Immunohistochemistry revealed localization of antigen in epithelial cells, lymphocytes and in antigen presenting cells of different visceral organs including liver, kidney, lungs etc.

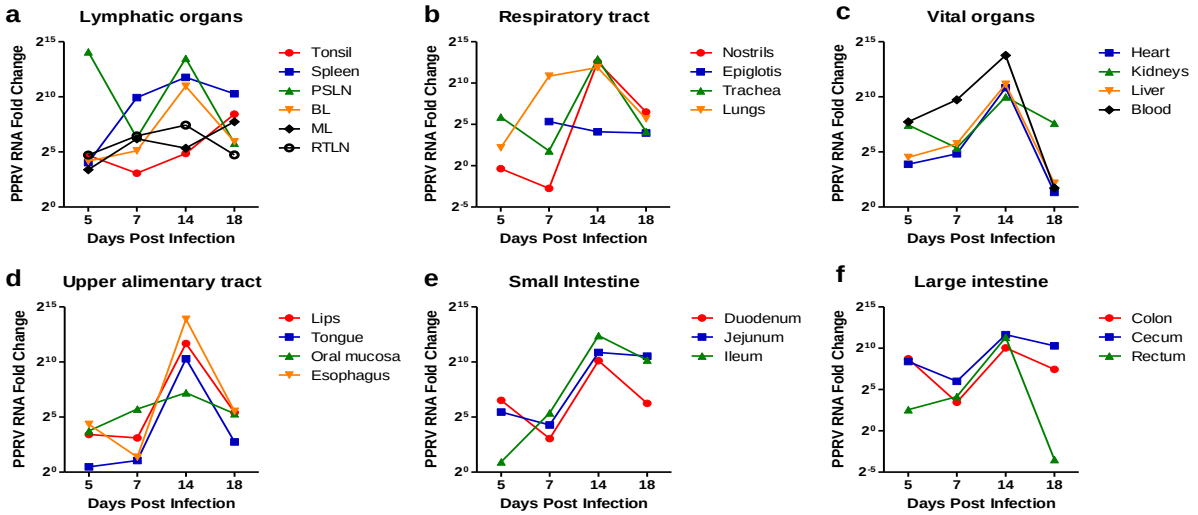

Fig. 2: Virus loads in different tissues of PPRV infected goats at different time point.

Based on the above findings, we elucidated pathogenesis of PPR disease as follows: virus enters through orinasal routes. Virus enters into regional lymph nodes through lymphatic channels. After initial replication in the regional lymph nodes, the virus moves to lungs followed by the alimentary and respiratory tracts and enters into blood (first viremia), localizes in various tissues including visceral organs and massive replication occurs and releases plenty of viruses in circulation (second viremia). The important visceral organs like liver and kidney sustain damage during virus replication. Virus releases from the host through secretions after the first viremic stage and then continues till convalescence. Diarrhea is developed due to enteritis during or at the end of second viremic stage and temperature falls to sub-normal levels due to dehydration and liver, kidney and respiratory dysfunction and animals die. A small percentage of animals may survive depending upon previous health status, but remain immunosuppressive.

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MOLECULAR EPIDEMIOLOGY OF FMD VIRUS SEROTYPE O IN THAILAND DURING 2017-2019

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Introduction

Foot and mouth disease (FMD) is recognized as the most important animal disease from economical point of view. The causative agent is FMD virus (FMDV), which belongs to genus *Aphthovirus* in the family *Picornaviridae*. FMDV has seven immunologically distinct serotypes (O, A, C, Asia1, SAT1, SAT2, SAT3). Additionally, study of nucleotide sequence of the VP1 region of the FMD virus has shown that this regionis very important for molecular epidemiology. It showed that the FMDV could be classified to many topotypes that indicate the geographic outbreak; Middle East-South Asia (ME-SA), South East Asia (SEA), Euro-South-America (Euro-SA), Asia, etc. In Thailand, FMDV serotypes O and A are common, but FMDV serotype Asia1 has not been found for more than twenty years. Aprevious study of molecular epidemiology on FMDV serotype O in Thailand showed that was the commonviruses,specificallyO/Mya-98/SEA and O/PanAsia/ME-SA sub-types.A recent molecular epidemiological work new the FMD virus serotype O lineage (O/Ind2001/ME-SA),which is commonly found in the Indian Subcontinent. This lineage was classified into five sub-lineages (a, b, c, d and e),butonly two sub-lineages have been found in South East Asia (Bachanek–Bankowska et al., 2018). One was Ind2001d sub-lineage was that found in Vietnam in 2015 and Lao PDR in late 2015. The other was Ind2001e sub-lineage that was found in Myanmar and Thailand in late 2016. Thus, the objective of this study was to perform a molecular epidemiology of FMDV serotype O for emerging or re-emerging virus-lineage outbreaksin Thailand during 2017-2019.

Material and Method

Viruses:Totals of 113~~152~~, 190~~176~~ and 147~~41~~ were collected in 2017, 2018 and 2019, respectively. All of them were tested by ELISA typing and real-time RT-PCR to confirm FMDV and the serotype of virus. Then,109~~57~~ viral fluid samples (filtered 10% PBS tissue sample suspension) of FMDV type O were used for investigationby nucleotide sequencing.
RNA: Total RNA was extracted from viral fluid samples using Trizol®LSReagent (AMBION, USA) according to manufacturer's protocol.

RT-PCR and Sequencing protocol

Reverse transcription (RT) and Polymerase chain reaction (PCR):The extracted RNA was reverse transcribed and amplified using NK61 and 1C-609 primers set specific for the VP1 gene and mixed with LightCycler® multiplex RNA virus master (Roche, USA) according to manufacturer'sprotocolina C1000 Touch™ Thermal Cycler (Bio-rad, USA). The reaction was performed for an initial incubationcycleat 50°C for 30 min and 95°C for 15 min followed by 35 cycles of PCR (95°C for 1 min, 60°C for 1 min and 72°C for 2 min), a final 5-min incubation at 72°C and a cooling at 4°C.

Nucleotide Sequencing (Knowles and Samuel, 1998): PCR product was purified withtheQIAquick PCR purification kit (QIAGEN,Germany) and labeled with BigDye®terminator v3.1 cycle sequencing kit (Applied Biosystems, USA) and NK72 primer. Excess BigDye®terminator was removed by Centri-Sep™columns(Applied Biosystems). The labeled sample was dried,resuspended with HiDiformamide (Applied Biosystems) and then subjected to nucleotide sequencing by using ABI3500 Genetic Analyzer (Applied Biosystems).

Phylogenetic analysis:Nucleotide sequencing results were analyzed and aligned using BioEdit software version 7.2.5. Phylogenetic analysis based on the multiple alignment of partial VP1 gene sequence (639 bp) was performed using MEGA software version 6.0 by the neighbor-joining method with 1,000 replications of bootstrap values.

Result

The phylogenetic tree showed three lineages of FMDV serotype O (Ind2001e/ME-SA= 89~~41~~ samples, PanAsia/ME-SA= 14~~10~~ samples and Mya-98/SEA= 6 samples). O/Ind2001e/ME-SA was predominant and these virus isolates were closely related to O/ME-SA/Ind2001e samples from Myanmar and Thailand in 2016. In addition, O/PanAsia/ME-SA virus isolates were related to sample from Cambodia in 2015 and O/Mya-98/SEA virus isolates looked like the previous virus isolates in South East Asia. (Figure 1).

Conclusions

The molecular epidemiological investigation of FMDV type O, which caused the outbreaks in Thailand during 2017-2019 revealed that FMDV serotype O was still geneticallyto previous~~previously~~ virus isolates from the region.To determine antigenic variation, some virus isolates of those were tested via vaccine matching with the current Thai vaccine seed strains.The results of vaccine matching show a good match with the Thai vaccine strain (O/Udornthani/87).

Keywords: molecular epidemiology, FMD virus, serotype O, Thailand

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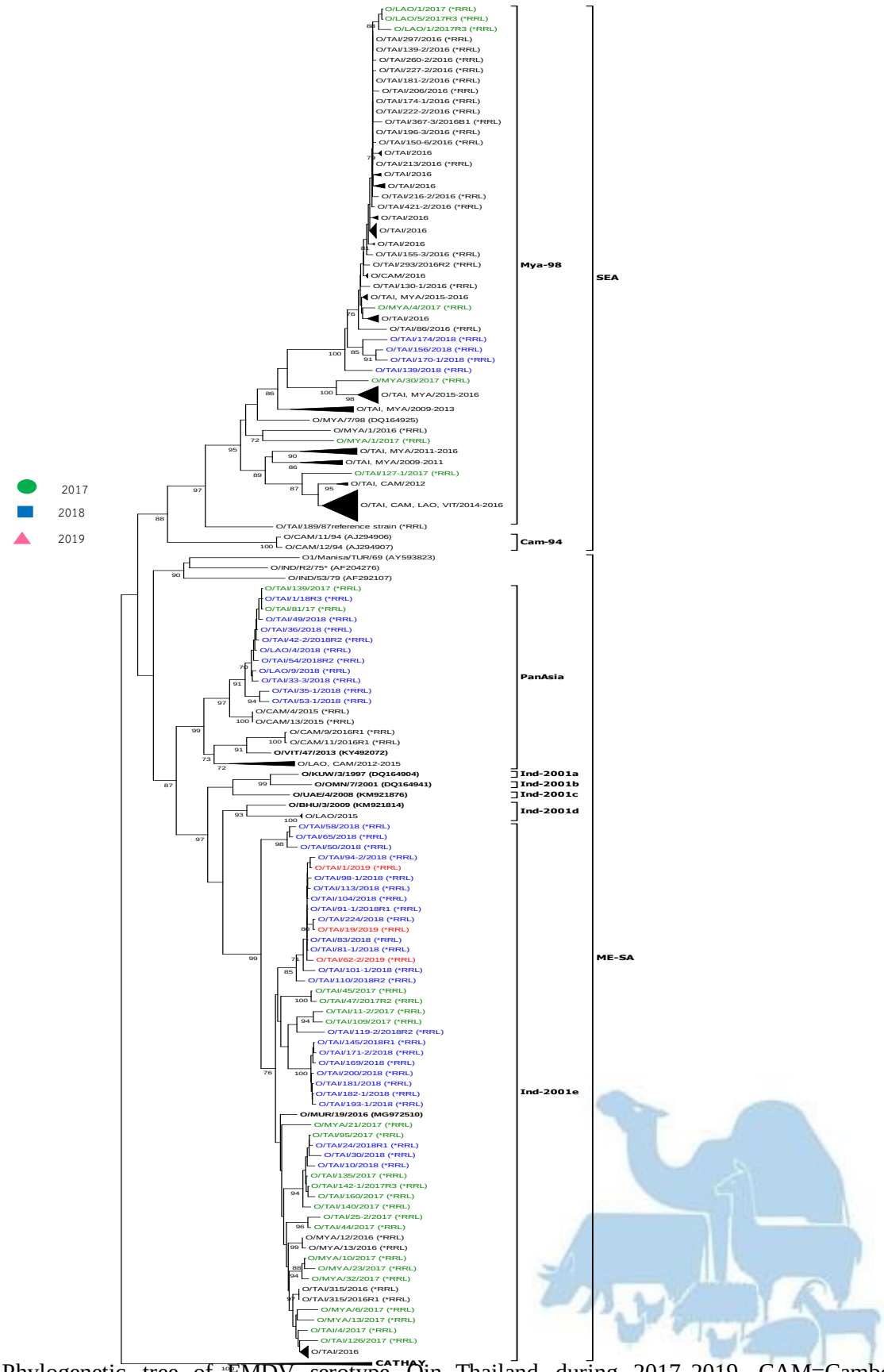

Figure 1. Phylogenetic tree of FMDV serotype O in Thailand during 2017-2019, CAM=Cambodia, LAO=Lao PDR,MYA=Myanmar, TAI=Thailand and VIT=Vietnam

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DETECTION OF EQUINE INFECTIOUS ANEMIA IN NATIVE THAI PONY FROM NORTHERN PART OF THAILAND

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Keywords: Equine Infectious Anemia, native Thai pony, northern Thailand

Introduction

Thai Pony breed is a native horse of Thailand usually found in the northern part of country. They are used for transportation, agriculture and nowadays in tourism for pulling carriages. From their DNA pattern indicates that the native Thai pony has DNA features that suggest they are closely related to the horses of Mongolia. They are promoted to be the "Heritage" horse in the country by the local pony foundation, which attempts to preserve this ancient breed. However, apart from promotion as a Heritage breed, disease control is also an important strategy for native breed conservation. Equine Infectious Anemia (EIA) is an important equid disease caused by a retrovirus in the genus *Lentivirus*. This study aimed to generate the nucleotide primer sets for detection of EIA virus in blood samples and tissues of euthanized seropositive horses to confirm the disease status and using to study molecular characteristics and phylogeny of viruses.

Materials and methods

Six seropositive horses (with AGID test) for EIA were euthanized. EDTA-blood and tissues including liver, spleen, lymph node, lung, heart, and kidney samples were collected from those horses for further testing. Total nucleic acids were extracted from samples using a spin column-based extraction kit. This study designed primers to amplify the 647-bp partial fragment of the *gag* gene by using the Primer3 program (http://frodo.wi.mit.edu/primer3/). The results of PCR detections in each sample were compared. Moreover, the amplicons were sequenced and processed for sequence alignment. A phylogenetic tree was generated based on the neighbor joining statistic with 1000 bootstrap replications by using MEGA software (version 10.0.5).

Results and discussion

The PCR assay with primers developed in this study detected viral DNA/RNA in all six seropositive horses, however, variation occurred with each sample type. Viral nucleic acids were detected in all EDTA-blood samples, indicating blood as the sample of choice for EIA detection using the PCR assay. In addition, internal organs including spleen, liver and lung were somewhat appropriate samples to investigate the virus (Table 1). Among six PCR positive horses, two of them were selected for sequencing and phylogenetic study. Based on the *gag* gene, the phylogenetic analysis showed that Thai pony isolates were most closely related to EIA virus Goshun 482v and Tokyo 548v isolates (accession no AB675093 and AB675094, respectively) which were found in native Japanese ponies. In addition, the EIA viruses in this study were in the Asian cluster, indicating that there are similar viruses circulating in native pony breeds in this continent (Fig 1).

Table 1. Comparison of PCR detection for EIA virus in each sample type.

Horse no.	Sample						
	EDTA-blood	Spleen	Liver	Lung	Lymph nodes	Heart	Kidney
1	+	+	++	+	-	-	-
2	++	++	++	++	++	++	++
4	++	++	-	-	-	-	-
5	++	++	++	++	+	-	-
7	++	-	+	-	-	-	-
8	+	+	-	-	-	-	-

++: strong positive
+: weak positive
-: negative

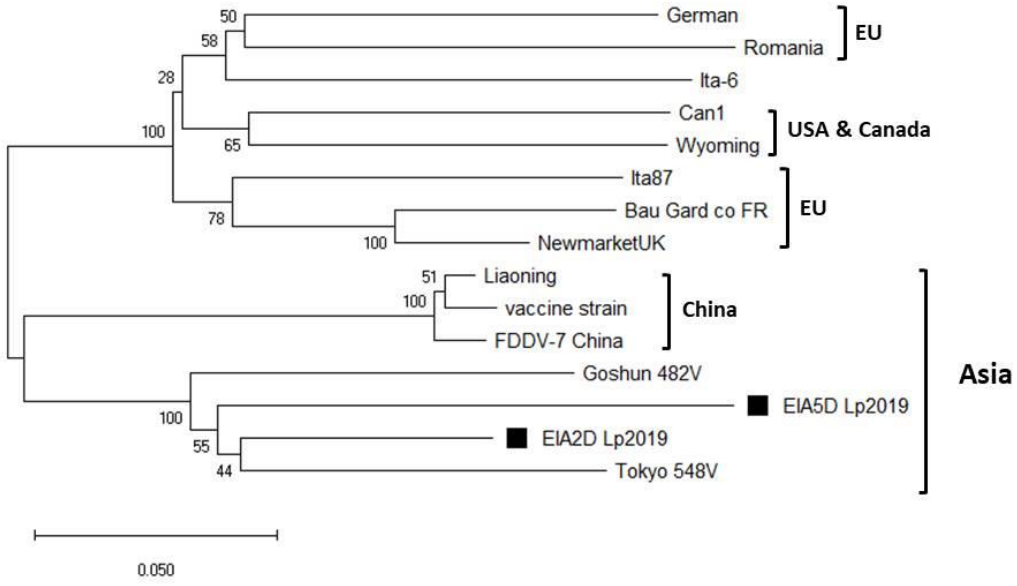

Figure1. Phylogenetic analysis of Thai native ponies in this study ( ) and other EIA isolates based on the partial fragment of *gag* gene.

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SEROLOGICAL EVIDENCE OF FOOT AND MOUTH DISEASE VIRUS TYPE A & ASIA 1 INFECTION IN LOCAL GOATS IN NATOGYI AND MYINGYAN TOWNSHIPS IN MYANMAR

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Abstract

Foot and mouth disease (FMD), one of the most important transboundary animal diseases, is endemic in Myanmar particularly in the central parts. Serotype O Foot and mouth disease virus (FMDV) is frequently detected from cattle. Prevalence of FMDV serotype O in goats was also reported. The occurrence of FMD in cattle due to serotype A and Asia 1 were reported in Myanmar. However, there are limited report on the occurrence of FMDV due to serotype A and Asia 1 in goats, the maintenance host for FMDV. Therefore, the objective of this study is to explore the occurrence of FMD due to Type A and Asia 1 in goats in Natogyi and Myingyan townships of Myanmar. Totally 450 serum samples were randomly collected from the goats (238 from Natogyi and 212 from Myingyan) during July to August 2017. The goats were not vaccinated against FMDV Type A and Asia 1. Serotype specific antibodies to A and Asia 1 were detected by commercial available Enzyme linked immunosorbent assay (ELISA) kits. As the results, 79 of 450 samples (17.56%) and 5 of 450 (1.11%) samples were seropositive to FMDV serotype A and Asia 1, respectively. The positive samples of FMDV serotype A and Asia 1 were also confirmed by FMDV nonstructural proteins (NS) specific blocking ELISA. Specific antibodies to NS protein of FMDV were detected in 54 of the 79 serotype A positive samples and 4 of the 5 serotype Asia 1 positive samples. The presence of specific antibody to FMDV NS protein confirmed the presence of natural infection in goats. Our study clearly indicates that the occurrence of FMDV Type A and Asia 1 in local goats from Myingyan and Natogyi townships in Myanmar.

Keywords: Foot and Mouth Disease, Myanmar, Blocking ELISA, NS ELISA.

Introduction

Foot and mouth disease (FMD) is a highly infectious and economically devastating disease of livestock. FMD is caused by Foot and Mouth Disease Virus (FMDV), of the *Aphthovirus* genus in the *Picornaviridae* family. There are seven different serotypes FMDV: such as O, A, C, Asia 1, Southern African Territories [SAT] 1, SAT 2, and SAT 3. The endemics of FMDV Type O, A and Asia 1 in Myanmar are well documented (Gleeson, 2002; Di Nardo et al, 2011; Andel *et al*., 2019; Blacksell *et al*., 2019). The occurrence of FMD due to serotype O in cattle and buffalo was frequently reported (Maung *et al*., 2019). The occurrence of FMD due to serotype Asia 1 virus in cattle was reported in the Rakhine State of Myanmar in 2017 (Bo *et al*., 2018). However, most of the reports on FMD outbreak were mainly based on the occurrence in cattle. There are limited reports on the occurrence of FMDV due to type A and Asia 1 in goats, the maintenance host for FMDV. Therefore, the objective of this study is to explore the occurrence of FMD due to Type A and Asia 1 in goats in Natogyi and Myingyan townships of Myanmar.

Materials and Methods

Totally 450 serum samples were randomly collected from the goats (238 from villages of Natogyi and 212 from villages of Myingyan) during July to August 2017 (Figure 1). Serum samples were collected based on flock size (5 from 20-30, 15 from 30-50 and at least 30 from>50 flock size). The goats were not vaccinated against FMDV serotype A and Asia 1. Specific antibody to FMDV serotype A and Asia 1 were detected by commercially available serotype-specific Enzyme linked immuno-sorbent assay (ELISA) kits for serotype A and Asia 1 (PrioChek, USA). Seropositive samples of FMDV Type A and Asia 1 were also confirmed by FMDV nonstructural proteins specific blocking ELISA (NS ELISA) (PrioChek, USA). The data were analyzed by 2×2 contingency test and $p<0.05$ was considered as significant.

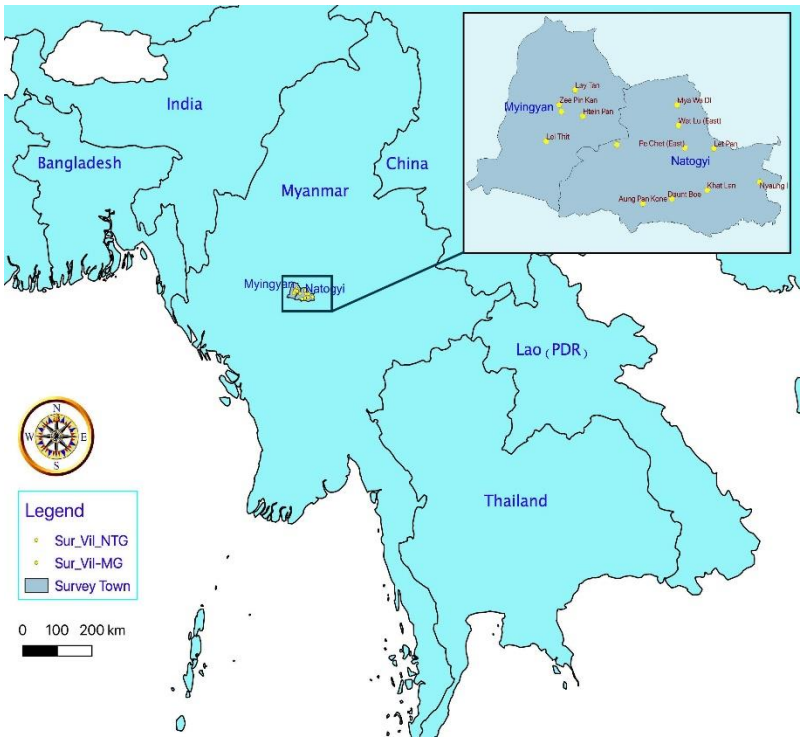

Figure 1. The map illustrating location of the villages and the townships for the survey area. Since FMD outbreaks were mainly reported from central Myanmar, Natogyi and Myingyan townships are selected as the survey areas.

Results

As the results of serotype-specific ELISA, 79 of 450 samples (17.56%) and 5 of 450 (1.11%) samples were seropositive to FMDV Type A and Asia 1, respectively. Interestingly, there was no sample with seropositive to both serotypes.Specific antibodies to the both serotypes were detected in the both townships. Specific antibodies to NS protein of FMDV were detected in 54 of the 79 serotype A-positive samples and 4 of the 5 serotype Asia 1-positive samples (Table 1).

Table 1. The seropositivity to FMDV in goats from two townships

FMD Serotype	Township	No. of tested sera	No. of positive sera & %	Confirmation by NS ELISA & %
Type A	Natogyi	238	11 (4.62%)	10 (4.20%)[b]
Type A	Myingyan	212	68 (32.08%)	44 (20.75%)[a]
Type A Total	Both Townships	450	79 (17.56%)	54 (12.00%)[A]
Asia 1	Natogyi	238	1 (0.42%)	1 (0.42%)[a]

Asia 1	Myingyan	212	4 (2.36%)	3 (1.42%)[a]
Asia 1 Total	Both Townships	450	5 (1.11%)	4 (0.89%)[B]

[a,b & A, B] = The data within the same column with the different superscripts are significantly different at $p<0.05$ (2×2 Contingency test).

Discussion

FMD is usually relatively mild or subclinical in goats. Many infected goats have such mild symptoms that they are easily missed on clinical examination. However, they are still infectious to other livestock. Goats are capable of spreading infection for up to nine months after being infected, even though the disease may have passed without any clinical signs being seen (Matthews, 2016). The presence of specific antibody to FMDV NS protein confirmed the presence of natural infection in goats (Uttenthal *et al*., 2010).

The occurrence of serotype A was significantly higher ($p<0.05$) than that of serotype Asia 1 in goats in the two townships. The occurrence of serotype A in Myingyan township was significantly higher ($p<0.05$) than that of Natogyi township. In the present study, seropositivity percentage were relatively lower when compare with the previous study by Phyoe *et al*., (2017), who found that overall prevalence of 46.8% in sheep and goats by LPB ELISA against FMDV serotype O. It may probably due to detection of specific antibody to serotype O, the major prevalence serotype in Myanmar, in their study. It may also due to differences in sensitivity and specificity of the ELISA used in the different studies. The endemic of FMDV serotype O is well documented in the central Myanmar and FMDV serotype O was frequently detected. In conclusion, our study clearly indicates that the occurrence of FMDV serotype A and Asia 1 in local goats in Myanmar. Since the goats may act as maintenance host for FMDV and are capable of spreading infection for long period, it is important to consider the presence of unvaccinated goats in an endemic area for proper control and eradication programmes of FMD.

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OCCURRENCE OF INFECTIOUS BOVINE RHINOTRACHEITIS AND ITS ASSOCIATED RISK FACTORS IN LOCAL CATTLE IN MYANMAR

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Abstract

Infectious Bovine Rhinotracheitis (IBR), caused by Bovine Herpesvirus-1 (BHV-1), is one of the most common bovine respiratory diseases. However, there is scarcity of report on the occurrence of IBR in the local cattle of Myanmar. Therefore, this study was conducted to investigate the occurrence of IBR and associated risk factors in local cattle by cross-sectional study in the Nay Pyi Taw Union Territory and Sagaing region in Myanmar from June to December 2019. A total of 387 serum samples of local cattle were collected from 12 farms (9 from Nay Pyi Taw 3 from Sagaing): 259 from Nay Pyi Taw and 128 from Sagaing. All cattle had no vaccination against IBR infection. Specific antibody to IBR was detected by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (ID.vet, France). The cow level occurrence of IBR seropositivity was evaluated by location, age, and sex. The data obtained were analyzed by Chi-square test using SPSS version16 and $p<0.05$ is considered as significant. Overall, there were 100% seropositivity based on farm level and 60.47% seropositivity based on cow level. The occurrence of IBR in Nay Pyi Taw (77.99%) was significantly higher ($p<0.05$) than that of Sagaing (25%). The occurrence of seropositivity of IBR in >6 years age group was significantly higher ($p<0.05$) than those of 2-4 and 4-6 years age groups. The IBR occurrence between male and female was not significant ($p>0.05$). The presence of the viral genome in the seropositive samples was also detected by BHV-1 gB gene specific polymerase chain reaction (PCR) assay using the specific primers. The results of PCR confirmed the presence of IBR infection in the local cattle. Our study clearly indicates the occurrence of IBR as natural infection in the local cattle in Myanmar.

Keywords: Infectious Bovine Rhinotracheitis, Bovine Herpesvirus-1, Myanmar.

Introduction

Infectious bovine rhinotracheitis (IBR), caused by bovine herpesvirs-1 (BHV-1), is one of the major diseases causing significant losses in cattle industry (Nandi et al., 2009; Nettleton and Russell, 2017). The disease in cattle is mainly manifested by fever, dyspnoea, nasal and ocular discharges and loss of condition. The prevalence of IBR reported by the neighbouring countries of Myanmar were 67% in Thailand (Kampa *et al.*, 2004), 35.8% in China (Yan *et al.*, 2008), 38% in India (Nandi *et al.*, 2011). Cow level IBR seroprevalence of 61.40% in the dairy herds has been reported in Myanmar (Kyaw Tin, 2019). However, there is very limited information on the occurrence of IBR in the local cattle of Myanmar. Therefore, the objective of this study was to investigate the occurrence of IBR and associated risk factors in the local cattle in Myanmar.

Materials and Methods

A cross-sectional study was conducted to investigate the occurrence of IBR and associated risk factors in local cattle in the Nay Pyi Taw Union Territory and Sagaing region in Myanmar from June to December 2019 (Figure 1). A total of 387 serum samples of local cattle were collected from 12 farms

(9 from Nay Pyi Taw 3 from Sagaing): 259 from Nay Pyi Taw and 128 from Sagaing. All cattle had no vaccination against IBR infection. Specific antibody to IBR was detected by commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (ID.vet, France). The occurrence of IBR seropositivity was evaluated by location, age, and sex. The data obtained were analyzed by 2×2 Contingency test (Chi-square test) using SPSS version16 and $p<0.05$ is considered as significant. The presence of the viral genome in the seropositive samples was also detected by BHV-1 gB gene specific polymerase chain reaction (PCR) assay using the specific primers (Fuchs et al., 1999). The primers used for amplification were (F: 5'-TAC GAC TCG TTC GCG CTC TC-3'; R: 5'-GGT ACG TCT CCA AGC TGC CC-3').

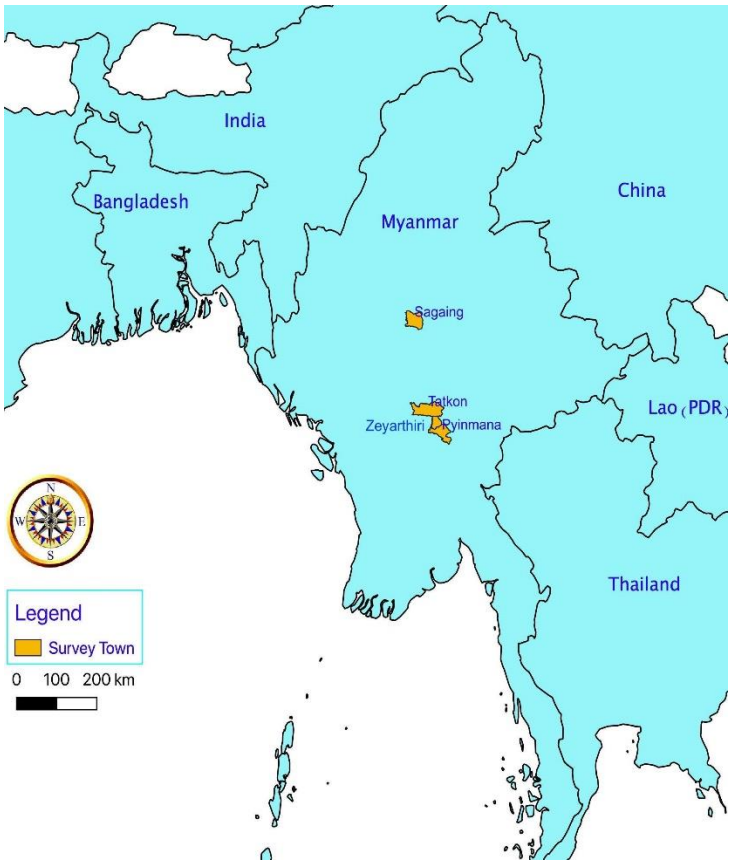

Figure 1. The map illustrating location of the survey area townships at Nay Pyi Taw and Sagaing regions. Since Nay Pyi Taw and Sagaing have relatively higher population of local cattle in comparison to the other regions, these regions were selected as survey areas.

Results

There were 100% seropositivity based on farm level and 60.47% seropositivity based on cow level (table 1). The occurrence of IBR in Nay Pyi Taw (77.99%) was significantly higher ($p<0.05$) than that of Sagaing (25%). Even in the Nay Pyi Taw, there were significant differences ($p<0.05$) in the occurrence of IBR among different townships. The occurrence of seropositivity of IBR in >6 years age group was significantly higher ($p<0.05$) than those of 2-4 and 4-6 years age groups. The IBR occurrence between male and female was not significant ($p>0.05$) (Table 2).

Table 1. The occurrence of IBR in local cattle at farm level and cow level

Level	No. of tested sera	No. of positive sera & %	Occurrence %
Farm	12	12	100 %

Cow	387	234	60.47%

Table 2. The occurrence of IBR in local cattle of different age groups from four townships

Hypothesized factors	Parameter	No. of tested sera	No. of positive sera & %	Significant level
Location	Sagaing	128	32 (25.00%)[b]	P<0.05
	Nay Pyi Taw	259	202 (77.99%)[a]	
Township level	Sagaing	128	32 (25.00%)[c]	P<0.05
	Tatkonn	60	44 (73.33%)[b]	
	Zay Yar Thiri	16	16 (100.00%)[a]	
	Pyinmana	183	142 (77.60%)[b]	
Age groups	2-4 year	137	58 (42.33%)[c]	P<0.05
	4-6 year	148	84 (56.76%)[b]	
	>6 year	102	92 (90.20%)[a]	
Sex	Male	147	91 (61.90%)[a]	NS
	Female	240	143 (59.58%)[a]	

[a,b,c] = The data within the same column with the different superscripts are significantly different at p<0.05 (Chi-square test). NS = Not significant.

Discussion

In this study, 100% farm level and 60.47% cow level occurrence of IBR were observed. All cattle in this study had no vaccination against IBR infection. The PCR results also confirmed the presence of the viral genome of BHV-1 in the collected sera. Therefore, the result of the present study indicates that the presence of IBR natural infection in the local cattle in the Nay Pyi Taw Union Territory and Sagaing region. However, all the local cattle of the survey study apparently healthy at the time of sampling. It indicates the occurrence of IBR as a latent infection in local cattle. It may lead to clinical disease after any stress condition. Location and age are the associated risk factors for the occurrence of IBR in local cattle. Significant variation (p<0.05) in the occurrence of IBR was observed among different townships of Myanmar. The older the cattle, the longer the contact time with BHV-1, and the more occurrence of IBR.

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IMPLEMENTATION OF THE BIOSAFETY AND SECURITY POLICY AND QUALITY MANAGEMENT SYSTEM (IN ACCORDANCE WITH ISO17025:2017) IN THE NATIONAL SCIENTIFIC CENTER "INSTITUTE FOR EXPERIMENTAL AND CLINICAL VETERINARY MEDICINE"

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Background

The National Scientific Center "Institute for Experimental and Clinical Veterinary Medicine" (NSC IECVM) is the National body for veterinary research. It is the leading establishment for execution of the National Program for veterinary research of NAAS "Biosafety and epizootic wellbeing", where the area of Biosafety & Security (BS&S) is included as the one of priorities. This abstract is devoted to BS&S, physical security and laboratory testing quality management (under ISO17025:2017) policies implementation.

Material

National, regional, and international requirements for BS&S were analyzed. SOPs, guidelines and handbook for BS&S policy, ISO17025 quality management policy was developed in 2015-2017 (adopted to ISO17025:2017 edition requirements in 2019) have been developed. Implementation of the policy was executed via its integration in the institute's general security platform and on-site workshops for NSC IECVM laboratory personnel.

Results

The NSC IECVM BS&S policy has been developed on the basis of further BS&S and diseases control normative basis gap analysis. The national normative documents were compared with international regulations for BS&S and animal diseases' control.
The policy, developed within DoS-UA project GTR2-15-61311-0. It included: a) BS&S policy handbook, b) security and access control policies, c) BS&S dedicated SOPs (disinfection, decontamination, spill-off, waste management etc.). It has been implemented in the institutional level via director's orders and trainings/workshops, as well as integrated in NSC IECVM laboratories' ISO17025 policy.
ISO17025 required laboratory testing quality management system has been developed in 2015-2017. It covers 6 divisions dealing with viral and bacterial diseases of animals and poultry, using molecular (PCR and sequencing), virological (isolation, typing), microbial (culturing, biochemical properties testing), and serological testing (IF-test, CFT, HI, ELISA and others), as well as necropsy and pathological lesions examination concerning cattle (Bovine), sheep and goats' (Caprine), porcine (Suidae), and poultry viral and bacterial diseases for animal testing in farms and backyards. The techniques were covered with the quality handbook, workflow and testing SOPs, adopted or developed and approved in the institutional level. The ISO17025-correspondance of the quality management have been clarified by the National accreditation authority and certified with certificate of competence in

2017. The quality management system has been modified to correspondence to ISO17025:2017 after its upgrade in 2019.
The physical security updated included the perimeter and guard station renovation, video surveillance system installation. Institute has been supplied by the diesel generator for alternative power supply, repositories were supplied by storage devices for secured maintenance of viruses and bacteria from Institute's collections. RG3 laboratories were supplied with digital locks, negative pressure ventilation system, video surveillance, and fire alarm.

Conclusion

International requirements' harmonized BS&S and physical security policies, as well as quality management system for serological, molecular, virological and microbiological testing under ISO17025:2017 requirements have been developed and implemented in institutional and laboratory levels (NSC IECVM and its RG3 laboratories).

Perspective vision

The improvement of the research level of NSC IECVM in pathogens testing requires further BS&S upgrades and implementation of GLP for animal based testing to fit the OIE/FAO/WHO full-diagnostics complex cycle. The animal acute and challenge experiments will improve institute's capacity in animal and zoonotic diseases diagnostics in Ukraine and Eastern Europe. This capacity building declares the needs for the future development and implementation of the technical support projects for animal facility construction and accreditation for infection-associated in vivo trials. At the moment institutional top management and divisions are intensively developing the necessary policies and SOPs for animal study implementation and paying the strong attention for searching for investments for constructional part of this ambitious project.

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DETECTION OF AFRICAN SWINE FEVER VIRUS ANTIGEN FROM WILD BOAR, IMPORTED PORK AND PORK PRODUCTS IN MALAYSIA

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African swine fever virus (ASFV), the sole member of the *Asfaviridae* family, is the aetiology agent for African swine fever (ASF). A highly contagious transboundary disease, ASF affects domestic and wild pigs of all ages for which there is no vaccine nor treatment (Beltrán-Alcrudo *et al.* 2017). In Asia, ASF was first reported in Liaoning province in China back in early August 2018. It spread rapidly throughout China and into neighbouring countries like Mongolia, Viet Nam, Cambodia, Democratic People's Republic of Korea, Lao People's Democratic Republic, Philippines, Myanmar, Republic of Korea, Timor-Leste, Indonesia, Papua New Guinea and India (OIE 2021). Although humans are not affected by ASF, the mortality rate in pigs are up to 100% (Beltrán-Alcrudo *et al.* 2017), causing substantial socio-economic implications. Since November 2018, Veterinary Research Institute (VRI) has been instrumented to conduct surveillance and referred case testing for ASF to prevent the introduction of ASF into Malaysia. The various agencies that VRI works closely together includes Malaysian Quarantine and Inspection Services (MAQIS), State Department of Veterinary Services and Department of Wildlife and National Parks Peninsular Malaysia (PERHILITAN).

Up to 31 December 2020, a total of 1,126 samples from 463 cases received from various agencies were tested for ASFV (Table 1). Conventional polymerase chain reaction with primers from King *et al.* (2003) were used for the ASFV detection. Samples tested includes confiscated or imported pork and pork products (belly, rib, shoulder, sausages, canned pork products, cured meat, bone and *etc*), blood and swabs from commercial pig surveillance program, organs (kidney, tonsil, liver, lymph node, brain, skin), blood and ticks from wild boars surveillance program. So far, we detected two positive ASFV samples from imported pork products. Sanger sequencing and nucleotide sequence homology analysis confirmed both samples were ASFV with high sequence identity to various ASFV isolates including isolates from China.

Early detection and rapid diagnosis of ASF are crucial steps towards effective disease control. Active monitoring and surveillance, enforcement of ban on pork and pork product imported from countries hit with ASF, continuous public awareness and strict biosecurity measures are needed to mitigate the risk for ASF introduction into Malaysia.

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Table 1: Summary of samples received and tested for ASFV from November 2018 till 31 December 2020

Categories	Total Cases	Total Samples	Country of Origin/Sender	Total Samples	Type of Samples
Commercial Pork & pork products	**339**	**512**	Australia	18	Meat, Product
			Belgium	4	Meat
			Canada	1	Meat
			China	35	Bone, Meat, Product
			Denmark	41	Meat
			France	6	Meat
			Germany	40	Meat, Product
			Hong Kong	1	Meat
			Indonesia	10	Product
			Italy	13	Meat, Product
			Korea	4	Product
			Malaysia	149	Blood, Organ, Product, Swab
			Myanmar	1	Product
			Netherland	26	Bone, Meat
			New Zealand	1	Meat
			Poland	4	Meat
			Singapore	40	Meat, Product
			Spain	69	Meat, Product
			Taiwan	1	Product
			Thailand	4	Meat
			United Kingdom	5	Meat
			Unknown	22	Animal Feed, Meat, Product
			USA	9	Meat
			Viet Nam	8	Meat, Product
Wild Boar Surveillance	**124**	**614**	DVS Johor	5	Pooled organ
			DVS Kedah	5	Pooled organ
			DVS Melaka	128	Blood, Pooled organ, Tick
			DVS Negeri Sembilan	74	Pooled organ
			DVS Perak	75	Blood, Pooled organ
			DVS Pulau Pinang	38	Pooled organ
			DVS Terengganu	28	Pooled organ

			RVL Bukit Tengah	15	Pooled organ
			RVL Johor Bharu	18	Pooled organ
			RVL Kota Bharu	138	Blood, Pooled organ
			RVL Kuantan	90	Blood, Pooled organ
Grand Total	**463**	**1,126**		**1,126**	

DVS= Department of Veterinary Services; RVL=Regional Veterinary Laboratory

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CAPRINE LENTIVIRUS CONTROL IN SLOVENIA

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Abstract

Caprine arthritis and encephalitis (CAE) is a debilitating disease of goats that significantly affects welfare and reduces the profitability of goat farming. In the presentation, experiences with management, diagnostics and the control programme in Slovenia will be presented.

Keywords: goats, caprine arthritis and encephalitis, virus, diagnostics, control programme.

Introduction

The goat population in the world has been steadily increasing since 1960, and this has been particularly noticeable in the last 10 years. According to FAO (2021), the current goat population is more than 1.1 billion. The most important limiting factor in maintaining economically viable goat production is adequate control of infectious and parasitic diseases (1,2). One of the most insidious and devastating endemic, persistent and slowly debilitating chronic diseases in goats is caprine arthritis and encephalitis (CAE), which is present in most countries of the world, including Slovenia, and is caused by Caprine arthritis encephalitis virus (CAEV). CAE causes major economic and animal welfare concern in the goat industry. The disease was first recognized in 1974 and there is currently no effective treatment or vaccine against it (3).

Caprine arthritis encephalitis virus (CAEV)

CAEV belongs to Genus *Lentivirus*, Family *Retroviridae* of the Order *Ortervirales* according to ICTV (2021). The virus is genetically closely related to Visna/Maedi virus (VMV) of sheep and cross-infections of both viruses between species are evident. Therefore, many authors use the term Small Ruminant Lentiviruses (SRLV). It can also infect sheep and wild ruminant species, including ibex (*Capra ibex*), chamois (*Rupicapra rupicapra*), mouflon (*Ovis orientalis orientalis* group), and mountain goats (*Oreamnos americanus*). The virus is very labile in the environment (3).

Pathogenesis

CAEV infects monocytes and macrophages in many tissues, inducing inflammatory (immune-mediated) lesions. The infection leads to a multisystemic, progressive inflammatory disease. The mechanism(s) responsible for this variability in host-specific tropism and pathogenicity are not yet fully understood. But most likely, host (e.g., immune system characteristics), virologic (genetic characteristics of the virus), and environmental factors (e.g., dose at exposure, animal density, time of year, etc.) dictate the outcome of infection. Clinical signs often appear late in the course of the disease and are often not specific to the infection, such as emaciation. SRLV does not cause an immunodeficiency syndrome (3).

Disease transmission

The main route of transmission is through the consumption of infected colostrum or milk. The disease can also be transmitted by direct contact between animals, as virus-infected monocytes or macrophages are shed in body fluids such as saliva, tears, urogenital secretions, faeces, and/or respiratory secretions. Less effectively, CAEV is transmitted transplacentally and iatrogenically. The most common ports of entry are the respiratory and gastrointestinal mucosa (4).

Clinical signs

Asymptomatic carriers of the virus are common. The clinical disease has five possible forms, all of which have also been noted in Slovenia.
Arthritis is the most common form of the disease and usually occurs in adult animals. Sometimes yearlings or even animals as young as 6 months of age also show this form. The disease can progress rapidly and lead to non-weight bearing or cause only lameness for many years. Infection leads to fluid accumulation, destruction of the joint surfaces, and eventually joint ankylosis causing loss of mobility. Tendon sheaths and bursae may also be inflamed. Goats are afebrile with initially good appetite. About 22% of seropositive goats are expected to develop clinical arthritis, which is also an important welfare problem (5).
Interstitial mastitis, also known as hard udder due to induration, results in low production of milk. Even if the hardening disappears a week or two into lactation, production remains low.
Interstitial pneumonia presents initially with a dry cough, followed by dyspnea and weight loss.
Encephalitis most commonly affects kids 2 to 6 months of age. Sometimes kids as young as 1 month of age and even older goats can develop this form. Initial lameness and ataxia that develop within a few days into hemiplegia or tetraplegia, circling, hyperesthesia, blindness, and recumbence with torticollis (indicative of disease of the higher centers, especially the midbrain). The kids are otherwise bright, alert and have a good appetite. Sometimes a tremor may be noted. In adults, knuckling of the pasterns, circling, progression to paresis, and paralysis are observed.
Progressive emaciation may occur alone or accompany other forms (6).

Diagnostics

Currently, there is no treatment for SRLVs and no successful vaccination. Diagnosis can be made based on clinical presentation and necropsy. Confirmation of the disease is based on laboratory diagnosis. Agar gel immunodiffusion test (AGID) and enzyme-linked immunosorbent assay (ELISA) are the most common methods for detecting specific antibodies that can detect a broad spectrum of viral strains and are used as screening tests. ELISA is generally a more sensitive technique than AGID. Complementary tests, such as Western blotting (WB), are used to confirm or deny the results of screening tests and to clarify indeterminate results. Polymerase chain reaction (PCR) methods are used to detect the provirus genome in blood and tissue (3). The major disadvantage of serological methods is the slow seroconversion of infected animals. Antibodies to CAEV are usually present in sufficient concentration to be detected no earlier than 6 months after natural infection, but it may take up to 2 years after infection (8).

Economic impact of the disease

CAE has a negative impact on several performance parameters (reduced production, growth rate and longevity), resulting in economic losses. Lactation length, milk yield and milk fat, milk protein, dry matter and lactose content are significantly reduced in infected goats and this reduction in yield increases with lactation number (7–9). In addition, animals in advanced stages of the disease have a significantly reduced body weight at slaughter and may therefore not be fit for consumption (10).
Epidemiological situation in Slovenia
The first evidence of the disease in Slovenia dates back to 1996, when antibodies against CAEV were detected in a goat flock. In 2013, 1292 goats from 30 flocks were tested and 357 (27.6%) CAE-seropositive animals were detected in 20 flocks (6). No systematic testing has been done since then, but

the situation in the field suggests that the problem is even greater today. For the detection of antibodies against CAEV in serum samples, a commercial ELISA test Chekit-CAEV/MVV Screening ELISA Test Kit (IDEXX Laboratories, Switzerland) is routinely used in Slovenia. The test uses whole virus as antigen. If CAEV infection is suspected at necropsy, tissue samples (lung, mediastinal lymph nodes, spleen, carpal joint tissue, and brain) are collected and tested for the presence of CAEV DNA using an in-house real-time PCR (11,12).

Voluntary control program for CAE in goats

In Slovenia, a national voluntary control programme (financed exclusively by the owners) for CAE was implemented in 2016. All the requirements for obtaining free flock status are prescribed in the Rule for recognition, acquisition and maintenance of CAE free goat flock status. Status is granted if the flock complies with the conditions of the rule and all animals over 6 months of age are serologically negative on two consecutive tests not less than 12 months apart. If all animals are not negative, an eradication programme is established by a local veterinarian that includes biosecurity, periodic clinical examinations, serological testing, kids management, and culling of positive animals. CAE-free status is maintained by annual serological testing of all animals older than 12 months (Figure 1). If three consecutive annual tests are negative, the flock may be tested every second year. A list of all negative flocks in Slovenia is publicly available, but to date no breeder has decided to join the official programme. There are breeders who control the disease unofficially.

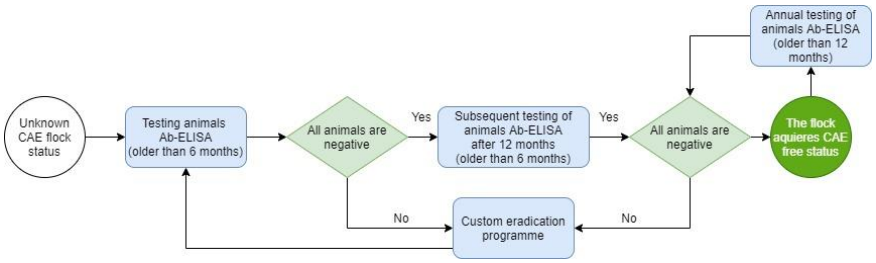

Figure 1: Scheme of the Slovenian caprine arthritis and encephalitis control programme

Some reasons for the low interest in the programme are the high cost of laboratory tests (6,04 EUR per test + tax), the cost of official sampling by licensed veterinarians, the stricter official control of the flock (all animals must be individually identified and their origin registered), and extra care for biosecurity. Since there are no flocks with negative status in Slovenia, the purchase of replacements and bucks is much more expensive and limited. To promote the program, co-financing by the state or breeders' organizations would be necessary, as has been shown in other countries (10).

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APPLICATION OF RADIOISOTOPES IN VETERINARY MEDICINE: METABOLISM OF RADIONUCLIDES IN ANIMALS AND DOSIMETRIC CHARACTERISTICS OF THE EXPOSURE

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Introduction

Advances in nuclear medicine and radiation technology create the conditions for the development and expansion of the application of radioactive isotopes and ionising radiation in veterinary medicine in tasks related to increasing animal production. To date, extensive experimental information has been obtained on the metabolism of radionuclides. These data can be used to improve diagnosis, treatment and prevention methods in farm animals. At the current stage of research, detailed biokinetic analysis of this information is required with the aid of new methods and mathematical modelling tools that have been tested and adopted for the tasks in human radiation safety and the development of new medications [1, 2].
The aim of this research was to develop mathematical models of the metabolism of radioactive isotopes, dosimetric models and assessment of the possibilities of their application for operational diagnostic testing of thyroid pathologies in dairy cows in iodine deficiency regions taking ^{131}I as an example.

Materials and methods

The input data for the model of transport in the gastrointestinal tract development and metabolism of radioactive isotopes in farm animals were the experimental materials of the computer database "Archive of Experimental Data", which is being formed at the RIRAE. The database mainly includes information from the RIRAE research published as the scientific reports other published sources of the Institute.
Based on the analysis of the studies carried out for cattle, a general model of radionuclide metabolism with different organotropicity has been developed. Figure 1 presents a possible variant of the ^{131}I model, which is widely used in veterinary medicine to diagnose a number of animal diseases and metabolic disorders in iodine deficiency conditions. It is worth also noted that ^{131}I is the main radionuclide causing thyroid lesions in case of the nuclear reactor's accidents.
This model was used to determine the dynamics of ^{131}I in the thyroid and milk of dairy cows for scenarios of radionuclide intake in the form of radioactive solid particles (compartments q1, q3, q4, q6), in the form of condensation forms of airborne deposition (compartments q2, q5, q7) and parenteral injection (compartment q8).
The q9 compartment represents a thyroid gland - a hormone synthesis tissue and a thyroglobulin depot. The q10 compartment is an extrothyroid tissue of an organic fund and tissue of an inorganic fund of animal iodine. Calculations were performed by solving a system of differential equations in analytical form, as well as using numerical methods of the PTC Mathcad Prime 4.0 multifunctional interactive computer system.
Absorbed doses were determined on the basis of precision Monte Carlo modelling of energy transfer to biological tissues by ionizing radiation using a modern MCNP5 computational code and the IAEA-recommended nuclear data library TENDL-2019 (TALYS, EUROPE 2019) [3].

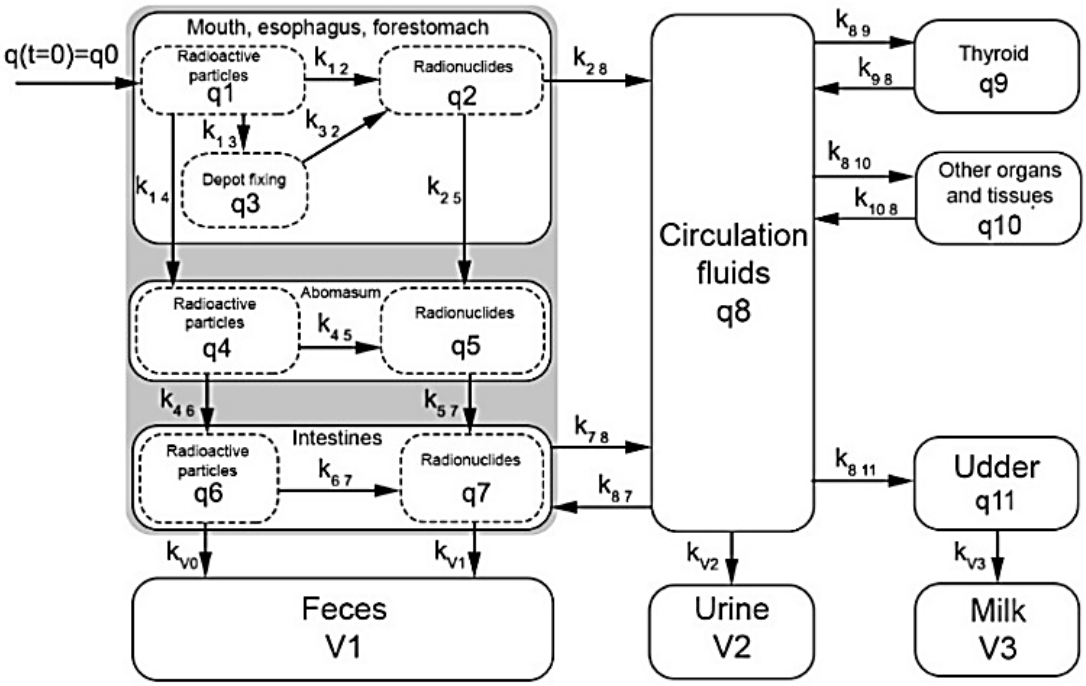


Fig. 1 Compartment model of ^{131}I metabolism in dairy cows.

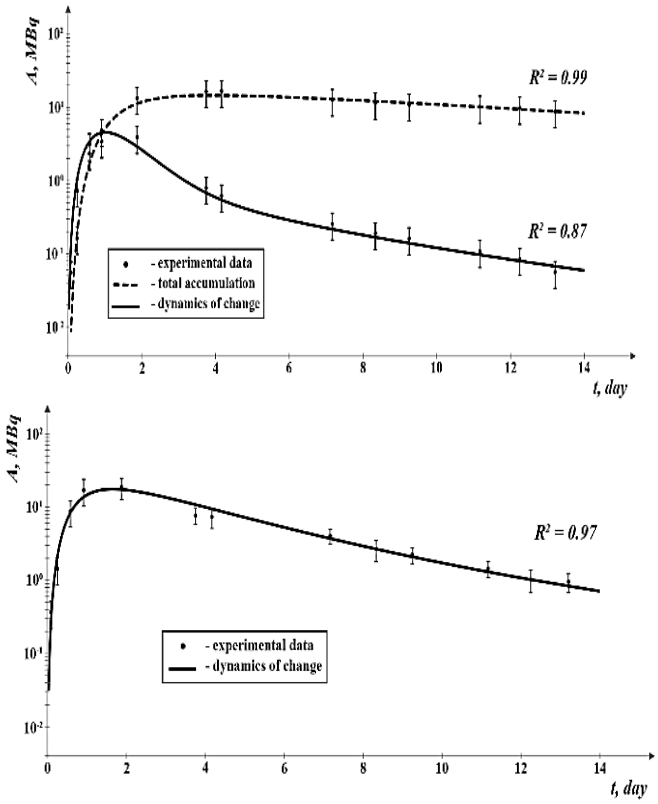
Results and discussion

Radioactive isotopes used for diagnostics shall meet a number of requirements: they should have a short half-life and low radiotoxicity, provide a possibility to register their radiation and have characteristic biological properties (organotropicity) to be used for examination of different systems and organs. Table 1 presents the biokinetic parameters for the ^{131}I metabolism model, obtained by fitting the parameters to the experimental data, using statistical criteria of adequacy of mathematical modelling [4].

Table 1: Biokinetic parameters obtained based on a compartment model of radionuclide metabolism in dairy cows (Fig. 1)

Biokinetic parameters, d^{-1}	**Value**	**Biokinetic parameters, d^{-1}**	**Value**	**Biokinetic parameters, d^{-1}**	**Value**
k_{12}	10	k_{46}	0.5	k_{810}	1
k_{13}	0.12	k_{57}	24	k_{108}	0.1
k_{14}	1.0	k_{67}	10	k_{811}	0.5
k_{25}	0.7	k_{78}	4	k_{V0}	2.5
k_{28}	0.5	k_{87}	0.3	k_{V1}	2.5
k_{32}	10	k_{89}	1.2	k_{V2}	1.5
k_{45}	10	k_{98}	0.5	k_{V3}	2

Fig. 2 provides data on the dynamics of ^{131}I in milk and thyroid obtained using data from Table 1, and the determination coefficients (R^2) showing satisfactory agreement between the calculated and experimental data.



A B

Fig. 2. Dynamics of ^{131}I content in cows milk (A) and in the thyroid (B) after a single intake of radionuclide with feed

A measure of the radiotoxicity of radioactive isotopes is the absorbed dose of ionizing radiation accompanying radioactive decay. An important result of this work is the conversion factors for the activity of radioactive isotopes into the average absorbed dose rate in animal organs and tissues (Bq → Gy/s) presented in compartment models [5, 6]. It has been also shown that under conditions of radioactive contamination with ^{131}I, the destruction of the parenchymatous tissue of the thyroid gland occurs at average absorbed doses (~150-200 Gy) formed during ~3-5 months.

The developed model can be used for different applications unrelated to radioactive contamination. In particular, it can be implemented in the studies of the animal diet optimization in the iodine deficient regions using the potassium iodide-based feed additives.

Using the results obtained, an algorithm of the software has been developed for the rapid diagnostic testing of animal health and decision support for optimisation of the animal production in the iodine deficit regions.

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FACTORS EMERGING INFECTIOUS DISEASES DURING SMALL RUMINANT DRIVE

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In Tajikistan is carried out pasture keeping of small ruminants. The specificity of animal husbandry in the Republic of Tajikistan is such that every year in spring, for summer and autumn, over 3.5 million sheep and goats drive along the same routes to winter pastures. During this period, on small ruminant driving routes, animals cross the farms bordering with neighbor countries, which have a special immune background.

Due to the climatic changes in the region, as well as the animal and human migrations, many transboundary animal diseases (TADs), including those with zoonotic impact have seriously affected livestock of Tajikistan.

Sheep and goat pox, salmonellosis, brucellosis, *AI* and other infectious diseases, many of which in countries with continental climate were considered exotic, have re-appeared during 2014 - 2017 causing severe economic losses for the farmers and serious public health and food safety considerations.

In recent years, among TADs, the most common in Tajikistan are *sheep and goat pox*, *PPR*, *brucellosis*, *salmonellosis* and *CCPP*. Between all these dangerous infectious diseases in 2020, also the great economic damage to livestock farms of the republic was caused by *salmonellosis*. Salmonellosis in epizootic form is registered, especially in sheep-breeding farms of Khatlon region. Disease in ewes caused abortions and birth of unviable lambs. As a result of monitoring in sheep farms, it was found that salmonellosis in unfavorable cases causes somewhere about 5 - 6% death of ewes and 11% death in newborn young animals (Picture 1 and 2: the clinical signs of salmonellosis).

Picture 1. Internal organ with salmonellosis in lambs

Picture 2. The death of lambs with salmonellosis

The animals got sick with salmonellosis after being driven from summer pastures to winter pastures and return. The peak of the disease was registered in spring (late February and early March).

In positive samples, as a result of differentiation, different serotypes of salmonella were isolated.

Table 1: Results of investigated samples in sheep breeding farms of Khatlon region

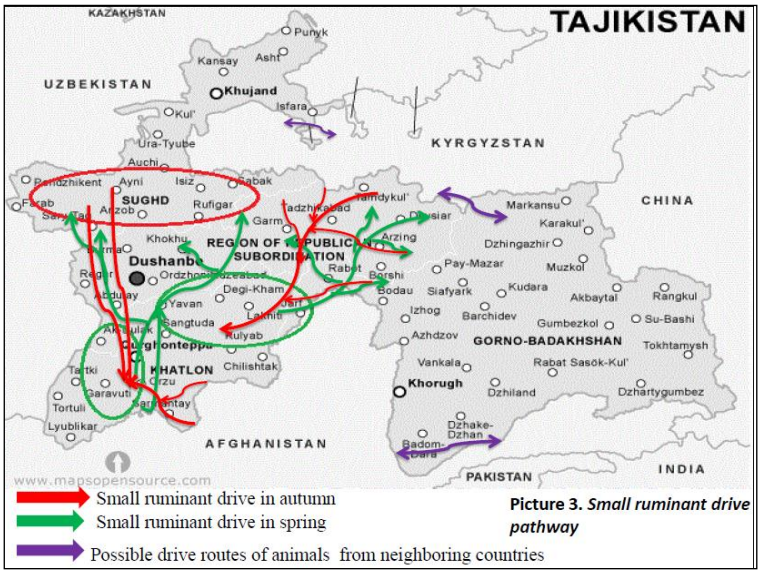
N°	Name of farms	Amount of samples	Positive samples	%

1	Tebelay	378	45	11.1
2	Bakht-82	335	12	3.6
3	Muhammad	231	19	8.2
4	Samar	125	7	5.6
	Total	1069	80	7.5

Allocated and differentiated *Salmonella abortus ovis*, *Salmonella typhimurium* and *Salmonella enteritidis* from sheep.

It should be noted that the landscape, location, climatic conditions and the way of keeping animals play an important role in the spread of TADs.

It has been found that in terms of biosafety, the surrounding neighboring countries form a significant threat to the country. As 95% or more than 5 million of sheep and goats privately owned in the country, mainly kept in farmsteads or graze on seasonal pastures throughout the year.



Picture 3. *Small ruminant drive pathway*

The distribution of livestock in the regions and districts of the republic is different. So, in the cities and districts of republican subordination (as of 01.01.2021) 1494374 sheep and goats, in Sughd region - 1590131, in Khatlon region - 2425622, in the Badakhshon mountainous autonomous region 358328 sheep and goats were registered. It depends on the area of agricultural land and pastures in a particular region of the country.

The main areas of winter pastures of the republic (659.1 thousand ha) are located on the territories of Khatlon (557.5 thousand ha) and Sughd regions (101.6 thousand ha).

Not only animals of farms of this region return to winter pastures of Khatlon region, but also from the cities and districts of republican subordination and Sughd region, which have different immunological status and are potential carriers of various infections.

In Khatlon region, winter pastures are located on the territory of 16 rural areas out of 24. The largest areas of pastures in Kulob valley are located in the districts of Dangara (109.2 thousand ha), Temurmalik (64.8 thousand ha), Shamsiddin Shokhin (22.8 thousand ha) and Farkhor (20.3 thousand ha).

In Vakhsh valley of Khatlon region, the largest areas of winter pastures are located in Shaartuz (107.6 thousand ha), Kabadiyan (89.2 thousand ha), Vakhsh (60 thousand ha), Nosiri Khusrav (55.1 thousand ha) and Dzhaikhun (40.9 thousand ha.) districts. Most of the districts and their winter pastures border with neighbor countries.

Winter pastures have not enough grass due to low rainfall in the spring-autumn period of the year, frequent droughts and a large number of grazing animals.

From the total area of agricultural land of the republic (4.1 million ha), 76% consist of pastures and meadows.
Flocks mainly migrate from the Khatlon region to the summer pastures of the cities and districts of republican subordination and Darvoz district of the Badakhshon mountainous autonomous region, which borders with neighbor countries and return to winter pastures (Picture 3.).
During the cattle drive, flocks pass through 100 to 400 km. Pathways for small ruminant drive often run through mountain villages, where animals are often exchanged and sold, which contributes to the spread of many infectious diseases.
In addition, there is formal cross-border cattle drive to seasonal pastures, especially sheep and goats between neighbor countries, as well as cross-border exchange and sale of animals.
Thus, the distant pasture management of sheep and goat breeding, the joint keeping of sheep and goats in flocks is traditionally practiced in the country.
There is a joint use of pastures and places for watering animals by residents of several villages, transboundary movement of animals to pastures or the exchange and sale of animals between neighboring countries.
Another factor in the spread of infectious diseases is the presence of wild animals, rodents, reptiles, migratory waterfowl, wild birds, other animals that can cross the border pastures and other environment factors.
Taking into account the peculiarities of animal husbandry in Tajikistan and the need to combat these dangerous diseases, investigate the characteristics of the course of the disease by using nuclear derived techniques and monitoring the spread of pathogens of TADs, such as PPR, sheep and goat pox, salmonellosis and brucellosis, etc. are very important.

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IAEA-CN-281-275

SURVEY OF CONTAGIOUS BOVINE PLEUROPNEUMONIA AND CONTAGIOUS CAPRINE PLEUROPNEUMONIA IN MIDDLE-BELT REGION OF NIGERIA.

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Introduction

Contagious bovine (CBPP) and caprine pleuropneumonia (CCPP) are OIE-listed diseases because of the socio-economic impact they have, mainly, on smaller holdings often on marginal land in Asia and Africa (1). Despite some successful attempts in Nigeria at controlling CBPP in the 1970s there is substantial evidence that the disease is endemic in many parts of the country (2). CCPP, on the other hand, has been suspected based on clinical and pathological signs but has not been confirmed by laboratory methods (3). Furthermore, there have been no surveys to show its distribution in the country.

Materials and methods

Following the purchase of equipment, test kits and reagents and refurbishment and training, a mycoplasma laboratory was established and commissioned at Ilorin University. The Idexx competitive ELISAs and latex agglutination tests for the serological detection of the causative agents of CBPP, *Mycoplasma mycoides* subsp *mycoides*, and CCPP, *M. capricolum* subsp. *capripneumoniae* was used for screening sera in the state. Diagnostic media was bought from Mycoplasma Experience Ltd (UK) for mycoplasma isolation. A PCR test for M mycoides was carried out on 7 isolates.

Results and conclusions

For CBPP, between 6 and 135 samples were taken from each of 11 abattoirs and the percentage of positive sera varied between 0 and 13.5%; however, where more than 40 samples were taken seroprevalence was seen to be between approximately 7 and 14%. Later, 10 cattle were chosen from each of these four abattoirs with high seroprevalence for more detailed examination. CBPP-positive cattle (4/10) were found at Ilorin East based on clinical, gross pathological, cultural and serological criterion (fig. 1). *M. m. mycoides* was identified based on specific staining in diagnostic medium. Cattle from the other three abattoirs were clinically and pathologically negative but some were seropositive and yielded mycoplasmas though these were not considered specific. The PCR test was positive for 6 of 7 isolates isolates (fig. 2). In parallel, 10 goats were examined for CCPP at each of the same abattoirs. Again, while confirmation is still being carried out on isolates, goats examined at two abattoirs were positive for CCPP based on clinical, pathological and laboratory tests (fig. 3). *M. c. capripneumoniae* was identified based on specific staining in diagnostic medium which represents the first isolation of *M c. capripneumoniae* in Nigeria (fig.4). Culturally positive goats were also seropositive suggesting CCPP is widespread in Kwara state though number of goats examined was small (fig. 5). A serological survey using the cELISA of over 300 goats kept extensively gave an average seroprevalence of 4.4%. Finally, the cELISA was used to identify various risk factors associated with CBPP infection in cattle herds in Kwara state. Herd size was the most significant factor with herds greater than 100 more likely to have a higher percentage of positive cattle. Evidence was seen that some breeds, in particular the White Fulani and Adamwa Gudaly, had higher infection rates for CBPP though not significantly. Cattle gender and seasonality were not significantly linked to susceptibility.

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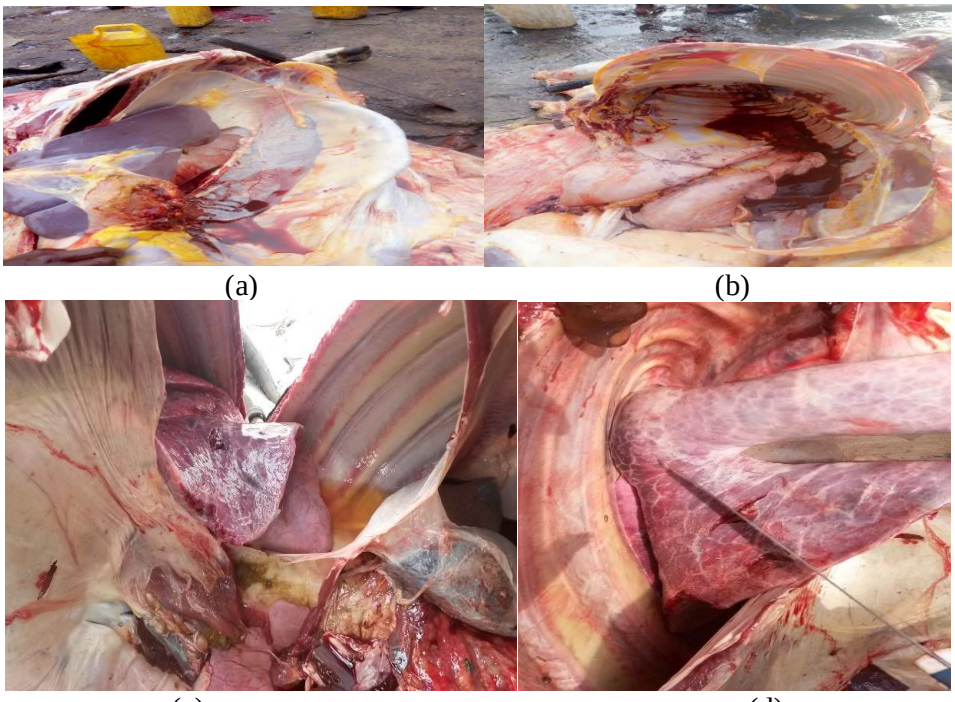

(a) (b)

(c) (d)

Fig 1: Cattle lungs showing fibrin deposition (a); areas of focal necrosis in the lung (b); pleural fluid in thoracic cavity(c); sequestrum (d) suggestive of CBPP

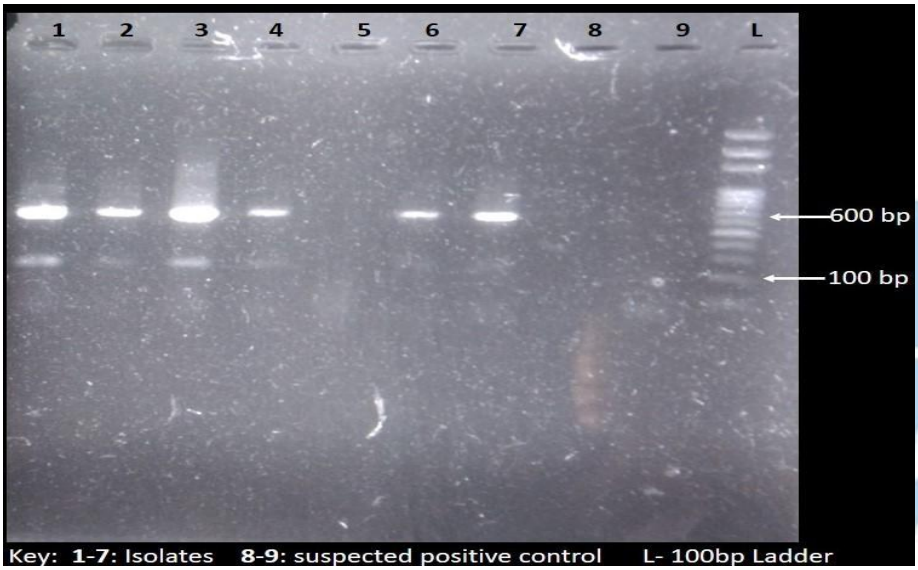

Fig. 2: PCR test results showing positive bands for *M mycoides* lanes 1-4 and 6-7)

(a) (b) (c)

Fig. 3: Goat lungs showing severe congestion (a), fibrin deposition (b) and pleural fluid containing a lot of blood (c) suggestive of CCPP

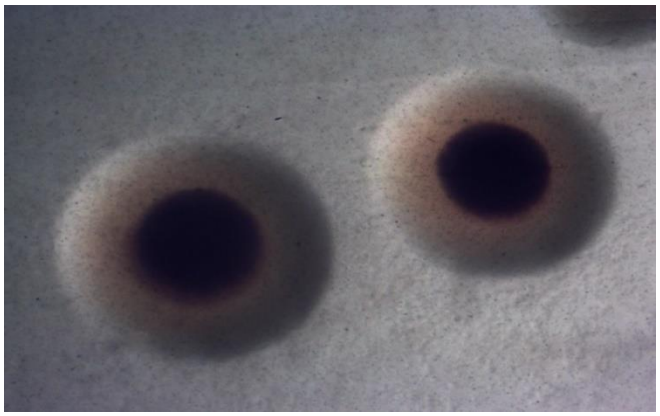

Fig. 4: Mycoplasma colonies showing specific staining indicative of *M. c. capripneumoniae*

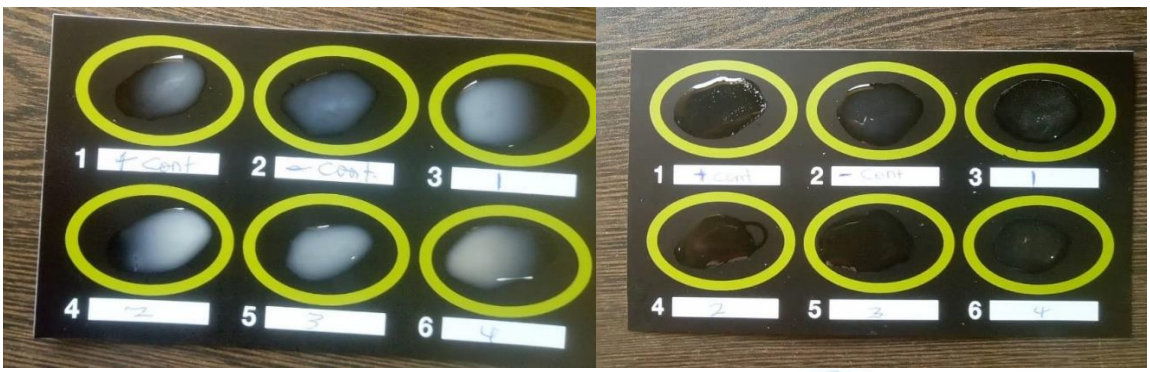

Fig. 5: Latex agglutination test for CCPP. Left hand card showing negative results; right hand showing positive agglutination

IAEA-CN-281-285

ROLE OF MIGRATORY WILD WATERFOWL IN SPREADING OF INFECTIOUS DISEASES

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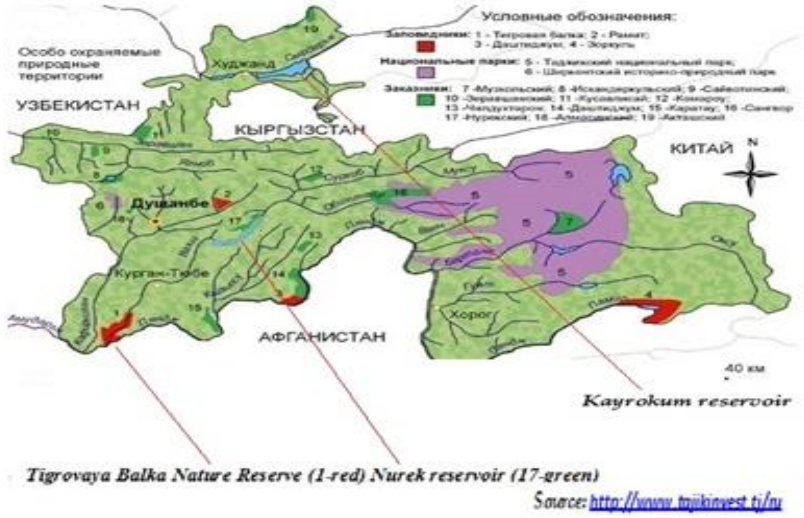
An important branch of agriculture in Tajikistan is poultry farming, which takes a significant place in solving the problems of the needs of the population in dietary and ecologically clean food and ensuring food security.
In the poultry industry of the republic, infectious diseases pose a serious danger, the spread of which is facilitated by frequent violations of regulations on poultry farming.
Migratory wild waterfowl (WWF) play a special role in the spread of infectious diseases of viral and bacterial etiology, including AIV. Most influenza viruses cause no or very mild illness in wild birds, but cause severe illness and death in domestic birds.
AI is a zoonotic disease and can also be transmitted from animals to humans. Human infections with the H5N1 avian influenza strain are rare, but in infected cases the consequences can be serious, sometimes even fatal.
The H5N1 and H9N2 viruses have similar characteristics, which increases the possibility of a new, pathogenic for humans. In view of the fact that Tajikistan is located in the South-Eastern part of Central Asia and the route of migratory WWF from many countries lies through the territory of the republic. Therefore, conducting scientific research on a sample of feathers, cloacal faeces, using sustainable isotope analysis, according to the IAEA scientific contract №17452 "Collection and use of samples of faeces, swabs and feathers of WWF for the diagnosis and identification of HPAIVs and studying the migration route of birds the primary and timely goal of scientists of the Institute of Veterinary Medicine of TAAS (IVM TAAS) (under the CRP D32030 Use of Stable Isotopes to Trace Bird Migrations and Molecular Nuclear Techniques to Investigate the Epidemiology and Ecology of the Highly Pathogenic Avian Influenza).
To achieve the goals investigation was carried out in various reservoirs and temporary environment of WWF in the republic.
Samples were collected in Khatlon and Sughd regions in the first half of 2013 to 2017.

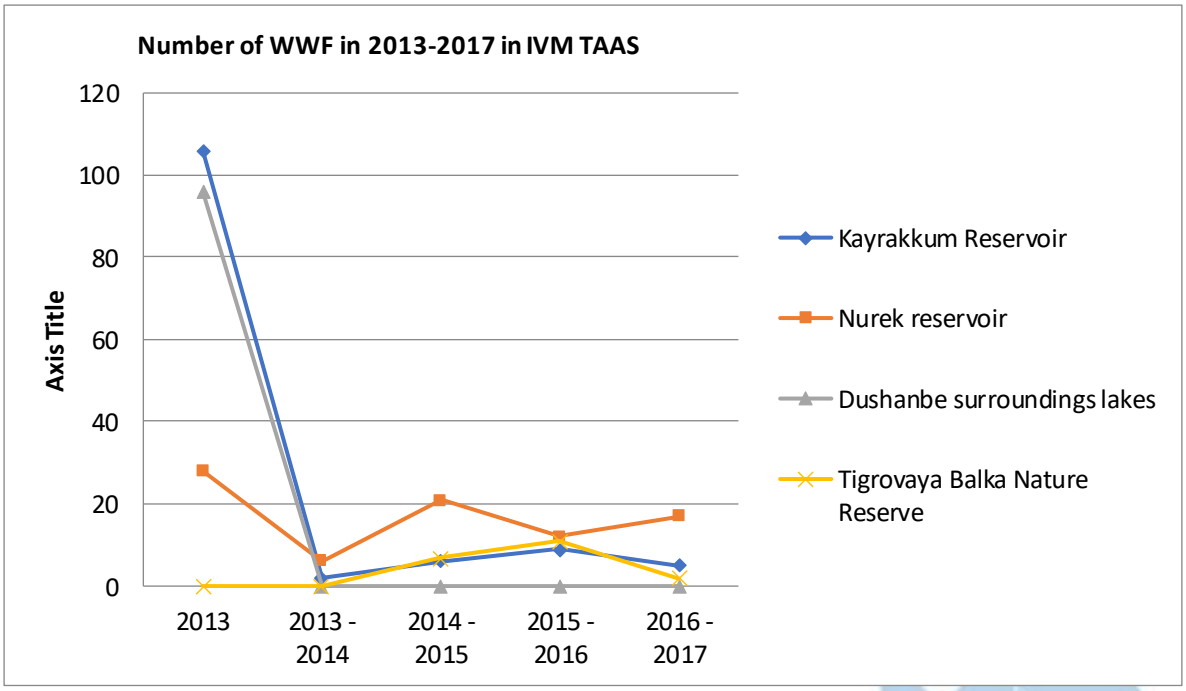
The research sites-places of concentration of a large number of WWF,/the seasonal migration of birds in February and May in the spring, and from August to November - in the fall in Tajikistan 2014-2015.



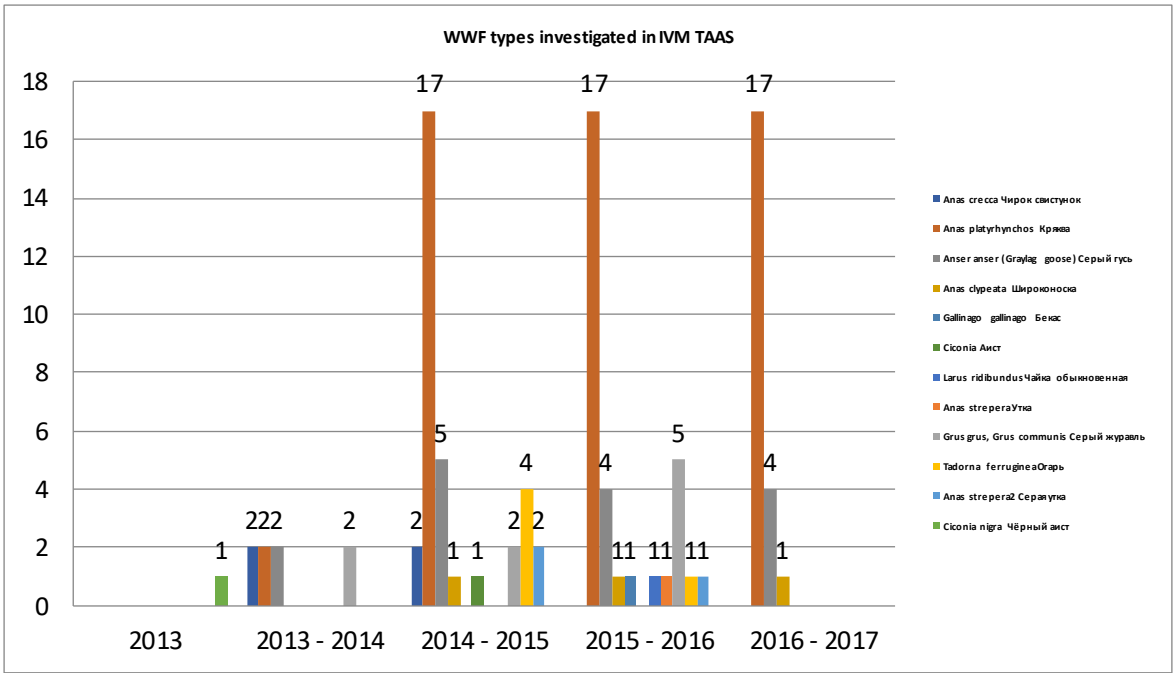
Tigrovaya Balka Nature Reserve (1-red) Nurek reservoir (17-green)

Source: http://www.tajikinvest.ti/ru

Trips were made to catch and shoot waterfowl in the districts, near Nurek and Karakum reservoirs and the Tigrovaya Balka nature reserve.



The shelling of WWF was carried out directly in the water, and near-water synanthropic birds were caught at watering holes or on the banks of reservoirs. The samples collection produced immediately after the extraction of birds, some of them in the field and some of them in the laboratory. The samples from the field had been delivered in a special container to virology lab for sampling and storage. All collected fecal samples and smears were frozen in a minus refrigerator (- 18^{0}C).
In 2013 – 2017 different bird species were investigated in Nurek and Karakum reservoirs, as well as in the surrounding area of Dushanbe. Samples of feathers and cloacal feces of these birds were collected, which was examined in the laboratory of virology in order to extract DNA and RNA from these samples.



In the investigation of all samples, positive reaction was not identified. For a detailed study, all collected samples in a special container were packed and sent to the IAEA laboratories in Seibersdorf, Austria for further research and extraction of DNA, RNA and genetic studies. According to the PI650 and regulation UN3373 all the feathers were wiped with 70% alcohol and dry heated at +100 °C for one hour before the shipment.

Thus, monitoring and experience in combating the most common avian diseases accumulated in recent years has shown that in order to develop and conduct effective antiepizootic and anti-epidemic measures, it is necessary to build a system of constant monitoring of the circulation of the influenza virus based on the use of laboratory methods for accurate and rapid identification and characterization of circulating strains of influenza A virus, in order to determine their genetic relationship, the degree of pathogenicity and detection of mutations.

Integrating information on WWF migration through stable isotope analysis and detecting AIVs in faecal and environmental samples will contribute to understanding the epidemiology and ecology of the long range transmission of AIVs, using non invasive methods.

The global community is facing unprecedented challenges in the long range spread of infectious animal and zoonotic diseases. Migratory WWF are important contributors in the long range transmission of diseases.

Synanthropic birds also can be another factor in the spread of infectious diseases between migratory waterfowl and poultry farms.

The current situation with the rapid spread of infectious diseases requires that every effort be made to prevent and diagnose pathogens, especially zoonotic diseases, effectively and within the framework of international agreements and conventions.

In this direction, the IAEA has provided IVM TAAS with modern diagnostic equipment (PCR) for timely and accurate diagnosis and detection of zoonotic diseases, which helps to determine the origin of strains and prevent the spread of AIVs and other infectious diseases.

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EVALUATION OF DNA BARCODING OF FAECES AS PART OF A NON-INVASIVE APPROACH TO ACTIVE WILD-BIRD SURVEILLANCE FOR NOTIFIABLE AVIAN INFLUENZA AND AVIAN PARAMYXOVIRUS INCURSIONS

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Wild-birds are responsible for the maintenance and migratory dissemination of avian influenza viruses (AIVs), particularly the H5Nx highly pathogenic (HP)AIVs which remain a cause of concern. H5Nx HPAIVs originated from East Asia and have incurred repeatedly into Europe, the Middle East and sub-Saharan Africa in recent years, and caused economically-damaging poultry outbreaks. Wild-bird surveillance has been largely passive, whereby swabs from carcasses are collected for laboratory testing, but there remains a need for active wild-bird surveillance to investigate H5Nx HPAIVs dissemination by apparently healthy wild birds. As an alternative to trapping or shooting, faeces collected at wild-bird locations are first tested for AIV and avian paramyxovirus type 1 (APMV-1, which include the Newcastle disease viruses) by virus-specific RealTime PCRs. Virus-positive faeces may then be investigated by DNA barcoding to identify the species of origin to provide valuable epidemiological information and potential early-warning concerning the spread of these notifiable avian pathogens. Wild-bird speciation by faecal DNA barcoding has featured within an IAEA Project (17501-CRP D32030), and is now being evaluated in the UK as part of a proposed non-invasive approach to active wild-bird surveillance for AIV and APMV-1. Different barcoding protocols for analysing wild-bird faeces have been compared, and our presented findings will guide the organisation of faecal sampling in the UK: Autumnal wild-bird migration later in 2021 may be the next risk period for introduction of a fresh wave of H5Nx HPAIV to Europe / the UK, so a thorough evaluation of barcoding during 2021 is both timely and important.

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DETECTION OF ANIMAL RABIES IN BANGLADESH

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Rabies is a highly fatal zoonotic disease caused by Rabies virus under the genus Lyssavirus of Rhabdoviridae family that affects all worm blooded animal including human (Samad, 2013) and are characterized by encephalitis and neurological signs. The fatality rate is close to 100% but 100% preventable through vaccination. Rabies is the 10th biggest cause of death due to infectious diseases Worldwide. The annual death toll is around 50 000–60 000, with 99% occurring in tropical developing countries. Worldwide, 87 countries contain Rabies, high in Asia. The disease is endemic in Bangladesh (Hossain *et al*. 2011) but there is no report of laboratory confirmed cases for decades. In Bangladesh, domestic dogs are the source of over 99% of human infections (WHO, 2013). During the period of 2010–2012, 3425 rabies deaths in domestic animal populations (cattle: 2845; goats: 547; sheep: 13) were reported (Mondal & Yamage 2014) where dogs, cats and other wild animals are not included. Rabies is a serious concern in Bangladesh due to large stray dog population in the country. Bangladesh has taken rabies eliminate plan with four strategies: advocacy, communication and social mobilization (ACSM), modern treatment for dog bite, mass dog vaccination (MDV) and dog population management (DPM). Animal rabies diagnosis is based on Clinical signs & symptoms but no lab confirmation for the decade.

Objectives: In this context the study was conducted

a) to establish rabies diagnosis using Direct Fluorecence antibody test.
b) Diagnosis of field rabies cases in animals.

Methodology

A total of 8 whole head of suspected animals with neurological signs and /or history of dog bite were received/collected from different regions of Bangladesh. Sample collections were done by the field veterinarians having pre-prophylaxis and with adequate PPE that were sent to Central Disease Investigation Laboratory of Department of Livestock Services, Bangladesh. Brain tissues (brain stem and rostral part of cerebellum) were collected at CDIL through foramen magnum without opening the skull using straw as described by Lamamoto et al., 2011. Slide preparation, staining with monoclonal antibody conjugate and microscopy were don following the CDC protocol for rabies diagnosis (CDC, 2006) Briefly, impression smear/slide of brain stem and cerebeller tissues were prepared separately, fixed in ice cold absolute acetone and stained with fujirabo monoclonal antibody-fitc conjugate (Fujirubio Diagnostics, Inc. USA) and finally examined under fluorescent microscope with fitc filter to visualize the fluorescence of rabies virus or its protein in the smear. Results were interpreted.

Results

Clinical History

Clinical History, Location of sample collection, biting history etc are given below in table 1.

Table-1: Clinical history with some demographic data

Sample No	Species	Biting history	Clinical feature	Location in The country
1	Cattle, 7 month of age	Bitten by rabid dog 17 days back from the death	Profuse salivation, incoordination, progressive ataxia	Mohammed pur, Magura
2	Dog	Biting history unknown	Profuse salivation, attacking tendency	Rajapur, Jhalakathi
3	Adult cattle	Biting history unknown	Profuse salivation, incoordination, progressive ataxia	Vandaria, Piruspur
4	Fox	Biting history unknown	attacking tendency, bitten few human	Shakhipur, Tangile
5	Goat	Bitten by rabid dog 34 days back from the death	Profuse salivation, incoordination, progressive ataxia	Savar, Dhaka
6	Cattle, 7 month	Biting history unknown	Profuse salivation, incoordination, progressive ataxia	Gupalpur, Tangile
7	Cattle 4 month	Biting history unknown	Profuse salivation, incoordination, progressive ataxia	Shibpur, Narsingdi
8	Adult Goat	Biting history unknown	Profuse salivation, incoordination,	Manikgonj

Direct Flourescent Antibody test result

The test was successfully established in Central Disease Investigation Laboratory, Department of Livestock Services, Dhaka, Bangladesh. Seven samples out of 8 samples were positive on DFA test. One sample was negative for rabies on DFA with repeated DFA test. Results are shown in Table -2. The positive samples yielded Fluorecence on fluorecescent microscopy (Figure: 1).

Table – 2: DFA test result

Sample No	Species	Test result on DFA		Repeated test result for initial negative sample	
		Brain stem	cerebelum	Brain stem	cerebelum
1	Cattle, 7 month of age	+	+		
2	Dog	+	+		
3	Adult cattle	+	+		
4	Fox	+	+		
5	Goat	+	+		
6	Cattle, 7 month	+	+		
7	Cattle 4 month	+	+		
8	Adult Goat	-	-	-	-

"+" indicate positive for Rabies on DFA; "-" indicate negative for rabies on DFA test

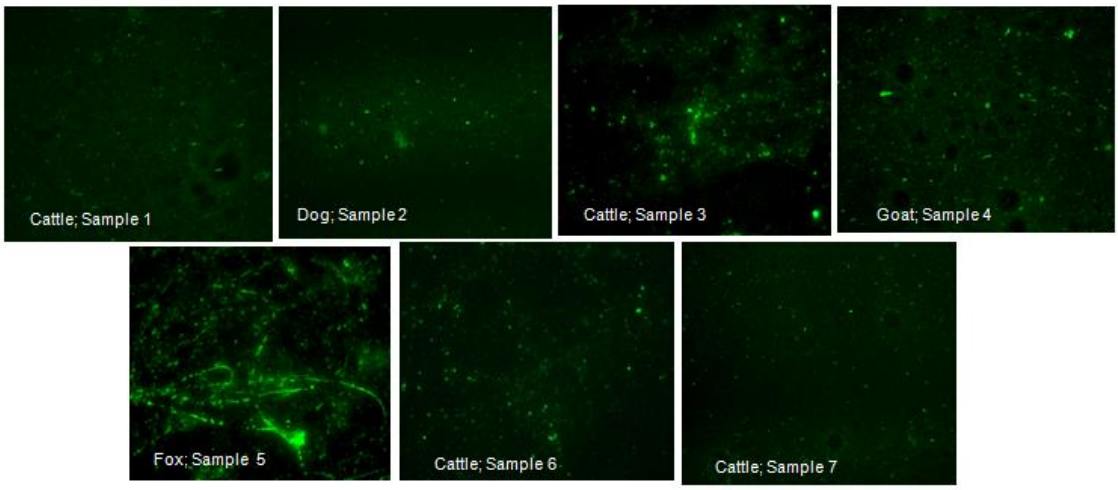

Figure-1: Fluorescence under Fluorescent microscopy using FITC filter using 40X objectives

Discussion

Direct Fluorescent Antibody test is an internationally acceptable test for rabies diagnosis. CDIL successfully employed this method for the detection of Rabies in the brain tissues of rabid animals. It was the first cases of laboratory confirmed rabies detected in wild animal (Fox) in Bangladesh. No previous report of rabies in wild animals in Bangladesh have been reported. This result implies that wild animal should also be addressed adiqualtely for the successful rabies elimination program in Bangladesh.

Acknowledgement: FAO, Bangladesh for reagents and technical assistance, PARB project of DLS for supplying Fluorescent Microscope (Nikon Eclips E 200).

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CHARACTERIZATION OF THE FOOT AND MOUTH DISEASE VIRUS IN BURKINA FASO : OUTBREAKS OF 2018

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Keywords: FMDV, Characterization, Burkina Faso

Background

Foot and mouth disease is a viral disease that affects cattle, buffaloes, pigs, sheep, goats and a variety of wildlife. The disease can seriously affect animal production and significantly disrupt regional and international trade in animals and their products.
The disease is enzootically in Burkina Faso. From January 2014 to June 2018, 384 outbreaks were reported to the World Organization for Animal Health (OIE). In 2018, the severity of the disease caused heavy losses on farms. There are seven immunologically distinct serotypes circulating around the world (O, A, C, SAT 1, SAT 2, SAT 3 and Asia 1). All these serotypes are found in Africa except Asia 1. In West Africa we find the serotypes O, A, SAT 1 and SAT 2.
The objective of this study was to determine the serotypes of the foot-and-mouth disease virus on the basis of outbreaks in four regions of Burkina Faso.

Study area

The study concerned 4 regions (Plateau central, Centre Ouest, Boucle du Mouhoun and Centre) on the 13 regions that make up the territory. Figure 1 shows the distribution of sampling sites.

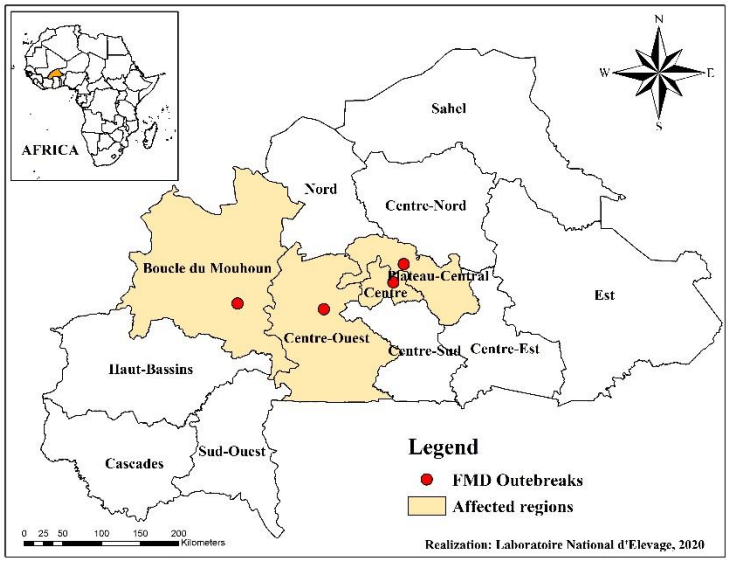

Figure 1: outbreaks of FMD in 2018 (Burkina Faso)

Methods

The samples were taken from farms with clinical suspicion of this disease. Only notified outbreaks was concerned. Samples concerned were vesical fluid and epithelial fragments made from sick animals. These areas were identified on the basis of bovine mortality alerts and presence of animals showing clinical signs (unbroken vesicles, tongue ulceration, etc.).

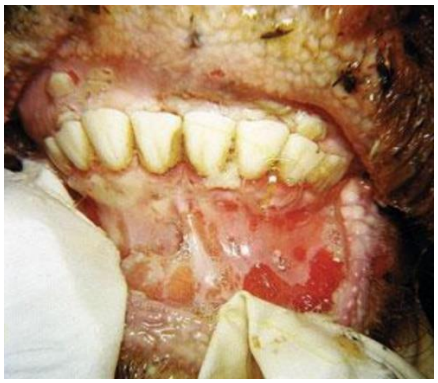

Figure 2: lesions of foot and mouth disease on bovine

Conditions for taking and transporting samples

The vesicular fluid was taken from animals with unbroken vesicles. The samples were then sent to the Laboratoire National d'Elevage no later than 24 hours after collection to be stored there at -80°C before shipment to the Pirbright Institute in England which is the OIE reference laboratory for foot-and-mouth disease for the characterization of the virus.
Samples were sent to The Pirbright Institute for sequencing.

Results

Characterization has shown that the isolated FMD virus belongs to serotypes O and to the topotype EA-3. Phylogenetic three show the result of characterization. Genotyping report mention the same serotype and topotype for Senegal, Mauritania, Gambia, Algeria in 2018 and Nigeria in 2016 and 2014.

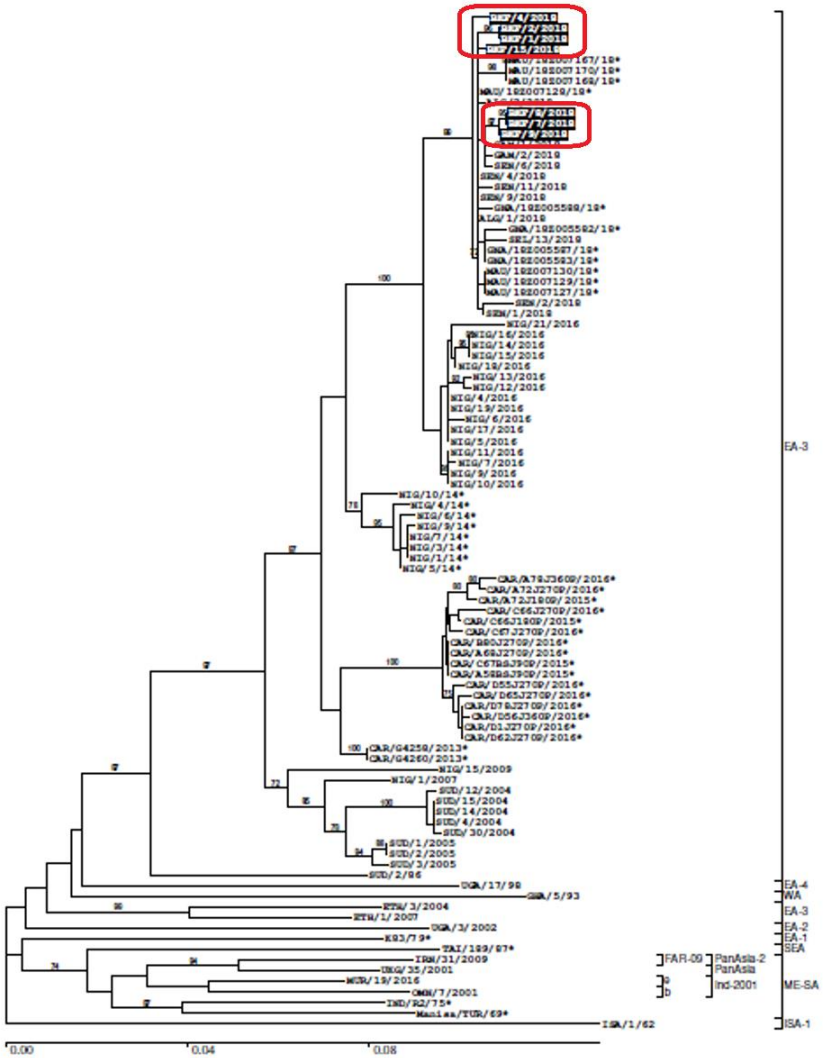

Figure 3. Phylogenetic analysis of FMD from Burkina: Neighbour-joining tree, of VP 1 gene region.

Identification of samples serotypes show serotype O, topotype EA-3. This serotype is circulating in many countries in west african region **(Teye et al., 2019).** This serotype is panzootic in west africa Region **(McLachlan et al., 2019; FAO, 2017).** From 1990 to 2013 serotypes O, A SAT1 et SAT 2 were found in Africa **(Paton et al., 2009).** Contact with wildlife, farming systems and cattle movements are very important in the transmission of virus **(Rweyemamu, 2008).** In Burkina Faso mean farming method is transhumance with a very low application of vaccination. Also mixed livestocks including many species favorize the maintain and propagation of the virus.
Investigation in all outbreaks each year is necessary to determine all serotypes circulating in the country.

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SEROPREVALENCE OF BRUCELLOSIS AMONG LIVESTOCK DURING 2014 TO 2018 IN THAILAND

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Abstract

Introduction

Brucellosis is a highly contagious zoonotic disease caused by *Brucella* spp. The 3rd FAO-APHCA/OIE Asia-Pacific Regional Workshop on Brucellosis Diagnosis and Control held in 2010 [1] stated that Brucellosis remains an important economic and public health problem in many countries, hence the disease situation in Asia-Pacific needs to be improved. In Thailand, *Brucella* spp. is frequently found in livestock, particularly, *B. melitensis* in goats and *B. abortus* in cattle and buffalo. The zoonotic transmission of brucellosis from animals to humans has been reported as an occupational risk in farmers who have a close contact with infected animals. This disease still remains a public health hazard. Due to the higher risk of getting infected by *Brucella* spp. among farmers, the Department of Livestock Development (DLD) was continuously conducted the activities of brucellosis surveillance across Thailand to monitor the disease status. The collaboration among the Bureau of Disease Control and Veterinary Services, the diagnostic laboratories for livestock included National Institute of Animal Health (NIAH), 8 Regional Veterinary Research and Development Centers (RVRDCs), and farm owners has supported the brucellosis surveillance program within country. The surveillance program included serum samples collection from livestock included dairy cattle, beef, buffalo, goat and sheep, then tested for antibodies specific to *Brucella* spp. using serological diagnostic methods. This study aims to investigate seroprevalence of brucellosis and to update the current situation of brucellosis in livestock in Thailand.

Materials and Methods

Serum samples from dairy cattle, beef, buffalo, goats, and sheep were submitted to National Institute of Animal Health (NIAH) and Regional Veterinary Research and Development Centers (RVRDCs) during 2014 to 2018. The total of tested herds and animals during 2014 to 2018 in livestock are showed in Table 1. All sera were screened by Rose Bengal Test (RBT) for the rapid diagnosis of brucellosis, the RBT positive samples were confirmed using Complement Fixation Test (CFT) and/or Indirect Enzyme-linked Immunosorbent assay (I-ELISA) followed the guidelines of OIE standard. The results from all laboratories were used to calculate brucellosis seroprevalence, standard deviation (SD) and 95% confident interval (CI) using Stata/IC vers.15.1 (College Station, TX).

Results

The seroprevalence of brucellosis in livestock are showed in Table 2. In dairy cattle, the highest seroprevalence at herd-level and animal-level were observed in 2014 which were 4.8% (95%CI: 4.3-

5.5) and 0.5% (95%CI: 0.4-0.5), respectively. In buffalo, the highest seroprevalence at herd-level and animal-level were observed in 2017 which were 3.7% (95%CI: 2.8-4.9) and 1.9% (95%CI: 1.8-2.0), respectively. In goats, the highest seroprevalence were 8.6% (95%CI: 7.9-9.5) at herd-level in 2017 and 1.1%, (95%CI: 1.0-1.1) at animal-level in 2014. In beef, the highest seroprevalence were 11.3% (95%CI: 9.9-12.9) at herd-level in 2016 and 1.9%, (95%CI: 1.8-2.0) at animal-level in 2018. In sheep, the highest seroprevalence were 15.8% (95%CI: 12.2-19.9) at herd-level in 2014 and 1.9%, (95%CI: 1.7-2.2) at animal-level in 2018.

Discussion

During 2014 to 2018, the overall of seroprevalences in dairy cattle and buffalo have been maintained in the low level. In contrast, the higher seroprevalence rates of brucellosis were observed in sheep, beef, and goats. This study was a passive surveillance study which is hard to control the bias of sample collection that caused differences in seroprevalence level among different animal species. In order to reflect the actual disease status, the active surveillance is recommended. Even though, the passive surveillance may not accurately represent the true disease status of the whole livestock populations, but it is cost efficiency and more practical for the long-term. The passive surveillance can be used as an initial general tool to monitor the trend of the disease, and then the active surveillance can be applied in the high prevalence species. This study showed that the differences of brucellosis seroprevalence trends in each species were changing over time. At herd-level, sheep had the highest seroprevalence relative to other species, while dairy cattle and buffalo had the lowest seroprevalence (figure 1). At animal-level during 2018, we observed that the seroprevalence of sheep and beef were higher than 1%, goat was around 1%, and dairy cattle and buffalo were remained lower than 1% (figure 2). We suggested that the herd-level prevalence should be the main focus in order to apply any disease control measures. However, together with animal-level prevalence monitoring and positive animal culling can be useful as a strategy to eliminate brucellosis across Thailand. This study shows that the brucellosis situation among livestock in Thailand can be improved according to the supports from various sectors. Specifically, the collaboration of laboratory network throughout the country, increase disease control program from the Bureau of Disease Control and Veterinary Services, as well as a good practice in farm management from the farmers helps to eliminate brucellosis in Thailand. In addition, the active surveillance should be performed in the individual species, particularly in sheep, beef, and goats, due to a high seroprevalence or in the infected area for potential brucellosis elimination in the future studies.

Keywords: Brucellosis, Livestock, Seroprevalence

Table 1: Number of Livestock in Herd and Animal Level during 2014 to 2018

Species	Unit	Number of tested				
		2014	2015	2016	2017	2018
Dairy Cattle	Herds	4921	5798	3950	4061	3670
	Animals	131437	121241	69230	82185	102451
Beef	Herds	2534	2722	1736	4253	3057
	Animals	36971	43640	41290	63529	58858
Buffalo	Herds	1012	2499	1656	1441	2688
	Animals	14911	24536	20675	18792	23393
Goats	Herds	5001	5065	4831	5101	6725
	Animals	170056	159164	148217	172775	169588
Sheep	Herds	374	428	367	367	526
	Animals	11954	12579	10213	11409	14274

Table 2: Brucellosis Seroprevalence, Standard Deviation (SD), and 95% Confidence Interval (95%CI) among Livestock during 2014 to 2018

Species	Unit	Seroprevalence (SD, 95%CI)				
		2014	2015	2016	2017	2018
Dairy Cattle	Herds	4.8 (0.3, 4.3-5.5)	1.8 (0.2, 1.5-2.2)	2.1 (0.2, 1.7-2.6)	1.9 (0.2, 1.5-2.4)	1.9 (0.2, 1.5-2.4)
	Animals	0.5 (0, 0.4-0.5)	0.1 (0, 0.1-0.1)	0.4 (0, 0.4-0.5)	0.2 (0, 0.2-0.3)	0.2 (0, 0.1-0.2)
Beef	Herds	10.7 (0.6, 9.5-11.9)	8.1 (0.5, 7.1-9.2)	11.3 (0.8, 9.9-12.9)	6.6 (0.4, 5.8-7.3)	7.9 (0.5, 7.0-8.9)
	Animals	1.9 (0.1, 1.8-2.1)	1.4 (0.1, 1.3-1.5)	1.3 (0.1, 1.2-1.5)	1.2 (0, 1.1-1.3)	1.9 (0.1, 1.8-2.0)
Buffalo	Herds	2.6 (0.5, 1.7-3.7)	2.6 (0.3, 2.0-3.3)	3.4 (0.4, 2.6-4.4)	3.7 (0.5, 2.8-4.9)	1.3 (0.2, 0.9-1.8)
	Animals	0.3 (0, 0.3-0.4)	0.5 (0, 0.4-0.6)	0.4 (0, 0.3-0.5)	0.6 (0.1, 0.5-0.8)	0.3 (0, 0.2-0.4)
Goats	Herds	6.7 (0.4, 6.0-7.4)	7.8 (0.4, 7.1-8.6)	6.8 (0.4, 6.1-7.6)	8.6 (0.4, 7.9-9.5)	7.9 (0.3, 7.3-8.6)
	Animals	1.1 (0, 1.0-1.1)	0.9 (0, 0.9-0.9)	0.8 (0, 0.7-0.8)	0.8 (0, 0.8-0.9)	0.8 (0, 0.7-0.8)
Sheep	Herds	15.8 (1.9, 12.2-19.9)	13.8 (1.7, 10.7-17.4)	11.4 (1.6, 8.4-15.2)	13.6 (1.8, 10.3-17.6)	15.6 (1.6, 12.6-19.0)
	Animals	1.6 (0.1, 1.4-1.8)	1.7 (0.1, 1.5-1.9)	1.8 (0.1, 1.5-2.0)	1.3 (0.1, 1.1-1.6)	1.9 (0.1, 1.7-2.2)

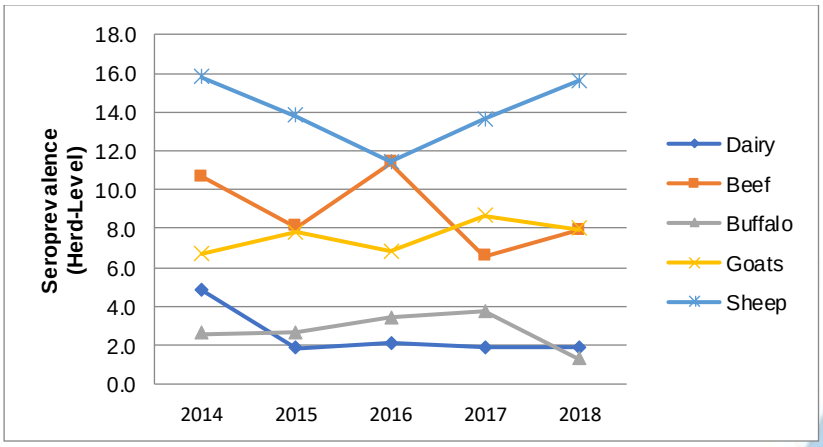

Figure 1: Seroprevalence of Brucellosis at Herd-Level in Thailand during 2014 to 2018

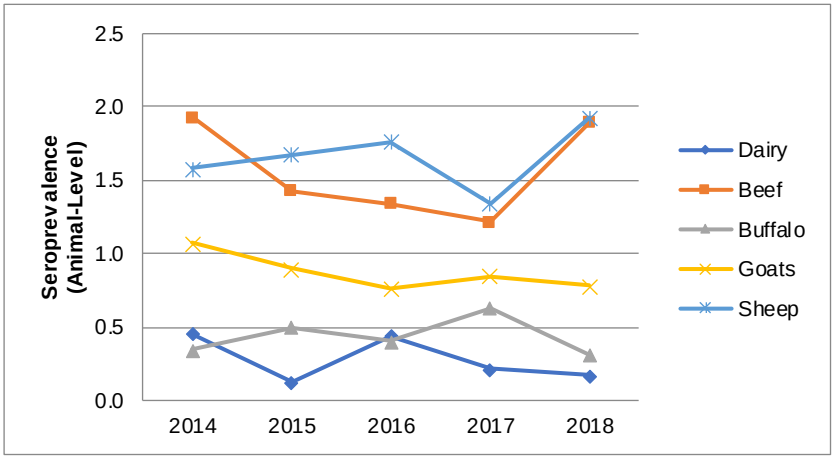

Figure 2: Seroprevalence of Brucellosis at Animal-Level in Thailand during 2014 to 2018

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EMERGENCE OF LUMPY SKIN DISEASE (LSD) IN BANGLADESH

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Introduction

Lumpy skin disease (LSD) is a viral disease of cattle, caused by Lumpy skin disease virus (LSDV) which belongs to the Capripox virus genus of the Poxviridae family (Babiuk et al., 2008). LSD is endemic in many African countries (Tuppurainen et al., 2011), circulate through the Middle-East region and emerging throughout to the rest of Asia (Abutarbush et al., 2003; Tageldin et al., 2004). LSD is one of the most economical important viral diseases of cattle due to loss of production, permanent damage of hides, infertility and death.

Objectives

This study was designed for the molecular detection of LSDV on field samples of cattle in Bangladesh.

Materials and Methods

Skin biopsies were collected from clinically infected cattle of different districts in Bangladesh. DNA extraction from skin samples were performed using DNeasy minikit (Qiagen, Germmany) according to the manufacturer recommendation.
For conventional PCR, primers were designed for p32 gene that is specific for capripox virus genus with the following sequence,
forward primer5'-CTAAAATTAGAGAGCTATACTTCTT-3´,
reverse primer 5'-CGATTTCCATAAACTAAAGTA-3´ to amplify 390 bp.
The thermal cycler (BIO-RAD, USA) parameters were: initial denaturation at 94 °C for 5 mins, 38 cycles of denaturation at 94 °C for 30 s, annealing at 50 °C for 30 s, extension at 72 °C for 30s ; and a final extension at 72 °C for 5 min. The products of PCR were separated by electrophoresis on 1.5% agarose gel in 1x TAE buffer.The image of the gel was photographed by gel documentation system (UVP Gel Studio, analytic jena, Germany).
For realtime PCR the method was adapted from Bouwden et al., 2008, for detection of all CaPV including Lumpy skin disease virus (Babuik et al., 2008). The assay uses a dual labeled fluorogenic probe and primers specific capripox virus CaPV 074.
CaPV-074F1: 5'-AAAACGGTATATGGAATAGAGTTGGAA-3'
CaPV-074R1: 5'-AAATGAAACCAATGGATGGGATA-3'
CaPV-074P1: 5'-FAM-TGGCTCATAGATTTCCT-MGBNFQ-3'

Results

The purified p32 genes of LSD virus were successfully amplified (amplicon size 390 bp) in all samples (Figure 1).
In realtime PCR all the samples also showed positive sigmoidal curves (Figure 2).

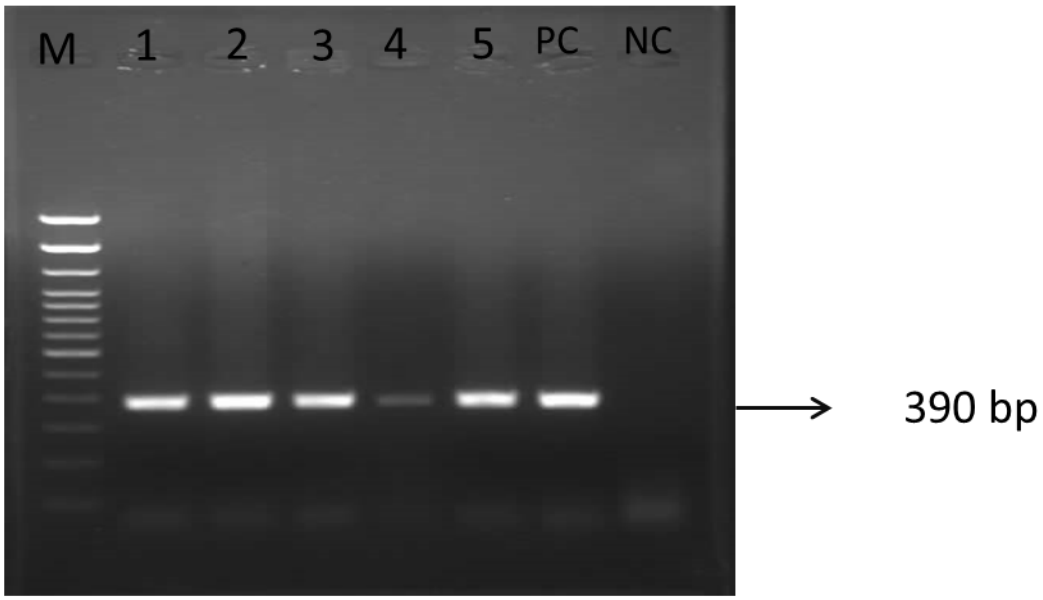

Figure :1. Electrophoresis picture showing the product of PCR analysis of P32 gene in LSDV. Lane M-Marker, 1-5 Field Sample, Lane PC:positive control, NC:negative control.

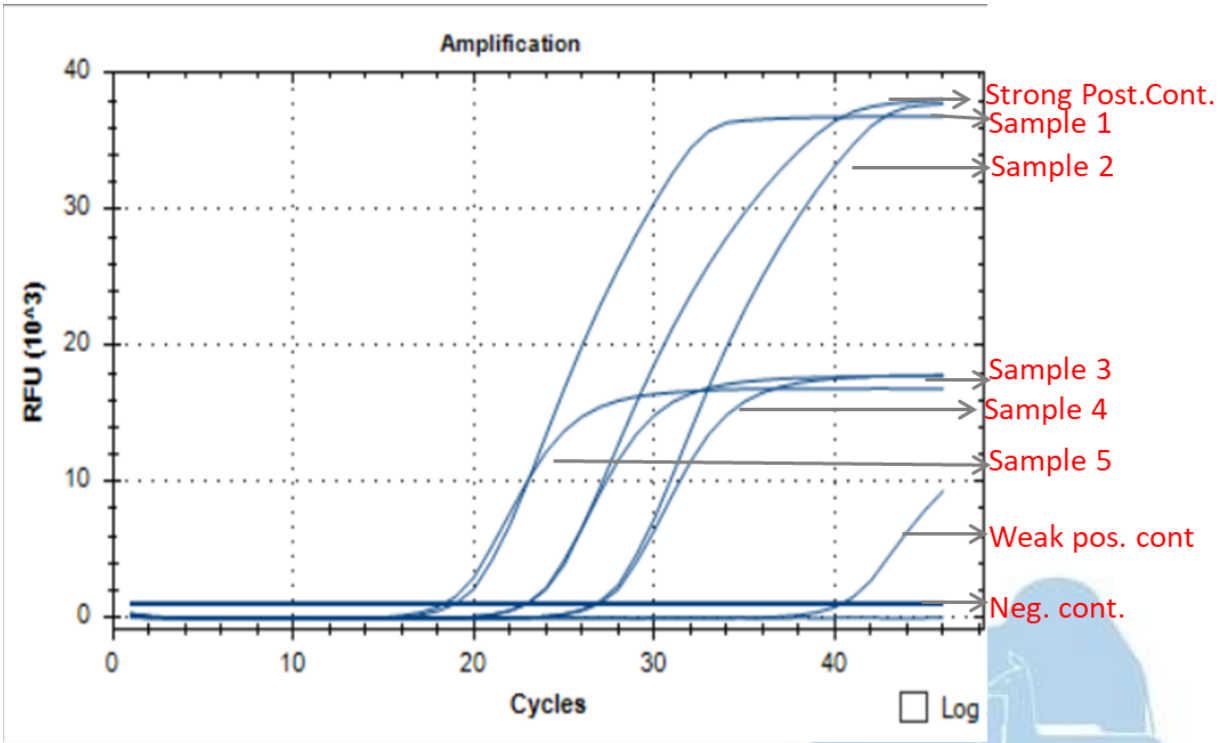

Figure: 2. Realtime PCR of LSDV samples.

Table 1: Cq value and result interpretation of Realtime PCR.

Sample	Cq value	Interpretation
Sample 1	18.34	Positive
Sample 2	26.71	Positive

Sample 3	22.90	Positive
Sample 4	26.97	Positive
Sample 5	18.99	Positive
Strong positive control	23.01	Positive
Weak positive control	40.38	Positive
Negative control	N/A	Negative

Discussion

The disease outbreak was severe in nature as cattle of many districts in Bangladesh were infected by LSDV. In Bangladesh, LSDV was detected for the first time at Central Disease Investigation Laboratory (CDIL), Dhaka from field samples collected from different parts of the country.

Conclusion

Both conventional and realtime PCR confirmed the presence of LSDV in Bangladesh indicating the emergence of this disease in Bangladesh. Sequencing and phylogenetic analysis are in progress.

Acknowledgement: Animal production and Health Laboratory, Joint FAO/IAEA Laboratories, International Atomic Agency for providing Primers, probe and positive control of LSDV.

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IAEA-CN-281-323

DIAGNOSTIC OF THE FIRST 2019 OUTBREAK OF FMD SEROTYPE O IN MOROCCO

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Foot and mouth disease (FMD) is a highly contagious transboundary animal disease that affects cloven-hoofed ruminants (cattle, swine, sheep, goats…). It is caused by a Picornavirus. The animals show fever and blister-like sores on the tongue, in the mouth, on the lips, between the hooves and on the teats. It can lead to severe production losses and major economic losses due to disruption in national and international trade.
In Morocco, after the episode of 2015, the disease reoccurred in early 2019 in the Region of Beni Mellal-Fquih Ben Saleh. After its confirmation on January 7, 2019 by the Regional Laboratory of Analysis and Research of Marrakech, belonging to the National Office of Food Safety, a national control plan has been launched to combat the spread of the disease in the country.

FMD Outbreak investigation and sample collection

A disease field investigation was carried out by the veterinary services on January 6, 2019, on a farm located in the province of Fquih Ben Saleh in central Morocco, where symptoms of FMD were reported. The herd consisted of 82 cattle and 55 sheep.

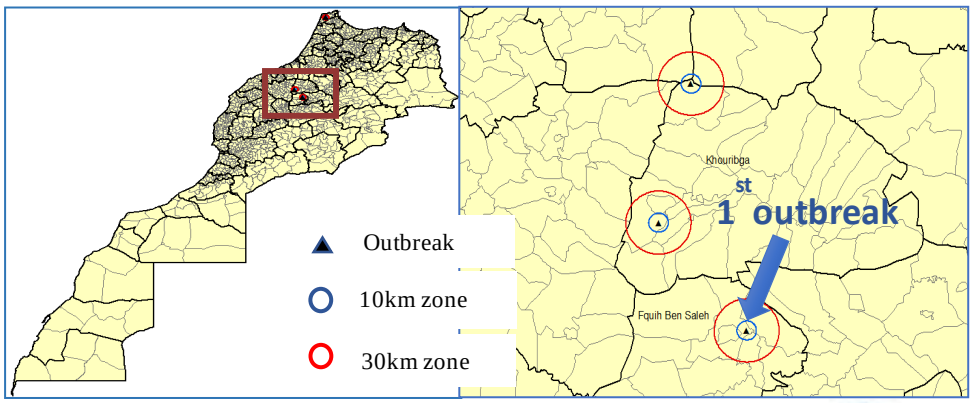

Fig 1: Location of the farm suspected of FMD

The symptoms appeared on 8 cattle, with clinical signs of fever, lameness, nasal discharge, salivation. There were also vesicular lesions in the buccal cavity (tongue, muffle, gum, palate) as well as erosive lesions. There has been one cattle death in the herd and no record or any history of vaccination against FMD for any of the cattle.
Sampling consisted on EDTA anticoagulated blood samples and nasal swabs from 4 of the affected cattle, and one vesicle from one affected cattle. Samples were immediately sent to the Regional Laboratory of Analysis and Research in Marrakech, belonging to the National Office of Food Safety (ONSSA), the official authority that manages animal health in Morocco. All sampled animals were 24 months old.

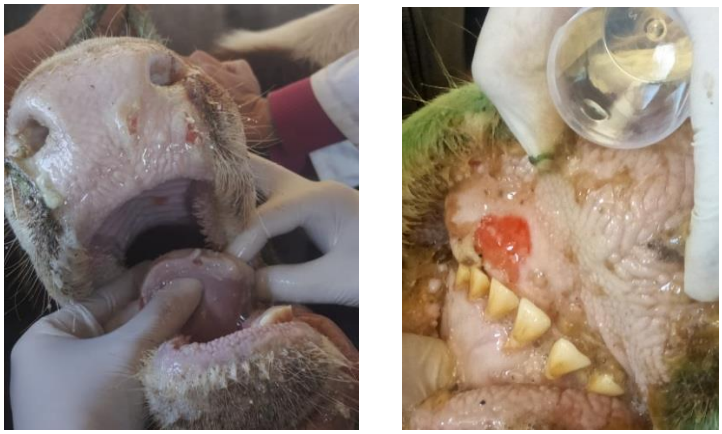

Fig 2: Buccal lesions, from vesicular to erosive lesions

Laboratory Diagnostic

Once received at the Regional Laboratory of Analysis and Research of Marrakech, the samples were immediately processed for the diagnosis of FMD by PCR. Under a Class II Biosafety Cabinet, the swabs were cut into a sterile Eppendorf tube containing 1mL of sterile phosphate buffer saline (PBS; 0.01M pH 7.4). After vortexing the tubes, they were centrifuged at 10,000 RPM for 3min at 4°C. The supernatant was then used for RNA extraction. Vesicular fluid and EDTA anticoagulated blood were used directly for RNA extraction.

The extraction of viral RNA was performed manually using the commercial kit Macherey Nagel Nucleospin RNA Virus according to the protocol of the manufacturer. 50 µL of RNAase free water was used to elute the extracted RNA. At the start of this step, 2 extraction controls were included: a positive field control from 2015 outbreak and a negative control for the validation of the experiment and for the detection of possible contamination during the extraction.

The method used to analyze RNA, described by Callahan JD et al., targets the 3D (RNA polymerase) coding sequence for tracking all of the seven FMDV serotypes. The rRT-PCR was performed using ABI7500 fast real time PCR system and the run was set according to the same method protocol. The amplification kit used was AgPath-ID™ One-Step RT-PCR Reagents.

The amplification protocol used the following conditions: Reverse transcription (one cycle), 50 ºC for 10 min, DNA polymerase activation 95°C for 5 min, followed by 50 cycles of 95 °C for 15 sec and 60 °C for 1 min. fluorescence was acquired at the annealing/extension steps in 60 ºC.

Two additional amplification controls (RNA negative and field positive extracts) were added at this stage to validate the experiment. When the Ct value was <40, the sample is considered positive.

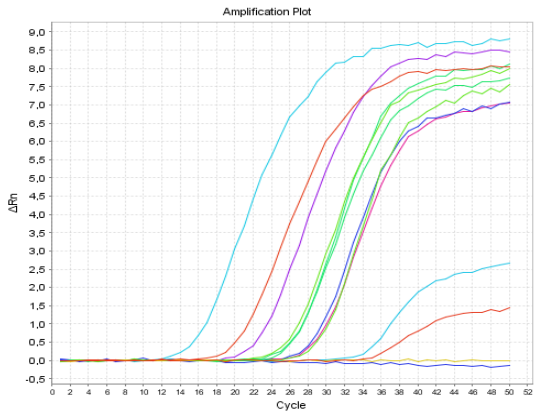

Fig 3: Amplification curves of the analyzed samples

Serotyping and phylogeny

After the confirmation of the etiology of the disease, other samples were submitted to anses FAO World Reference Laboratory for Foot-and-Mouth Disease (WRLFMD) for serotyping and Genotyping on January 16th, 2019. The results showed that this outbreak was caused by an FMDV Serotype O: Topotype O/EA-3, the previous epizootic of FMD, which occurred in 2015 (October 28 to the last case on November 28) was caused by the serotype O/India2001d. It is therefore the first time that the EA-3 topotype has been detected in Morocco.

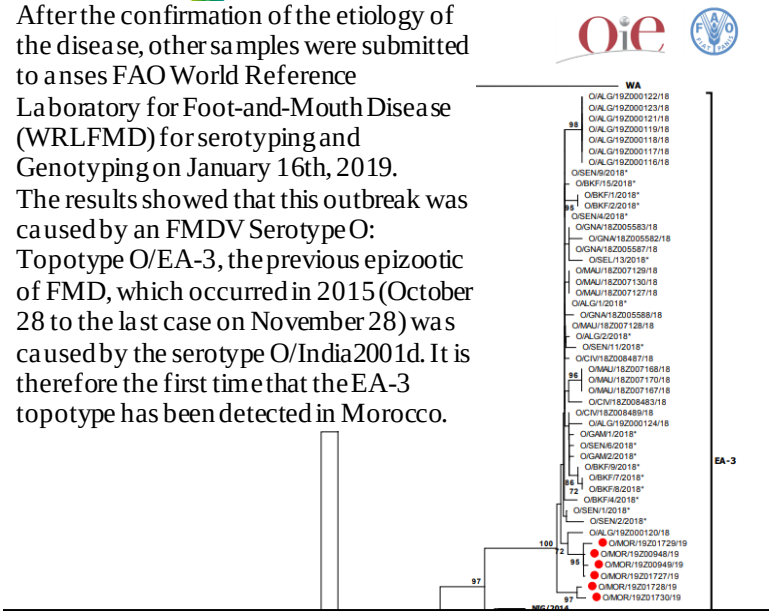

Fig 4: phylogenetic relationship between Moroccan FMDV isolate and other referenced isolates

Epidemiology

Region	Province	Outbreak	Cattle			Sheep	Goat
			Case	Death	Susceptible	Susceptible	Susceptible
Marrakech-Safi	El Kelaa des Sraghna	1	3		15		
	Rhamna	2	5	1	27		
Béni Mellal-Khénifra	Fquih Ben Saleh	4	12	1	98	88	
	Khouribga	2	9		64	463	36
Total		**9**	**29**	**2**	**204**	**551**	**36**

Fig 5: Synthesis of all the positive cases diagnosed in the two regions covered by the Regional Laboratory of Analysis and Research of Marrakech

Analysis of the data showed that the morbidity rate was 14.21% and that 2 out of the 204 susceptible animals died during the outbreaks with a mortality rate of 0.98%, which confirms the fact that most of the animals survive infection with FMDV.

Conclusion

Early detection of FMD and its rapid confirmation in the laboratory made it possible to rapidly initiate control measures to eradicate the disease over the country. The last outbreak in cattle was declared on 07/25/2019, resulting in 99 cattle cases in 36 outbreaks.

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IAEA-CN-281-325

THE ROLE OF FAO/IAEA VETERINARY DIAGNOSTIC LABORATORY (VETLAB) NETWORK IN CAPACITY BUILDING, TRANSFER OF TECHNOLOGY AND SHARING OF KNOWLEDGE.

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The International Atomic Energy Agency (**IAEA)** and the Food and Agriculture Organization (**FAO**) of the United Nations have joint laboratories called FAO/IAEA Veterinary Diagnostic Laboratory (**VETLAB**) Network that works in partnership with member states to perform a broad range of applied research and development activities.
The VETLAB network was initially developed to aid in the eradication of rinderpest by forming an eradication campaign through the development, evaluation, validation and transfer of selected diagnostic technologies, by the joint FAO/IAEA division of Nuclear Technologies in food and agriculture in close cooperation with the FAO animal production and health division. The campaign was formed because rinderpest was causing massive livestock and wildlife losses on three continents, which are Africa, Asia and Europe.

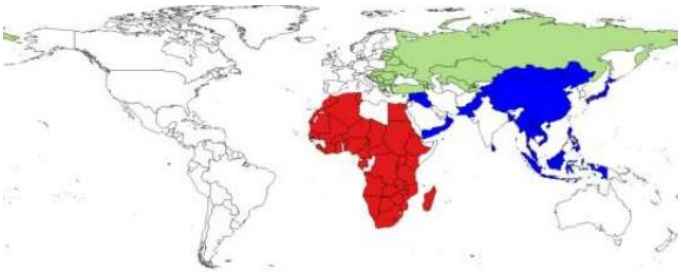

Figure 1: FAO/IAEA VETLAB Network member states

By 2017 the laboratory network had 44 African and 19 Asian national diagnostic laboratories sharing information and experiences. They also help with prevention, control and eradication of transboundary animal and zoonotic disease. It is a forum for the introduction and application of Quality Assurance systems to make sure that the laboratories produce internationally acceptable test results, they do this by doing laboratory accreditation of its member laboratories and offering proficiency testing exercises and inter-laboratory testing exercises. Participation in the proficiency testing exercises gives the laboratory confidence in their results and highlights short comings where they exist, allowing the laboratories to improve.
The VETLAB network carries out research and uses it for the development of its member countries. It also facilitates training programs and activities in support of member countries. They carry out both individual and group trainings of their member laboratories. The trainings are facilitated by field specific experts, to transfer knowledge.
The VETLAB network offers opportunity for countries facing similar challenges to work together and better coordinate activities, including training, information dissemination, and expertise, sharing experiences, as well as designing common disease control strategies. Therefore, network promotes country-to-country support, improves regional and national laboratory diagnostic capacity by providing technical advice/support, training on various diagnostic techniques for disease diagnosis.

By the aid of the FAO and IAEA and their support, several African laboratories have strengthened their diagnostic capacity, have had their facilities upgraded and are now able to produce quality and reliable test results. Examples of these are:

- The Botswana National Veterinary Laboratory and the Cameroon Laboratoire National Veterinaire have gotten their capacity improved and are now centres of excellence after this improvement. They have hosted training courses on disease diagnosis which were funded by both FAO and IAEA. The laboratory in Botswana is currently conducts external assessment for contagious bovine pleuropneumonia (CBPP) to countries in Southern African Development Community (SADC) region. Through the VETLAB Network support, Botswana has been granted the status of a World Organisation for Animal Health (OIE) reference laboratory for contagious bovine pleuropneumonia in May 2012.
- The laboratory in Cameroon is currently responsible for diagnosing African swine fever for Chad. These are good examples of country-to- county support.
- The National Veterinary Institute in Ethiopia, obtained the ISO 17025 accreditation in 2014 with support from the VETLAB Network. This international standard certifies that the laboratory is competent and able to produce accurate and internationally acceptable results. The National Animal Health Diagnostic and Investigation Centre in Ethiopia has also increased the number of accredited tests.
- Central Veterinary laboratory (CVL) Namibia as a member of the VETLAB network has also received many benefits from IAEA in terms of trainings and fellowships, consumables, equipment, the use of sequencing services and publications of research projects. CVL staff have attended trainings in molecular diagnostics, heavy metals and residue analysis, serological diagnosis of TADs and on ISO/IEC 17025 standard including proficiency testing. Several research projects funded by IAEA have been conducted at CVL and successfully published. All these have played a significant role in improving personnel skills, quality and capacity diagnostic services at CVL.

- Way forward is for CVL to continue being part of the IAEA VETLAB network, participate in the activities for improvement. CVL to show-case its capabilities to other IAEA Vet laboratories and offer support to other laboratories to grow in competency – by hosting or facilitating IAEA trainings. Furthermore, CVL will continuously strive to maintain the implementation of the ISO/IEC 2017 standard and its accreditation status by the Southern African Development Community Accreditation Standards (SADCAS), for reliable diagnosis and control of Zoonotic and TADs in Namibia and beyond.

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ZOONOTIC DISEASES AND COVID-19

IAEA-CN-281-111

MAPPING THE SPATIOTEMPORAL PATTERNS AND EPIDEMIOLOGY OF LIVESTOCK ANTHRAX IN UGANDA

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Background

Animals become infected with anthrax through consumption of anthrax spores from contaminated environments. Anthrax infection in animals is most commonly associated with sudden death, as they rarely show noticeable signs before death. For ruminants such as cattle and goats, only a small dose of the bacteria is required to become infected and die due to their high susceptibility to anthrax. Animals that have died from anthrax often bloat rapidly and bleed from natural orifices (nose, mouth, eyes, etc), discharging anthrax spores that contaminate the environment for decades. Vaccination of livestock against anthrax is the most effective way to prevent loss of livestock, interrupt the transmission cycle of anthrax, and prevent human infection.
Anthrax is endemic in most parts of Uganda, occurring throughout the year with negative impacts on the economy of the livestock industry and public health in Uganda. Besides being recognized as a global re-emerging zoonosis in recent years, Anthrax has drawn considerable attention due to its potential use as a biological weapon. Despite its potential effects on both livestock and public health, spatiotemporal distributions and epidemiology of the disease in animals and its potential driving factors in Uganda remain poorly understood. This paper discusses the distribution and epidemiology of livestock anthrax in Uganda. Findings in this paper are expected to compel the veterinary and medical authorities to not only maintain but also strengthen anthrax surveillance to mitigate its effects.

Methodology/Principal Findings

A retrospective epidemiological study and risk assessment of anthrax in Uganda was conducted using the national surveillance data of livestock anthrax from 2000 to 2019. Findings revealed the majority of livestock anthrax cases were located in Western and Central districts in Uganda. Also, four clustering areas with higher disease incidences were identified. Outbreaks mostly peaked in May or November. Correlations of monthly incidences of livestock with monthly average temperature, relative humidity and monthly rainfall were determined. Although vaccination of livestock remains an important measure in controlling anthrax, effective control is influenced by socio-economic, political, environmental and cultural complexities.

Conclusions/Significance

Livestock anthrax outbreaks in Uganda were largely influenced by seasonality and spatial clustering. Factors for spatial distribution of anthrax were livestock husbandry, livestock populations, climate and vegetation among others. Improved surveillance for livestock anthrax in the high-risk regions is critical for prevention of livestock and human infections

IAEA-CN-281-115

MAIN FACTORS INVOLVED IN THE EMERGENCE OR REEMERGENCE OF ZOONOSES, FUTURE THREATS AND THE STRATEGIC IMPORTANCE OF RESEARCH AND SURVEILLANCE IN BRAZIL

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Introduction

Human beings have always depended on animals for food, transportation, work and company. However, these animals can be a source of disease infectious diseases caused by viruses, bacteria and parasites, that can be transmitted to the human population (Seimenis, 2008). These diseases are called zoonoses (Brown, 2003). Agriculture and livestock are essential sectors for the economy of Brazil (Center for Advanced Studies in Applied Economics, 2009). As for the factors production, diseases are the biggest threats to stability of production systems, as their impact exceeds 20% of losses in livestock production all around the world. The socioeconomic impacts caused by animal diseases are also substantial, considering that currently 1 billion people rely directly on livestock production (Vallat & Wilson, 2003). The Food and Agriculture Organization of the United Nations (FAO) estimates that zoonoses are associated with to losses of over 30 million tonnes of milk annually, which, in turn, contributes to malnutrition and decreased disease resistance in children and the elderly (Seimenis, 2008). In addition to declines in productivity, countries lose trade opportunities because of their sanitary status and reduced international investment (Vallat & Wilson, 2003). The purpose of this paper is to address the main factors involved in the emergence and reemergence of zoonoses as well as future threats and the importance of the research and surveillance strategy in Brazil.

Factors for emergency or reemergence of zoonoses

Pathogen adequacy to new host species

Formerly resistant populations are becoming susceptible to previously harmless pathogens, which breaks the balance between immune defense and infections. This phenomenon occurs due to the aging of the world population and the use of therapies that modulate disease susceptibility through immunodeficiency or immunosuppression.

Food safety and industrialization

Emerging infectious agents may also be transmitted by food of animal origin. When bovine spongiform encephalitis emerged in cattle in the United Kingdom (Jacobson et al., 2009) several cases were reported of a human variant of the disease called “Creutzfeldt-Jakob (vCJD)”, caused by transmission of bovine spongiform encephalitis to people (Momcilovic & Rasooly, 2000). Thus, an emerging animal illness, quickly became an emerging zoonotic disease with hundreds of cases in the United Kingdom and, consequently, elsewhere in the world.

Pets and exotic animals

The presence of pets is very popular in homes around the world. Raising pets can be a risk for disease transmission, like scabies, salmonellosis and *Larva migrans*, and other parasites, viruses or bacteria present in these animals. Immunocompromised individuals are particularly at risk (Une & Mori, 2007;

Okulewicz & Bunkowska, 2009). Worldwide, an estimated 40,000 primates, 4 million birds, 640 thousand reptiles and 350 million of ornamental tropical fish are marketed per year (Karesh et al., 2005), generating an industry estimated international wildlife traffic of $6 billion (Check, 2004). Examples of zoonoses transmitted by these activities include rabies, tuberculosis, brucellosis, psittacosis (*Chlamydia psittaci*) and H5N1-HP (Chomel et al., 2007). Humans, especially children, can be exposed to zoonoses when attending fairs (including livestock), zoos (especially mini-zoos) and circuses where infections by *Escherichia coli* O157: H7, *Salmonella*, *Mycobacterium tuberculosis*, *Coxiella burnetii* and influenza virus already have been reported (Chomel et al., 2007; Vincent et al., 2009).

Change in management practices and livestock production

Many hypotheses point to the expansion of livestock as a source of pathogens for humans (Graczyk et al., 2000; Panda et al., 2008; Lejeune & Kersting; 2010). There is a growing demand worldwide for animal protein, with consequent increases in the rearing of animals in confinement, a predisposing situation for various diseases (Guo et al., 2015). The excess of confinement and feed processing may have led to the emergence of encephalitis bovine spongiform disease (Jacobson et al., 2009), also known as "mad cow disease. The mix of animals of different species and under stressful conditions also favored the emergence of SARS in Asia (Stavrinides & Guttman, 2004).

Transport of animals and infected people

In the 19th century, it took several weeks or months for disease agents to travel from one continent another. Today they can be transported to far-off lands in less time than the incubation period of many diseases (Brown, 2003). In addition, the globalization of technology, information and economy is creating forces for industries, cultures and organisms get connected. Geopolitical barriers practically do not exist anymore. An example of this is the appearance of the West Nile Virus on the east coast of the United States, where it caused outbreaks. The globalization of trade - tires imported from Japan - may have been responsible for the importation of this virus vector, *Aedes albopictus*, into the US state of Texas and for its subsequent spread within the country (Brown, 2003).
World trade has tripled in the last 20 years, and tourism is a growing sector in the global economy: one in four people visits another country each year (Brown, 2003). Currently, approximately 2.5 million people use airports each day and the destinations of more than 1 million of these trips are international, which accelerates the transmission of the pathogenic agents (Cutler et al., 2010). Ecotourism is the fastest growing tourism segment, on average 10% per year, and includes safaris, extreme sports, other types of tours (Chomel et al., 2007). Zoonoses associated with these practices include a variety of rickettsial diseases, brucellosis, hepatitis E, hantaviroses, leptospiroses, encephalitis transmitted by ticks and schistosomiasis (Cutler et al., 2010).

Interaction with wild animals

Agriculture may have changed the ecology of transmission of pre-existing human pathogens, having resulted in new interactions between humans and wildlife. One such interaction is domestication, which provided an enabling environment for infection of wild animal diseases in humans (Pearce-Duvet, 2006).

Action strategy, security measures and disease control

Predicting the onset or return of epidemics is difficult. However, the key points in preventing zoonoses are to perform early identification of pathogens in animals and respond quickly before the disease becomes a threat to the human population. At the University of Edinburgh in Scotland, 1,415 known human pathogens have been cataloged. Among these, 616 are also farm animal pathogens and 374 are known pathogens of carnivores (Cleaveland et al., 2001). 61.6% of human pathogens had a zoonotic

origin. Among animal pathogens, 77.3% were considered "multi-host", i.e. infecting more than one species. Among pathogens of carnivores, 90% were considered multi-hosts.
Primary detection and control is important, especially at "hot spots", which may include animal slaughtering facilities. Economic problems may arise if meat consumption is halted in countries at risk or following a positive diagnosis, such as occurred with the foot-and-mouth disease outbreak in the United Kingdom in 2001. That episode caused the loss of approximately 6 billion pounds sterling to the country, primarily to agriculture and tourism sectors (Thompson et al., 2002).
Support for the control of zoonoses is provided by organizations like the World Organization for Animal Health (OIE). Founded in 1924, the OIE currently has 180 member countries. The organization has a global network of 247 laboratories covering 117 diseases or topics in 38 countries, and 49 collaborating centers, covering 46 topics in 26 countries. However, the OIE does not act in isolation, there have been agreements with WHO since 1960 and FAO joined this group in 2006.
The surveillance of viruses and bacteria resistant to medication is also critical. Risk analysis and investment in animal health defense, training and response focusses on geographic areas where these threats are likely to emerge. Epidemiological research applied at the molecular level will have an immense value in the future to recognize associations between host genotypes and the pathogen. Human and animal health specialists should build an early detection network for disease at local, regional and national levels. Such a network requires diagnostic laboratories facilities, rapid response capability and actions for risk reduction.
Strategies should focus on the following actions: 1) detection of pathogens in wildlife (animals wild animals) that can cause disease in humans; 2) analysis and characterization of potential risks and methods transmission of specific diseases, including monitoring trade and transport of animals and products; 3) institutionalization of the "One Health" strategy in relevant health sectors with research for the development of new medicines to treat diseases such as resistant bacteria antimicrobials; 4) appropriate national outbreak response capacity, which involves the acquisition and distribution of antivirals, as well as the production of vaccines against pandemic diseases; and 5) risk reduction to prevent, minimize or eliminate the potential for emergence and transmission of new diseases.
In addition, the network must work on five planes, which focus on: 1) surveillance; 2) research and outbreak response training; 3) a laboratory network; 4) formulating strategies to contain the threat of diseases when assessing their behavior; and 5) preparation for disasters and pandemics according to each situation (United States Agency for International Development, 2009). It is vital to foster partnerships with organizations who have experience in wildlife monitoring (fauna), epidemiology and training in the field, as well as excellent infrastructure and good communication and planning in the national level. In Brazil, this would require the support of the Ministry of Agriculture, Livestock and Supply (Mapa); Ministry of Health; Ministry of Environment; among others, as well as the corresponding Secretaries of States and the Federal Council of Veterinary Medicine (CFMV).

Game meat

Game meat is consumed in manyregions especially in Central Africa and the Amazon Basin (Chomel et al., 2007). The foamy virus of apes is a very close zoonotic retrovirus already having infected people who had contacted fresh meat from non-human primates. Trichinellosis is associated with hunting, such as bear hunting, and recent severe cases of hepatitis E were associated with consumption of deer and wild pig. Other examples include diseases caused by the following parasites: protozoa, such as *Toxoplasma gondii*; trematodes, such as *Fasciola* sp. and *Paragonimus* spp., basketides such as *Taenia* spp. and *Diphyllobothrium* sp. and nematodes such as *Trichinella* spp., *Anisakis* sp. and *Parastrongylus* spp. (Chomel et al., 2007).

Land use and climate change

Climate warming, the exploitation of new agricultural frontiers and the introduction of rodents, mosquitoes and other vectors in urban areas changes the dynamics of disease transmission. An example is the Ebola virus, which was identified in 1976 in central Africa and re-emerged in early 2014 in West

Africa (Liberia, Sierra Leone, Ghana and Nigeria), with more than 20,000 cases since then. In the current epidemic, the index case was due to the exposure to a colony of insectivorous bats (*Mops condylurus*) (Marí Saéz et al., 2015). The reduction in the abundance of natural hosts makes vectors look for alternate hosts, which increases opportunities for the transmission of diseases, as has occurred in the human cases of borreliosis, or Lyme disease, erlichiosis and anaplasmosis (Cutler et al., 2010).

Obtaining of new virulence factors

New virulence factors include increased potential for invasion, diffusion, toxin production or resistance to antimicrobial drugs. Pathogenic agents, especially viruses, may undergo mutations or modifications to adapt to the human host. New viruses (emerging or reemerging) are capable of rapid transmission if there is host immune response or available vaccine. The emergence of viruses in bats Australia and Southeast Asia for example, caused diseases in other animals and humans, such as the Hendra, Menangle, Lyssavirus, and Nipah viruses. The last one emerged in Malaysia in 1999, where it decimated the swine industry and caused hundreds of deaths. Although pathogenic to pigs, this virus also causes severe disease in humans and kills 40% of the people infected (Brown, 2003).

Final Considerations

Research, combined with partnerships, is able to generate knowledge and troubleshooting tools for animal production chains Brazil. Knowledge, vigilance and cooperation are forces that must be united to propose strategies and prevent the country from being the cradle of an emerging disease, which can harm the economy and animal and human health. The strategy is already being outlined so that Brazil can defend against possible non-tariff barriers in international trade and avoid negative factors that may affect animal production. The main objectives are to produce with low cost by saving labor from the producer, decrease impact on the environment, and especially to prevent diseases and thus reduce their economic impact both on and off-farm and on imports, which will help guarantee a safe product for consumption.
The prevention and control of disease agents are animal health research priorities. In the case of the Brazilian Agricultural Research Corporation (Embrapa), these actions are divided into three streams: food security; health defense support, which includes emerging, exotic diseases and those already occur in the country that are risks for exportation; and endemic diseases for which the main problems are associated with economic losses. The idea is to protect the productivity and competitiveness of the production chains for beef, pork, chicken, goat, sheep, horse, and buffaloes; in addition those related to acquaculture (fish, crustaceans and molluscs) and those of other derivatives such as eggs and milk. The results obtained from the research and technologies generated by animal health projects should protect and ensure national livestock security through detection, prevention, control and treatment of animal diseases and their related pathogens.

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RABIES DIAGNOSIS IN ROMANIA ACCORDING TO THE 2018 EDITION OF THE OIE MANUAL

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Abstract

Rabies is a fatal zoonotic viral infection of the central nervous system with the causative agent Rabies virus, capable of infecting all mammal species. Until 2018 the most widely used test for rabies diagnosis was the Fluorescent Antibody Test (FAT), recommended by both WHO and OIE. According to the 2018 edition of the OIE Manual, the diagnosis strategy and methodology have been changed. For instance, new primary diagnostic tests for rabies have been added: the direct rapid immunohistochemistry test (dRIT) and pan-lyssavirus polymerase chain reaction (PCR) assays. In this paper we would like to explain the new OIE strategy and to highlight the advantages of these new techniques based on the Romanian National Reference Laboratory experiences, which has also been nominated recently as an OIE reference laboratory for Rabies. In Romania foxes are the main wildlife reservoir. Oral rabies vaccination (ORV) of this specie is the most effective method to control and eradicate rabies. Supported by co-financing program between Romania and European Union, successive ORV campaigns were conducted. From 2014 a multiannual program of ORV is performing. The vaccination area of this study involved of the entire Romanian territory (237.500 km2). The vaccination of foxes is carried out by air distribution of baits from 8 aircraft (number of 5325200 baits with an approx. 25 baits/km2), with a distance between flight lines of 500 meters and 150 meters altitude by avoiding the territories of localities, water surfaces, highways, etc. Estimated surface suitable for aerial vaccination is approximated at 213.375 square kilometers. Around localities and areas difficult to reach by plane it is done at manual distribution (number of 75400 of baits, approximately 25 baits per km2). The data are recorded on Geographical Identification System (GIS) using Geographical Positioning System (GPS). At 45 days following vaccination campaign, there shall be performed the hunting of foxes in order to assess the efficiency of vaccination; for this purpose, there shall be shot 4 foxes/year/100 km2. Samples of tooth and surrounding alveolar bone are tested by specific fluorescence to detect tetracycline deposits. Immune response is assessed using the indirect enzyme-linked immunosorbent assay (ELISA) method. There are 5 county laboratories in Romania that are able to perform these tests. All positive samples to rabies antigen by FAT technique (Fluorescent Antibody Test) are tested in order to discriminate between wild and vaccinated strains using molecular biology techniques which is performed at the Institute for Diagnosis and Animal Health only. Regarding passive surveillance each receptive animal found dead or with modified behavior is tested by FAT. There are 40 county veterinary laboratories accredited for this test in Romania. During the period 2017-2020 there were find 14 positive cases all over the country. Five out of them were wild animals and 9 domestic animals. Phylogenetic analysis demonstrated that all 14 field isolates from Romania found positive by hnRT-PCR belong to the classical rabies virus (genotype 1) and are all closely related. The program of oral vaccination of foxes has to continue, based on the increasing number of positive rabies cases in domestic animals in the analyzed period as well as the cooperation of vaccination programs between neighboring countries is very important.

Keywords: Rabies, diagnosis, fluorescent antibody test, RT-PCR**.**

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PROTECTIVE EFFICACY OF GAMMA IRRADIATED AVIAN INFLUENZA SUBTYPE H9N2 IRANIAN ISOLATE ANTIGEN ON BROILER CHICKEN

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Abstract

Immune response to gamma irradiated inactivated Avian Influenza H9N2 Iranian isolate antigen was evaluated in broiler chickens. Neutralizing antibody titration and lymphocyte proliferation assays were measured by using HI technique and cell proliferation ELISA kits and showed more protective response in the group with gamma irradiated AIV H9N2 antigen plus 20% trehalose. Irradiated AIV vaccine plus ISA70 and formalin vaccine plus ISA70 induced protective neutralizing antibody titration. Virus shedding for five vaccinated groups during 15 days post challenge was negative, while being positive for the positive control group. Gamma irradiated AIV H9N2 antigen is a good candidate for vaccine preparation and trehalose can be used as a protein stabilizer for vaccine formulation.

Introduction

The most widespread influenza virus in poultry is Low Pathogenic Avian Influenza Virus (LPAIV) H9N2 subtype (1). H9N2 subtype was reported in the industrial poultry populations of Iran in 1998 (2). A variety of strategies can be used for prevention and control of AIV infection in chickens, including increasing biosecurity, culling of infected animals, and vaccination (3). In this study AIV H9N2 subtype was inactivated by gamma irradiation and Montanide oil was used as an adjuvant with the inactivated antigen. In addition, trehalose was used for vaccine formulation (5), as a factor against stressors like heat, cold, oxidation and desiccation. Trehalose is a white, odorless powder; it is a non-reducing homo-disaccharide in which two glucose units are linked together (4).

Materials and Methods

AIV H9N2 subtype strain, A/Chicken/IRN/Ghazvin/2001 was used in this research. The optimum dose of gamma radiation for complete inactivation of virus samples was 30 kGy according to the procedure described by Salehi and et al (6). A Cobalt60 irradiator; the Gammacell 220 (MDS Nordion, Ottawa, Canada) was used for irradiation. Formalin vaccine was prepared by using the Razi Vaccine and Serum Research Institute protocol. Montanide ISA70 as an adjuvant was mixed with formalin and irradiated AIV H9N2 antigen (70:30 V/V), also 20 % of trehalose (1 M) was added to irradiation inactivated AIV as an inactivated vaccine. Seventy 70 day-old broiler chicks (Ross 308) were purchased from the Hen & Chicken of Alborz Company (Iran), individually weighed and randomly distributed in seven groups. The first group was injected with irradiated vaccine plus ISA70 subcutaneously (SC). The second group was injected with formalin vaccine plus ISA70 SC. The third group was injected with irradiated vaccine plus trehalose SC. The fourth group was inoculated with irradiated vaccine plus trehalose intranasal (IN). The fifth group was inoculated with formalin vaccine IN. The sixth group was inoculated with

sterile PBS as negative control and the seventh group (as a positive control) was inoculated with 100 EID_{50} of AIV H9N2 as nose drops. The negative control and vaccinated groups were housed in two separate places. The positive control group was inoculated and housed in an isolated room. Vaccination was done at the first and 15th days post-hatch. The vaccinated (and negative control) groups were challenged with 100 EID_{50} of AIV H9N2 (IN) two weeks after the second vaccination. Blood samples were collected from the wing vein at 1, 15, 30 and 45 days post-vaccination and sera samples were stored in -70 °C for the neutralizing antibody response assay by hemagglutination inhibition (HI) test. The spleens of the vaccinated chicken were removed aseptically at 15, 30 and 45 days for the lymphocyte proliferation assay and cytokine assay by real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) and specific primers for cytokines. Tracheal swap samples were collected at 1, 30, 32, 36, 38, 42, 45 days and placed in RNX-Plus solution (TRIzol) for total RNA extraction and virus shedding was evaluated by QPCR according to the protocol of Wee Theng Ong, et al. (2007) and a specific primer pair for H9 (7). The sequence of the H9 gene of AIV subtype H9N2 strain, A/Chicken/IRN/Ghazvin/2001 was deposited in the NCBI database with accession number FJ794817.1 (1741 bp). H9 Forward and H9 Reverse primers and FASTA format of H9 gene (FJ794817.1) were aligned by MegAlign software, the PCR product size was about 256 bp. RNA Mini Kit (Bio&Sell, Germany), Easy™ cDNA Synthesis kit (Parstious, Cat A101161, 50 reaction) and QPCR Mix EvaGreen kit (Bio&Sell, Germany) were used for RNA extraction from splenic lymphocytes and tracheal swap samples and real-time qRT-PCR.

Results

The titration of AIV subtype H9N2 used in this research was about $10^{8.5}$/ml EID_{50} before irradiation. The results of the neutralizing antibody titration and stimulation index for lymphocyte proliferation assay were measured by HI technique and Cell Proliferation ELISA kit, BrdU (colorimetric) Roche Cat. No. 11647229001 and are shown in Table 1.

Table 1: Neutralizing antibody titration and stimulation index

No. group	Vaccine	Antibody Titration		Antibody Titration & SI			
		1 d	15 d	30 d		45 d	
	PreImmune	0		AB	SI	Ab	SI
1	irradiated vaccine plus ISA70-SC		2.33±0.57	4.33±0.57	1.14	4±1	1.13
2	formalin vaccine plus ISA70-SC		2±1	4.66±1	1.07	4±0.5	1.08
3	irradiated vaccine plus trehalose-SC		2.66±0.57	4.75±0.95	1.20	4.35±0.5	1.24
4	irradiated vaccine plus trehalose-IN		3.33±0.57	4.75±0.57	1.25	4.5±0.57	1.52
5	formalin vaccine IN		2.25±0.95	4.25±0.5	0.95	3.66±0.57	1.16
6	Negative Control		0	0	0.89	0	0.93
7	Positive Control	Virus inoculation was done at 60 day and sampling at 65 day				0	

The cytokine assay was done by qRT-PCR for measuring IFN-γ and ΔΔCt showed there was significant increase between irradiated vaccine plus trehalose (groups 3 and 4) and other groups ($p<0.05$).
Virus shedding results are shown in Table 2. A serial dilution of cDNA for AIV H9N2 subtype was used to draw the standard curve. According to the Ct for standard dilutions and electrophoresis results for QPCR products, the Ct of negative samples was more than 20. Therefore, virus shedding for five vaccinated groups during 15 days after challenge was negative and it was positive for the seven group (positive Control).

Table 2: QPCR results (Ct) of tracheal swap samples to evaluate virus shedding

No. group	Vaccine	1 d	30 d	32 d	36 d	38 d	42 d	45 d	65 d
	Pre-Immunization	UND							
1	Irradiated vaccine plus ISA70-SC		34.62	36.30	38.59	37.62	32.48	33.72	
2	Formalin vaccine plus ISA70-SC		29.73	38.22	36.64	35.44	30.72	33.19	
3	Irradiated vaccine plus trehalose-SC		34.06	34.06	37.28	39.48	35.03	33.01	
4	Irradiated vaccine plus trehalose-IN		34.88	34.97	37.31	34.45	32.80	33.52	
5	Formalin vaccine IN		35.19	36.30	UND	37.78	29.83	30.56	
6	Negative Control		UND			UND		UND	
7	Positive Control								10.98

UND: Undetermined

Conclusion

Gamma irradiated inactivated AIV subtype H9N2 antigen is a good candidate for vaccine preparation. Montanide ISA70 can be used as an adjuvant for AIV vaccine formulation. Trehalose, as a when added to the vaccine as a protein stabilizer, can increase the protective efficacy for irradiated AIV subtype H9N2 vaccine. We observed more antibody and greater lymphocyte proliferation response in groups treated with such vaccines.

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AVIAN INFLUENZA RESISTANCE IN GUATEMALAN NATIVE CHICKENS

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Summary

The present study was carried out to determine resistance to avian influenza in Guatemalan creole (native) chickens. For this purpose, an avian influenza virus previously characterized as low pathogenic H5N2 called SM12/15 was inoculated into two groups of 10 native chickens from the Mexico-Guatemala border region and a group of 10 chickens from a commercial genetic line that functioned as a control. The three groups were raised in isolation areas of the Regional Animal Health Reference Laboratory -LARRSA-; the experiment was conducted in isolation units within the laboratory. For the use of experimental animals, the ethics committee of the Faculty of Veterinary Medicine of the University San Carlos de Guatemala approved the protocol. At the beginning of the experiment and prior to inoculation at three weeks; HI test (hemagglutination inhibition) was performed to confirm the absence of antibodies against avian influenza. For 7 days the symptoms and injuries were observed and compared. Using the ANOVA test, there was no significant difference between symptoms determined by reduction in weight and lesions. Tracheal excretion was also determined at three days in each of the groups, observing that in the native chickens the excretion was approximately one Log2 lower when compared with the control. The main finding was that none of the Creole chickens excreted virus at 7 days while 40% of commercial chicken continued to excrete viruses.

Keywords: Influenza, native chickens, Guatemalan, resistance

Introduction

The information on avian influenza in Latin America is limited (Afanador,2017). In a meta-analysis, the report shows evidence of avian influenza in almost all Latin American countries; with Mexico being the country with the highest number of reported cases, followed by Chile and Argentina. The majority of the investigations are the product of epidemiological surveillance; 43.7% correspond to migratory wild birds, 28.1% to local wild birds and only 28.3% correspond to farm chickens; Afanador points out the need for more studies at the regional level.

In Central America there is a reference of H5N2 avian influenza virus in chickens only in Guatemala and El Salvador; both viruses descend genetically from the circulating virus during 1994 in Mexico (Senne, 2006). The first report of H5N2 subtype influenza of low pathogenicity in Guatemala was made in 2000 (Serrano,2001) identified by the USDA as A / CK / Guatemala / 45511-1 / 00, secondary to an influenza outbreak of the same subtype in Mexico in May 1994 (Senne, 2006). The Policies of MAGA (Ministry of Agriculture, Livestock and Food) - during 2000 required vaccination in a controlled manner and only in regions with serological evidence of viral circulation, using the vaccine (H5N2 A / Ck / Mexico / CPA232 / 1994). After the year 2,000, sporadic outbreaks of H5N2 influenza of low pathogenicity have occurred mainly at the border with Mexico; the same vaccine strain has been used in the country for 19 years, without reports to date of high pathogenicity although previous studies have determined that vaccines used in Guatemala fail to reduce viral excretion. (Serrano 2019)

Tike Sartika (2011) reports that Creole chickens in Indonesia have the ability to resist viral infections. In his observations he attributes to the Mx proteins, induced by interferon (IFN) an inhibition effect on

RNA virus replication. In the first decade of the year 2000, resistance to high pathogenicity virus was reported in Thai chickens, which was attributed to haplotype B 21 of the MHC, however Hunt and Swayne (2010) reported that the presence of haplotype B 21 did not protect chickens from death before the challenge of a high pathogenic influenza virus, the authors suggest that epistatic factors could cause resistance in Creole birds. In Guatemala there have been no previous studies on resistance in Creole chickens.

Materials and Methods

The poultry census carried out by MAGA in 2013 revealed that the most vulnerable communities to the illegal transfer of Mexican poultry products and therefore to avian influenza in the Mexico-Guatemala border zone are in Democracia, the Guacamayas village. We decided to sample the Ceibas and Guaylá farmhouses in the aforementioned Guacamayas village. Only clinically healthy chickens born from birds within the community were included in the experiment. These were male or female birds serologically negative to avian influenza, which have phenotypic characteristics of Creoles; feathered or bare-necks, and feather color other than white. The virus for the challenge was the one that presented the highest pathogenicity among the isolates during 2015 and 2016; based on the results of the intravenous pathogenicity index IPV 0.33. (Serrano, 2018)

Experimental infection in chickens

The two groups of twenty Creole chickens obtained from Ceibas and Guaylá villages, and one corresponding to the control group with chickens of a commercial line (SAN), were challenged with the selected virus, by using an intranasal drop with $10^{6.2}$ DIE50 / 0.1 ml (Beato Maria, 2009) (Lonneke Vervelde, 2011).
The birds were monitored daily to observe symptoms., Tracheal and cloacal swabs were collected at 3 and 7 d.p.i (days after infection) to quantify viral excretion (3 days) and determine excretion (7 days); at which time they were also weighed. At the beginning of the experiment, serology was performed to confirm seronegativity of birds to avian influenza (exclusion criteria); the antibody titre was determined by the Hemoaglutination Inhibition test (HI), to ensure absence of titles and to compare results with the challenged group. Titles of 1: 8 or less were considered negative (Kapczynski Darrell, 2013)
At the conclusion of the experiment, the euthanasia of the chickens was carried out with a dose of Xylazine, the necropsy of the birds of each group was performed. The amount of virus excreted will be determined by the classical method (Reed and Munch test) of viral titration in embryonated eggs. (Kapczynski Darrell, 2013) (David Swayne, 2008). With the data collected, a comparison of the means of the different experimental groups and controls was carried out using the ANOVA test to determine differences in the means and the variances of at least one of the groups observed.

Results

The severity of the symptoms was assessed by the effect on the weight of the challenged birds. When using the ANOVA test, there were no differences in weights in the three groups evaluated, Guayla, Ceibas and Control.
The predominant lesions observed post challenge mainly consisted of tracheitis and hemorrhagic thymus. Pneumonia was observed only in the control group; splenomegaly was observed in 40% of native chickens. The target organs referred to by Swayne (1999) with low pathogenicity virus are the lungs, lymphoid and visceral organs that contain epithelial cells such as kidney and pancreas. When the ANOVA test was used to compare the groups there was no difference between them.
The tracheal excretion determined at three days using the Reed and Muench method was $10^{5.2}$ DIE50/0.1 ml in the control group, while $10^{4.1}$ DIE50/0.1ml in Guaylá chickens and $10^{3.9}$ DIE50/ml in Ceibas birds. The reduction observed when compared with the control group corresponded to approximately 1 logarithm. (0.97 in Guaylá and 1.19 in the case of Ceibas), The literature consulted

refers that post vaccination immune birds reduce by 2 logarithms the inoculated virus titer. Taking this information into account, Creole birds are not resistant to the challenge virus (SM12 / 15 H5N2).
Table No.1 Titration of tracheal and Cloacal viral excretion in the three study groups (dpc = days post challenge

Group	Tracheal and Cloacal Isolation (Number/total) 3 day	Cloacal Isolation (Number/total) 7 day	TrachealTitle 3 dpc DIE50/ ml
Ceibas	10/10	0/10	$10^{3.9}$
Guayla	10/10	0/10	$10^{4.1}$
Sham	10/10	4/10	$10^{5.2}$

At 7 days, none of the native birds excreted virus via tracheal or cloacal routes, while in the control (commercial line) 40% of the birds continued to excrete virus cloacally. Similar to the present study, Hernández (2016) when evaluating two genetic lines - the 0 resistant line and the C-B 12 sensitive line - reported that in Line 0, the excretion decreased from the third day, being negative at the week. When Hernandez challenged lines 0 and C B12 with a low pathogenicity virus (A / turkey / England / 647/77) he found differences in the period of excretion, which he called "restricted time curse".
In this study we conclude that the three groups of chickens (i.e. native vs. commercial) behaved in a similar way, and that the susceptibility is similar in Guatemalan Creole birds and commercial genetic lines. However, it is clear that the reduction in excretion after 7 days seen in native birds suggests that these birds were less affected. Similar studies should be performed to determine the reduction (Log 2) from three days until ten days, since in the present work only the third day was quantified.

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CONTINUING CIRCULATION OF A NEW REASSORTANT CLADE 2.3.2.1A HIGHLY PATHOGENIC AVIAN INFLUENZA H5N1 VIRUS IN CHICKENS IN BANGLADESH

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Synopsis

The poultry sector is an integral part of agribusiness and livestock farming in Bangladesh, creating employment opportunities, improving food security and enhancing the supply of quality protein and thereby contributing in country's economic growth and poverty elimination. This promising sector is hampered by disease outbreaks, especially avian influenza (AI). AI viruses (AIV) belong to genus Influenza A virus under the family *Orthomyxoviridae*. AIVs can be divided into two distinct groups on the basis of their ability to cause disease and certain molecular and biological properties of the virus: highly pathogenic avian influenza (HPAI) virus and low pathogenic avian influenza (LPAI) virus [1]. HPAI virus subtype H5N1 is extremely infectious and causes a systemic viral disease of poultry that affects both domestic and wild bird populations including chickens, turkeys, ducks, domestic geese, quail, pheasants, partridge, psittacines, gulls, shorebirds, seabirds, emu, eagles, and many captive and domestic animals [2,3]. In Bangladesh, HPAI virus of H5N1 subtype was first detected in 2007 [4]. During the first few years, clade 2.2 viruses were circulating among poultry in Bangladesh before the introduction of clade 2.3.2.1a virus in 2011 [5]. The clade 2.3.2.1a viruses further evolved through genome segment reassortments that started in 2013. Since 2015, the reassortant clade 2.3.2.1a virus had been detected during surveillance in live bird markets (LBM) and wet lands [6]. The emerged virus is a segment reassortant that acquired PB2, PB1, PA, NP and NS genes from LPAI viruses, mostly of non-H9N2 subtypes but retained HA, NA and M genes of the old clade 2.3.2.1a viruses. Nevertheless, the HA gene of these new viruses was 2.7% divergent from that of the old clade 2.3.2.1a viruses that circulated in Bangladesh [6]. In 2017 we detected the reassortant virus also from field outbreaks in chickens, ducks, goose and turkeys [7]. In the present study we performed genetic characterization of five recent H5N1 HPAI viruses from commercial layer chickens detected in 2018 (n=3) and 2019 (n=2) based on full genome sequencing.

This study was a part of our respiratory disease syndrome surveillance programme with the objective of pathogen search. Swab or tissue samples collected from layer flocks with respiratory symptoms were examined by a panel assay for different respiratory pathogens. Samples testing positive for H5N1 HPAI virus were subjected to full genome sequencing using universal [8] and custom designed primers. The raw sequence data were first checked for quality and then edited and assembled with the Bioedit (www.mbio.ncsu.edu/BioEd it/bioedit.html) and MEGA7 (www.megasoftware.net) software. The nucleotide sequences of all eight gene segments were subjected to a phylogenetic analysis along with related sequences representing each clade downloaded from the GenBank and GISAID databases. Multiple alignment was performed with the Clustal W algorithm and a Maximum Likelihood phylogenetic tree was constructed using respective best-fit models predicted for individual genes with MEGA 7 software. The stability of the nodes in the phylogenetic trees was tested by bootstrapping with 1000 replications.

Five samples turned out to be positive for H5N1 HPAI virus. Three samples were obtained from Sakhipur upazila, one in April 2018 and two in February 2019, and two other samples were obtained

from Bhaluka upazila in April 2018. The age of the chickens ranged between 27 and 87 weeks and the flock size of the affected farms ranged from 1100 to 1950. Morbidity of the affected flocks was relatively low (1.2%-6%), however, the mortality rates were very high (57%-100%) during the investigation period. Sudden death, depression, diarrhoea, cyanosis of comb and wattle were predominant clinical signs of the affected chickens. At necropsy, most of the infected birds showed typical lesions of HPAI, characterized by haemorrhages and congestion in different tissues including musculature, trachea and lungs; haemorrhages in pancreas, coronary and abdominal fat, ovarian follicle and caecal tonsils; enlarged liver and spleen and catarrhal enteritis.

Phylogenetic analysis based on full length gene sequences of HA gene placed all five viruses of the present study under clade 2.3.2.1a of H5N1 phylogeny. However, these viruses together with H5N1 viruses reported from Bangladesh in 2015 and onward formed a separate cluster (new reassortant) under clade 2.3.2.1a (Fig. 1a). The viruses of 2018 and 2019 showed 97.4% to 99.9% sequence identity. The phylogenetic tree of NA and M gene sequences of all Bangladeshi H5N1 viruses broadly followed the topology of HA phylogenetic tree where all five isolates clustered under the new reassortant clade 2.3.2.1a viruses. Analysis of other internal genes showed that the five isolates of 2018 and 2019 were segment reassortant, containing PB2 (Fig. 1b), PB1, PA, NP and NS genes from LPAI viruses of non-H9N2 subtypes. These gene segments were closely related to the viruses detected recently from LBM, aquatic birds and field outbreaks. Gene constellation (Fig. 1c) revealed that reassortant viruses of clade 2.3.2.1a which have been detected since 2015 in LBM and field outbreaks are under continuous circulation among commercial poultry.

In conclusion, continuing circulation of the new reassortant clade 2.3.2.1a virus with apparent replacement of the older genotype suggests relative fitness advantage of the new reassortant virus to poultry population.

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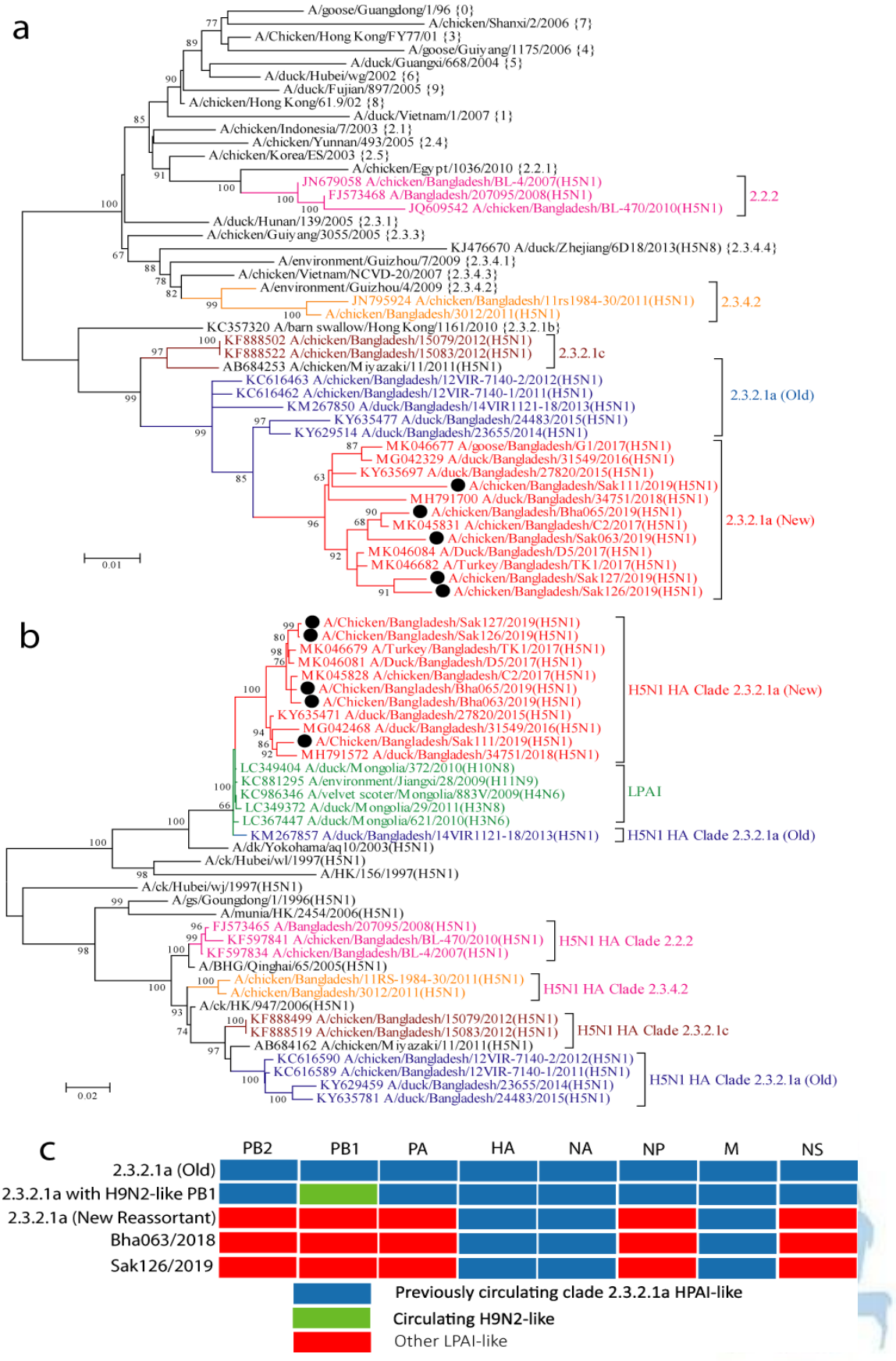
a
A/goose/Guangdong/1/96 {0}
A/chicken/Shanxi/2/2006 {7}
A/Chicken/Hong Kong/FY77/01 {3}
A/goose/Guiyang/1175/2006 {4}
A/duck/Guangxi/668/2004 {5}
A/duck/Hubei/wg/2002 {6}
A/duck/Fujian/897/2005 {9}
A/chicken/Hong Kong/61.9/02 {8}
A/duck/Vietnam/1/2007 {1}
A/chicken/Indonesia/7/2003 {2.1}
A/chicken/Yunnan/493/2005 {2.4}
A/chicken/Korea/ES/2003 {2.5}
A/chicken/Egypt/1036/2010 {2.2.1}
JN679058 A/chicken/Bangladesh/BL-4/2007(H5N1)
FJ573468 A/Bangladesh/207095/2008(H5N1)
JQ609542 A/chicken/Bangladesh/BL-470/2010(H5N1)
2.2.2
A/duck/Hunan/139/2005 {2.3.1}
A/chicken/Guiyang/3055/2005 {2.3.3}
KJ476670 A/duck/Zhejiang/6D18/2013(H5N8) {2.3.4.4}
A/environment/Guizhou/7/2009 {2.3.4.1}
A/chicken/Vietnam/NCVD-20/2007 {2.3.4.3}
A/environment/Guizhou/4/2009 {2.3.4.2}
JN795924 A/chicken/Bangladesh/11rs1984-30/2011(H5N1)
A/chicken/Bangladesh/3012/2011(H5N1)
2.3.4.2
KC357320 A/barn swallow/Hong Kong/1161/2010 {2.3.2.1b}
KF888502 A/chicken/Bangladesh/15079/2012(H5N1)
KF888522 A/chicken/Bangladesh/15083/2012(H5N1)
AB684253 A/chicken/Miyazaki/11/2011(H5N1)
2.3.2.1c
KC616463 A/chicken/Bangladesh/12VIR-7140-2/2012(H5N1)
KC616462 A/chicken/Bangladesh/12VIR-7140-1/2011(H5N1)
KM267850 A/duck/Bangladesh/14VIR1121-18/2013(H5N1)
KY635477 A/duck/Bangladesh/24483/2015(H5N1)
KY629514 A/duck/Bangladesh/23655/2014(H5N1)
2.3.2.1a (Old)
MK046677 A/goose/Bangladesh/G1/2017(H5N1)
MG042329 A/duck/Bangladesh/31549/2016(H5N1)
KY635697 A/duck/Bangladesh/27820/2015(H5N1)
A/chicken/Bangladesh/Sak111/2019(H5N1)
MH791700 A/duck/Bangladesh/34751/2018(H5N1)
A/chicken/Bangladesh/Bha065/2019(H5N1)
MK045831 A/chicken/Bangladesh/C2/2017(H5N1)
A/chicken/Bangladesh/Sak063/2019(H5N1)
MK046084 A/Duck/Bangladesh/D5/2017(H5N1)
MK046682 A/Turkey/Bangladesh/TK1/2017(H5N1)
A/chicken/Bangladesh/Sak127/2019(H5N1)
A/chicken/Bangladesh/Sak126/2019(H5N1)
2.3.2.1a (New)
0.01
b
A/Chicken/Bangladesh/Sak127/2019(H5N1)
A/Chicken/Bangladesh/Sak126/2019(H5N1)
MK046679 A/Turkey/Bangladesh/TK1/2017(H5N1)
MK046081 A/Duck/Bangladesh/D5/2017(H5N1)
MK045828 A/chicken/Bangladesh/C2/2017(H5N1)
A/Chicken/Bangladesh/Bha065/2019(H5N1)
A/Chicken/Bangladesh/Bha063/2019(H5N1)
KY635471 A/duck/Bangladesh/27820/2015(H5N1)
MG042468 A/duck/Bangladesh/31549/2016(H5N1)
A/Chicken/Bangladesh/Sak111/2019(H5N1)
MH791572 A/duck/Bangladesh/34751/2018(H5N1)
H5N1 HA Clade 2.3.2.1a (New)
LC349404 A/duck/Mongolia/372/2010(H10N8)
KC881295 A/environment/Jiangxi/28/2009(H11N9)
KC986346 A/velvet scoter/Mongolia/883V/2009(H4N6)
LC349372 A/duck/Mongolia/29/2011(H3N8)
LC367447 A/duck/Mongolia/621/2010(H3N6)
LPAI
KM267857 A/duck/Bangladesh/14VIR1121-18/2013(H5N1)
H5N1 HA Clade 2.3.2.1a (Old)
A/dk/Yokohama/aq10/2003(H5N1)
A/ck/Hubei/wl/1997(H5N1)
A/HK/156/1997(H5N1)
A/ck/Hubei/wj/1997(H5N1)
A/gs/Goungdong/1/1996(H5N1)
A/munia/HK/2454/2006(H5N1)
FJ573465 A/Bangladesh/207095/2008(H5N1)
KF597841 A/chicken/Bangladesh/BL-470/2010(H5N1)
KF597834 A/chicken/Bangladesh/BL-4/2007(H5N1)
H5N1 HA Clade 2.2.2
A/BHG/Qinghai/65/2005(H5N1)
A/chicken/Bangladesh/11RS-1984-30/2011(H5N1)
A/chicken/Bangladesh/3012/2011(H5N1)
H5N1 HA Clade 2.3.4.2
A/ck/HK/947/2006(H5N1)
KF888499 A/chicken/Bangladesh/15079/2012(H5N1)
KF888519 A/chicken/Bangladesh/15083/2012(H5N1)
H5N1 HA Clade 2.3.2.1c
AB684162 A/chicken/Miyazaki/11/2011(H5N1)
KC616590 A/chicken/Bangladesh/12VIR-7140-2/2012(H5N1)
KC616589 A/chicken/Bangladesh/12VIR-7140-1/2011(H5N1)
KY629459 A/duck/Bangladesh/23655/2014(H5N1)
KY635781 A/duck/Bangladesh/24483/2015(H5N1)
H5N1 HA Clade 2.3.2.1a (Old)
0.02
c
PB2
PB1
PA
HA
NA
NP
M
NS
2.3.2.1a (Old)
2.3.2.1a with H9N2-like PB1
2.3.2.1a (New Reassortant)
Bha063/2018
Sak126/2019
Previously circulating clade 2.3.2.1a HPAI-like
Circulating H9N2-like
Other LPAI-like

9.

Fig. 1: Phylogenetic analysis of HPAI viruses from Bangladesh based on complete HA (a) and PB2 (b) gene sequences, and gene constellations of HPAI H5N1 viruses isolated in Bangladesh under clade 2.3.2.1a (c). Bangladeshi isolates are marked with filled circle (●).

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PURPOSEFUL REHABILITATION OF THE ENVIRONMENT AS THE PRECONDITIONS FOR RECOVERING BRUCELLOSIS

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The situation in the country concerning animal brucellosis, arising from the microbial contamination of the environment as the background, can be considered as the initial stage of the environmental disaster. In order to rectify the situation and prevent an ecological disaster, radical measures need to be taken coupled with the wholesale modernization of the livestock sector.

Introduction

Human welfare and care for humankind's bright future is the main calling of the modern civilized world. To achieve this, a plethora of efforts is undertaken today, actively involving the scientific potential of different public strata and the efforts of experienced specialists. The spiritual, moral and physical well-being and productivity of labor is decisively dependent on the maintenance of ecological freshness of specific spaces designed for human habitation, dwelling and publicly beneficial activities.
In human habitation environs, there are multifaceted and numerous species of flora and fauna. Each individual animal and plant is surrounded by countless microorganisms, many of which have a detrimental effect on them or, to put it differently, may cause disease upon introduction. Of these the brucellosis infection is particularly problematic due to its ethiopathogenetic and immunogenic complications. The work on the theoretical and practical issues of brucellosis is ongoing intensely at multitude of scientific, medical and veterinary laboratories of many countries throughout the world.

Current Situation on Brucellosis

In recent years brucellosis has created a very difficult epizootic situation, caused by the failure to apply, for years, measures against it. The agents (Brucella) bring about a disease in people whose complete and final recovery becomes virtually unachievable. Frequently people remain handicapped and disabled. The disease is characterized by particular severity in children, sometimes even with lethal outcomes. It is noteworthy that the economic loss from this disease to our country's economy is basically unrecorded. Brucellosis remains a social and economic problem today and there seems to be no blueprint for a real resolution in near future.

Environmental Area Contamination

According to the judgement of Georgian and foreign researchers, presence of Brucella microbes in environmental areas and the emerging risk they entail to humans is caused by the collection of many direct and indirect factors. (B. Cherkasky 1990; K.Kapanadze; T. Shamatava 1981; S. Indiytskaya 1978; B.Sadikov, 1980). Here the leading role is assigned to the direct factor, namely, to the afflicted and Brucella-carrying animals and products derived from them.
Contamination of areas surrounding animals with especially dangerous Brucella pathogens is engendered by the interplay of many, direct or indirect, factors present in the ways of transmission.
Observations throughout the years have shown us, that the complexity of the epizootic processes caused by Brucellosis in our country's livestock sector, among many other circumstances, is directly related to

the conditions in animal keeping and their use. From such circumstances, the most important from zoonormatic standpoint are: suitability of livestock holdings; animal density per unit area; veterinary, sanitary and zoohygienic conditions of buildings and its adjacent and surrounding territories. In this respect, utmost significance shall be attached to the creation of risk-free areas throughout the entire transhumance period. As of now, these problems in the livestock field remain unresolved. The problem is particularly acute at smallholder (household) farms, where the majority of the livestock population is concentrated (M.Lomineishvili, T.Gavasheli, 2002; M.Lomineishvili et al. 2005; V.Basiladze 2015) and at which administration of effective sanitation measures is practically unrealizable.

From epizootic and epidemiological viewpoints, the factor of food and raw materials of animal origin must be mentioned in the abovementioned context. One of the noteworthy nodes in the transmission of brucellosis and environmental area contamination and thus in the harmful impact on humans in our reality is milk and cheese produced from unripened (new, unsalted) unpasteurized milk. Brucella are spread through milk for a long period of time. They are secreted from sheep and goat udders via milk for a period of 7-8 months and in certain individual cows this may last up to 7 years (P. Vershilova 1972)

Theoretical analysis and practical observations show us that the process of environmental contamination with the brucellosis agents is a complex process and includes:

1. Secretion of microbes from the diseased animal and their-long term presence at different environmental objects;
2. Introduction of Brucella in food and raw materials of animal origin following which they constitute a source of infection;
3. Subsequent migration and implantation of microbes into other healthy animals (including wild fauna) or human organisms resulting in pathological abnormalities.

This cycle of development may continue indefinitely, if no sanitation of the environmental area is undertaken. The intensity of all of this is determined by social conditions of human life and by their economic interaction with animals. This, in turn, causes serious ecological disorders in the form of microbial contamination, very negatively impacting human and animal health and impeding their welfare.

The aforementioned mechanism of the infectious transmission and spread represents a closed epizootic chain consisting of three links (Brucella, environmental area and a susceptible animal) without "breaking" of which it is impossible to recover from Brucellosis. The real way of breaking this chain would be to abolish the "Brucellosis link", meaning performance of sanitation of the environment from microbes. This can be achieved through modernization of the livestock sector.

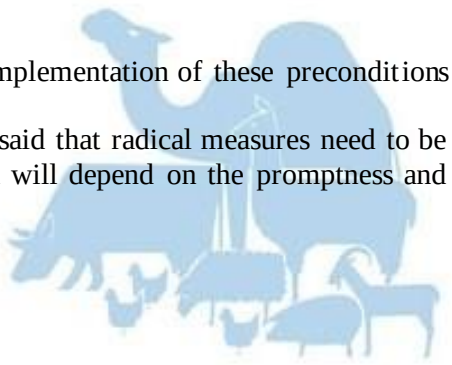
The inevitability of livestock sector modernization is primarily caused by the current complex veterinary-sanitary and hygienic conditions, as most animal holdings are irreversibly depreciated and have become an active source of infection.

To eliminate the brucellosis infection, the preconditions must satisfy a myriad of requirements, with the most worthy of mention being:

1. Recognition as a priority by the state and purposeful actions;
2. The feasibility of the objective;
3. Professionalism factor;
4. The guarantee of achieving the result – financial element.

Based on the analysis of the current situation, creation and implementation of these preconditions should be an achievable task in near future.

Therefore, by summarizing the presented materials, it may be said that radical measures need to be taken to eradicate animal brucellosis, the efficiency of which will depend on the promptness and completeness of modernization of livestock farms.



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PREVENTING TUBERCULOSIS DISEASE BY USING NUCLEAR TECHNIQUE

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Abstract

The viability of Mycobacterium tuberculosis strain DT (BKDT) against gamma radiation was studied to find the efficient and reliable lethal dose of the irradiation and determine the required dose to kill the micro-organism. In the study, 44 cultured bottles of the M. tuberculosis strain DT (BKDT) was exposed to a panel of 9 different doses from 1 kGy up to 8 kGy. The irradiated bacilli were subcultured onto MGIT as well as LJ tubes and the growth of the organism was checked by UV translumination and colony forming. All but one of the applied doses failed to kill Mycobacterium tuberculosis. We have shown that 8 kGy was potent enough to kill the organism and no biability was found at this dose.

Introduction

In 1900, Villard who was a French physicist physicist discovered gamma rays.
Gamma radiation is routinely used to inactivate viruses and in destroy bacteria medical products killing effects of UV on *Mycobacterium tuberculosis* has been identified years ago but there are not more studies on viability of Mycobacterial organisms irradiated by gamma rays. In this paper, we have assayed the use of gamma ray to kill this bacterium.

Material and methods

Bacterial strains

BKDT strain of *Mycobacterium tuberculosis* which is used in tuberculin production, was designated for the study.

Culture medium

The bacterium was cultured onto Dorset- Henley medium dispensed in 100 and 250 ml Bijou glass bottles. The inoculate were taken from 500 ml culture flasks containing 4 weeks old bacteria propagated on D-H medium. All the containers including of 36 small (20 ml) and 8 large bottles (200 ml) were inoculated with one loop full of bacteria and incubated for 8 weeks at 37 ^{oc}.

Irradiation

Gamma cell was used for the irradiation (gamma cell facility PX-30, Dose Rate = 0.65 Gy/Sec, Russian made). The gamma radiation was provided with 60 cobalt source (^{60} co). To perform irradiation, the bottles were transferred to the collaborator lab based at AMIRS. On reception they were set in 9 groups, three to five in each.
In each run, one group was exposed to gamma cell. After irradiation, the bottles were returned to the main Lab to perform sub- culture.

Recovery

On arrival all the irradiated bottles were sub- cultured onto one tube of MGIT (BBL-Difco, USA) as well as one slope of LJ medium (Merck, Germany). MGIT tubes and LJ slants were checked weekly for 16-24 weeks. Any change in tubes like colony forming were considered and checked.

Results

Altogether 34 culture bottles were irradiated. The bottles were divided into two groups. Table 1 shows details of the sets. The first set included bottles showed no growth at all in sub-culture indicating killing all the bacteria. This category depicted irradiation dose of 8 kg (Table). There were 2 bottles in this group. The second set consisted of bottles showed growth in some sub-cultured containers and no growths in others. This group consisted of 32 bottles.

Table: Effects of Different Gamma Radiation Doses on Mycobacterial Cultures

Irradiation dose (kGy)	2	2.5	3	3.5	4	4.5	5	5.5	6	6.5	7	8
Tota No . of Cultured bottles	4	1	2	3	4	3	4	3	1	1	6	2
Re-Cultured bottles with growth	1	1	2	3	4	3	3	2	1	1	0	0
Re- Cultured bottles with no growth	3	0	0	0	0	0	1	1	0	0	6	2

Discussion and Conclusion

One of applications of the nuclear technique is to control and prevent livestock baçterial diseases like mycobacterium tuberculosis. The lethal effect of gamma ray on micro- organisms has been identified in the past. Irradiation is an effective and safe technique that can prevent some food–borne diseases. Usually gamma rays with specific energies are produced by the spontaneous disintegration of radioactive isotopes or radioisotopes. We assume this method could be a concern in final steps of the PPD production process where we need to make sure there is no live mycobacterium in the product. In dairy industry, some recently published papers indicate surviving Mycobacterium paratuberculosis during pasteurization. M. Paratuberculosis is the causative agent of Johnes disease in ruminants and the suspected aetiology of Crohn's disease in human. Nowadays there are some debates on this issue that the classic way of pasteurization treatment is not potent enough to kill all the mycobacterial organisms particularly *M. paratuberculosis.* This is an important issue in terms of public health. We think gamma radiation may be utilized as a good alternative or supportive method in this respect. This is especially the case in areas where paratuberculosis is of high importance.

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DEVELOPMENT OF A MULTIPLEX REAL TIME POLYMERASE CHAIN REACTION ASSAY FOR DETECTION OF ZOONOTIC ABORTIVE AGENTS

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Abortions in livestock have great impact in the economy of many countries, mostly affecting animal health and productivity, as well as public health. In most cases, abortions do not present any premonitory signs making identification of the aetiological agent difficult. There are many techniques available for diagnosis of pathogens causing abortions. Generally, bacteriological isolation for these pathogens is hazardous, difficult, laborious and time-consuming (Baily *et al*., 1992; Bal *et al*., 1994; Maurin and Raoult, 1999; Limmathurotsakul *et al*., 2012). Serological tests exhibit considerable cross reactions (Godfroid *et al*., 2010; Dhama *et al*., 2015) and do not differentiate between natural infection and vaccination (Poiester *et al*., 2010; Horigan *et al*., 2011). Various molecular methods including end-point polymerase chain reaction (PCR) (Heinemann *et al*., 1999) and Quantitative PCR (q-PCR)/real-time PCR) have been have been developed and used over the years to detect pathogens causing abortions. Introduction of probe-based and fluorescent dye-based multiplex qPCR assays has greatly expanded the range of pathogens that can be detected in laboratories in a single PCR reaction to improve throughput (Sebastiani *et al*., 2018). However, probe-based qPCR assays require a labeled probe for each target to be identified, hence have high running costs.

In this study, a multiplex real time PCR for the simultaneous detection of four zoonotic abortive agents in sheep, goats and cattle; *Brucella* spp, *Leptospira* spp, *Listeria monocytogenes* and *Coxiella burnetii* using high-resolution melt (HRM) curve analysis technology was developed. The assay used dsDNA binding dye and primers that targeted the unique sequences in the insertion sequence (IS) element IS711 of *Brucella* spp, outer membrane, surface lipoprotein LiPL32 for *Leptospira* spp., IS1111 gene of *Coxiella burnetii* and ssrA gene in *Listeria monocytogenes*. Differences in fragment size and GC content were used as discriminating power. The designed primers were evaluated in monoplex and multiplex reactions using the plasmid harboring the target fragments for each pathogen as positive controls. Critical PCR parameters, such as primer concentrations, annealing conditions, heating and cooling conditions of the PCR products (temperature and time), reagents (amplification master mixes - analysis of several kits) were performed.

Our results showed that the assay that can identify each of the four different bacteria (*Leptospira* spp, *Brucella* spp, *Coxiella burnetii* and *Listeria monocytogenes*) based on differences in their melting temperature (Tm) of the PCR amplicons. Ongoing work involves validation of the assay and testing clinical cases.

Table 1 HRM primers designed

HRM primers	Primer sequence	Product size	GC content	Tm ˚C
BruHRM_F	*5'-AAGCCGGATAGAAGGCTTGA-3'*	101	54.1	83.0-83.2
BruHRM_R	*5'-CTGCATGCTGTTGTCGATG-3'*			
CoxHRM_F	*5'-AGGAGACACACCAACCGAGT-3'*	121	47.1	80.40-80.60
CoxHRM_R	*5'-GGTTGATGCTTATCGGGCTA-3'*			
LepHRM_F	*5'-CGGTTTAGTCGATGGAAACAA-3'*	77	43.2	75.6-75.8
LepHRM_R	*5'-GAACTCCCATTTCAGCGATT-3'*			
LisHRM_F	*5'-CGGTAACAGGCTTCCATTCA-3'*	93	42.9	77.4-77.6
LisHRM_R	*5'-GGGTCTCACTCTAAGTGGGCTA-3'*			

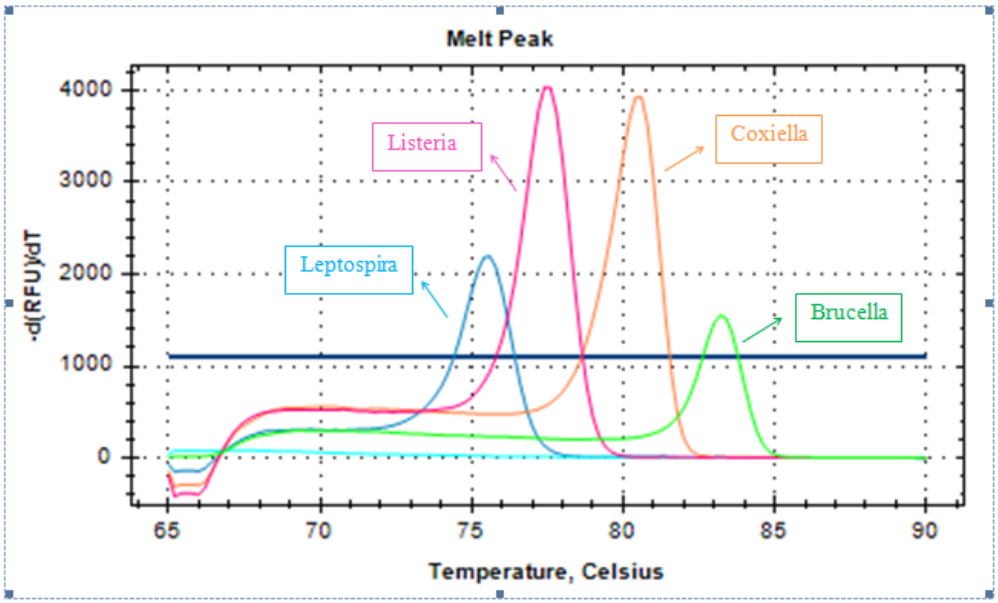

Figure1, showing the melting curve pattern of 4 different bacteria (Leptospira spp, Brucella spp, Coxiella burnetii and Listeria monocytogenes)

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ZOONOTIC TUBERCULOSIS: CHALLENGES AND OPPORTUNITIES FOR SRI LANKA

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Zoonotic tuberculosis is primarily caused by *Mycobacterium bovis.* This neglected topical diseases has relatively higher prevalence in developing countries than developed countries. Sri Lanka is a middle-income island nation in the Indian Ocean with a land extent of approximately 65000 km^2. Even though Sri Lanka is not among high disease burden countries for human tuberculosis, approximately 11,000 new cases are reported annually. Zoonotic tuberculosis has not been reported among humans in Sri Lanka. This may be due to underreporting as all acid-fast bacilli positive patients are routinely reported as having "bacteriologically confirmed TB" in Sri Lanka. However, we have recently reported the first two confirmed cases of tuberculosis among Sri Lankan elephants caused by *M. tuberculosis* strains that are similar to East-African-Indian lineage commonly circulating among humans in the country based on MIRU-VNTR typing. Similar findings have been reported from Nepal recently.

Our pioneering work confirmed bovine tuberculosis in Sri Lanka in 2012. Since then we have confirmed 18 fatal cases among Sri Lankan cattle. Most cases (n=15) were reported from Central province, which has most of the largescale dairy farms. Relatively high herd prevalence of reactive cattle was detected in twenty herds from the same province. One cattle carcass was condemned during meat inspection at a municipal abattoir. The affected animal was originating from North Western province. Remaining two cases were reported from a single farm in North Western province. No data is available on any wildlife reservoirs of *M. bovis* in Sri Lanka.

Genotyping of these 18 *M. bovis* strains revealed seven distinctive MIRU-VNTR patterns forming four clusters. The largest cluster had 9 strains; followed by three clusters which had two strains each. The remaining were single strains. Strains from Central province and North Western province clustered separately. Strains obtained from same farms shared similar MIRU-VNTR patterns except two cases. All nine strains in the largest cluster originated from two different farms in Central province and shared an identical MIRU-VNTR pattern. These two farms had a shared source of animals in the recent past. Similar results were observed from *M. bovis* strains originating form shared animal sources in other endemic countries such as Brazil, Tunisia and Mexico and Zambia. Interestingly, two orphan MIRU-VNTR patterns and two clusters each with two *M. bovis* strains were found in Central province. This indicates the occurrence of high genetic diversity among the circulating *M. bovis* strains in Sri Lanka and these findings are comparable with the results reported from other endemic countries. Physical distance between Central province and North Western province was reflected in the increased genetic distance between strains from these two provinces similar to previous observations from Mali, Nigeria, Cameroon, Brazil, Spain and South America Chad.

A subsequent preliminary survey undertaken in 2019 identified relatively low incidence of *M. bovis* infection among cattle processed at two abattoirs at Western and North Central provinces. *M. bovis* was

detected in 5.2% (6/115) of the lung samples by PCR while only one (0.87%) PCR positive sample had a granuloma. Previous prevalence studies from several endemic countries have reported >10% *M. bovis* in abattoir surveys. Complete cattle movement history records are not maintained by abattoirs in Sri Lanka. Therefore, this BTB incidence does not necessarily indicate actual disease burden in respective provinces. Most of the cattle processed at the abattoir in North Central province were from the free ranging herds that interact with wildlife at the boarders of the forests or even within the national parks These herds pose a potential risk for introducing *M. bovis* to wildlife or it may be an indication of carrying the infection from an already established wildlife reservoir.

The epidemiology and public health significance of bovine tuberculosis in Sri Lanka still remains largely unknown. A national level control program was initiated by the Department of Animal Production and Health since 2013. However, number of screening tests performed on live cattle in the country is not sufficient and the country lacks mechanism to compensate culled animals, seriously hampering the control efforts.

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WEST NILE VIRUS IN HORSES, PORTUGAL, 2016-2020

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Objective

This communication provides updated data about WNV circulation among horses in Portugal, pointing out the risk of human infections, in a One Health perspective.

Introduction

West Nile fever virus (WNV) is a mosquito-borne member of the family *Flaviviridae*, of the genus *Flavivirus*, included in Japanese encephalitis antigenic group. WNV enzootic transmission cycle involves mosquitoes as vectors and birds as amplifying hosts. Human and horses are dead-end hosts, since they develop low-level viremias (Komar et al 2003).
WNV is a neurotropic flavivirus for human and horses. The emergence, dispersion, and maintenance of a vector-borne pathogen are affected by the transmission efficiency, which depends on the convergence in time and space between competent vectors, competent vertebrate hosts, and the pathogen.
In European countries WNV activity is usually detected in late summer and early fall at urban sites near wetlands, where migratory birds and mosquitoes are concentrated at highest densities (Rappole and Hubálek, 2003).
WNV diagnosis usually rests on detection of IgM antibodies in horse serum or cerebrospinal fluid (CSF). A positive IgM test to WNV, in association with compatible clinical signs, confirms the diagnosis. Detection of WNV in field cases is hampered by the typically short duration and low level of the viraemia in horses.

Methods

Total RNA was extracted by using the BioSprint 96 workstation with the MagAttract 96 cador pathogen kit, according to manufacturer's instructions (Qiagen, Hilden, Germany). The samples were screened for WNV by real-time RT-PCR (RT-qPCR) targeting the NS2A gene (Barros et al. 2013). Equine sera were tested for WNV-IgM antibodies by capture ELISA (ID Screen West Nile IgM Capture ELISA, IDVET, Montepellier, France).

Results

Since 2016, twenty-three horses were diagnosed as WNV-positive on the basis of serological testing from a total of 165 horses, representing a sample positivity of 14%. One of the seropositive horses also tested positive by RT-qPCR. Although all animals described in table 1 had exhibited clinical manifestations compatible with WNV infection, some seropositive cohabitants did not developed any signs of disease. Follow-up information was available for eight horses. Four animals died or were euthanized, while the remaining four recovered from the disease.
With the exception of the last year, WNV infections occurred mainly during October through November. Also, most cases were detected in the south of the country, in predictable regions, close to wetlands and bird sanctuaries.

Year	Date	Sex	Age	RT-qPCR	IgM ELISA	Mortality
2016	August 26	Male	3	Negative (blood)	Positive	Yes
	September 8	Male	NA	Negative (blood)	Positive	NA
	October 7	Female	11	Negative (blood)	Positive	NA
	October 24	Female	3	Negative (blood)	Positive	Yes
	November 3	Female	2	Negative (blood)	Positive	NA
	November 8	Male	6	Negative (blood)	Positive	NA
	November 9	Female	10	Negative (blood)	Positive	NA
	November 18	Female	14	Negative (blood)	Positive	Yes
2017	October 3	Female	3	Negative (blood)	Positive	NA
2018	October 9	Male	26	Negative (blood)	Positive	NA
	November 9	Female	2	Negative (blood)	Positive	NA
	November 21	Male	12	Negative (blood)	Positive	NA
2019	October 14	Female	17	Negative (blood)	Positive	NA
	October 26	Female	10	Negative (blood)	Positive	NA
	October 27	Male	10	Negative (blood)	Positive	NA
2020	July 24	Female	1.5	**Positive (CSF)** Negative (blood)	Positive	Yes
	September 29	Female	10	Negative (blood)	Positive	NA

NA- data not available. Only the primary case from an outbreak is presented.

Conclusions and Relevance

From 2016 to 2020, the number of cases detected in horses has been 10, 5, 3, 3 and 2 per year respectively, reflecting the continued presence of the virus, and the need for awareness of the risks and recommended preventative measures by the horse owner.
West Nile virus is still considered a minor arbovirus in public health in Portugal, however countries with a temperate climate, such as Portugal, which anticipate important ecological changes due to future climate conditions, face a significant increase in risk for mosquito-borne disease emergences and outbreaks.
The systematic spatial and time scale of positive cases detected over the years would provide insights for the development of more efficient strategies for the surveillance, control and prevention of the infection.
Surveillance of WNV is therefore of utmost importance, since the identification of WNV cases in horses indicates the presence of pathogen and its vector in a given geographic area, reflecting the real risk of infection in human, a topic worthy of attention in the One Health perspective.

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ORAL DELIVERY PLATFORM FOR THE REDUCTION OF GLOBAL ZOONOTIC DISEASES

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Introduction

Zoonotic diseases are responsible for the death of 2.7M people, globally, a year, and the potential of the next "Disease X" remains a recurring threat. Orally delivered vaccines and therapeutics could address some, if not many, of these ongoing concerns, provided the platform has a high level of flexibility sensitive to differences in gut environment per species. This synopsis provides an overview of a platform approach that meets three critical criteria of targeted gut placement, immune response, and clinical relevance to challenge.

Platform Description

The described platform includes three distinct parts: 1) a physical substrate (i.e., consumable pellets, gelatines, or powders); 2) a bacterial expression system (expresses recombinant antigens or biologics); and 3) a chemical matrix-based enteric coating that may be configured per animal species and particular gut environment.
The development of the oral delivery platform for delivery of therapeutics and vaccines to improve animal health was developed with the specific goal to reduce the need for skilled veterinarians for administration, to reduce the cost associated with production, and to ultimately lead to wide-spread availability and implementation.

Targeted Gut Placement of Biologics

To determine release of biologics, an *in vitro* dissolution study of a recombinant OspA vaccine for Lyme disease was developed for mono-gastric animals and was incorporated into a chewable substrate. Simulation of the stomach, duodenum, and ileum showed the amalgam maintained structural integrity with predictable release of OspA antigen in the duodenum. The amalgam was completely dissolved at end of GI tract.
To test the platform's ability to orally deliver biologics in a ruminant (goat), rumen undegradable pellets were coated with barium sulfate incorporated into an enteric coating encapsulation chemistry for oral delivery. Solid delivery was compared to liquid barium sulfate administration using a gastric tube. Using X-ray imaging of the GI tract and the manure pellets, pellets and coating were shown to successfully bypass the rumen and release barium sulfate in the intestinal tract, with complete intermixing in the manure.
To show release from the encapsulation and successful uptake by the blood, Ivermectin was incorporated into the enteric coating. Using Ivermectin specific high-performancy liquid chromatography (HPLC) goat serum was tested at 6hr and 24hr post Ivermectin administration and showed successful uptake with serum concentrations following a dose-response curve. Solid administration led to a higher bioavailability of Ivermectin and a prolonged release profile.

Immune Response

The platform's ability to induce an immune response has been tested using a variety of different vaccines and species. Oral delivery to mice using the OspA antigen derived from *Borrelia burgdorferi*, induced consistent IgG responses and year-long protection against the causative agent for Lyme disease, *Borrelia burgdorferi*. Oral vaccination against *Eimeria* protects against parasites in poultry, parasites leading to coccidiosis and weight loss in chickens, showed significant reductions in gut lesions, increased weight gains, and decreased oocyst shedding. In goats, a proof of concept study using oral delivery of GFP induced strong IgG responses.

Sustainable farming

Anti-microbial resistance (AMR) remains a threat to sustainable farming. In response, an antimicrobial peptide was designed and expressed using a probiotic *Bacillus* carrier. *In vitro* trials showed that the peptide successfully inhibited both *E. coli* and *Eimeria* growth. Subsequent poultry trials showed complete protection from symptomatic disease in chickens infected with *Eimeria* parasites (compared to 20% body weight loss in untreated chickens).

Summary

The oral delivery platform as presented here is designed as a plug and play platform comprising of a bacterial carrier for expression of proteins and an enteric coating to protect these proteins from degradation in the hostile stomach environment. The platform can be adjusted for delivery in a variety of different species, and allows for quick incorporation of new proteins. The enteric encapsulation allows for long-term stability at high temperatures for ease of storage, and the oral delivery makes the therapeutics accessible to both smallholder and large industry farmers.

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DESIGNING SIRNA BASED ON NUCLEOPROTEIN GENE AGAINST THE INDONESIAN H5N1 VIRUS CLADE 2.3.2

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Introduction

The outbreak of the highly pathogenic avian influenza virus subtype H5N1 in Indonesia since 2003 has caused significant economic repercussions in the poultry sector as well as zoonotic threats for the public health sector. As the WHO report, the H5N1 human case in Indonesia up is 200 with 168 as fatal cases. Up to now, the treatment for the H5N1 infection has still used the neuraminidase inhibitors such as oseltamivir and zanamivir since most of the viruses are resistant to other antivirals such as adamantanes [1, 2]. However, several strains of influenza viruses undergo mutation causing resistance to the current antivirals [3, 4]. Thus, the RNA interference technology based on small interfering RNA (siRNA) provides an alternative for antiviral design, including influenza viruses [5]. Therefore, the objective of this study was to improve the siRNA based on the nucleoprotein gene (siRNA-NP) against the Indonesian H5N1 virus clade 2.3.2 that circulates and dominates in Indonesia in recent years.

Materials and Methods

The H5N1 virus

The H5N1 virus used in the study is the collection of the Indonesian Research Center for Veterinary Science, namely A/Chicken/Bali/NPD24/2017. The virus has been identified as the H5N1 virus by the RT-PCR tests based on the matrix, hemagglutinin and neuraminidase gene [6, 7]. The clade 2.3.2 classification was determined by the phylogenetic tree analysis of the hemagglutinin gene. Subsequently, the virus was propagated in the specific antibody-negative of 12 days old embryonated chicken eggs. The virus titer was calculated by the tissue culture infection dose 50/ml in the *Madin-Darby canine kidney* (MDCK) cells.

The siRNA-NP Design

The siRNA-NP was designed by software siDirect version 2.0 (http://sidirect2.rnai.jp/) using a consensus sequence of the nucleoprotein gene of the H5N1 viruses circulating in Indonesia from the NCBI Genbank database, namely siRNA-NP672 and 1433 (Table 1). The siRNA-NP1469 was included in this study as a control [8]. Subsequently, these siRNA-NPs were synthesized by the specification of the HP Custom siRNA w/o modification (QIAGEN, cat no 1027423).

Table 1. The siRNA-NPs designs used in the study

siRNA-NP	Orientation	Sequences
siRNA-NP672	Sense	UCCUCAAAGGGAAAUUCCATT

	Antisense	UGGAAUUUCCCUUUGAGGATG
siRNA-NP1433	Sense	UUUGACAUGAAUAAUGAAGTT
	Antisense	CUUCAUUAUUCAUGUCAAAGG
siRNA-NP1469	Sense	AUCUUAUUUCUUCGGAGTT
	Antisense	CUCCGAAGAAAUAAGAUCC

The In Vitro Challenge of siRNA-NPs

The effect of the siRNA intervention on the H5N1 infection was evaluated as an *in vitro* study in the MDCK cells. Briefly, the MDCK cells were grown in the Dulbecco's modified eagle medium (DMEM) at 37°C, 5% CO_2 with supplementation of fetal bovine serum, antibiotic and antifungal. The siRNA-NP (32 nM) was transfected into the MDCK cells using Lipofectamine®2000 (Invitrogen) and Opti-MEM (Gibco). Subsequently, after 24 hours the MDCK cells were infected with the H5N1 with MOI = 1. The analyses were performed at 24, 48 and 72 hours post-infection (hpi). The supernatant was harvested and evaluated for virus titer using the hemagglutinin test. On the other hand, the cells were analyzed for the expression level of NP gene.
The expression level of the NP gene was calculated by relative quantification using Livak's methods. The set primer for NP as target gene and γ-Actin as housekeeping gene was designed by the primer-blast software (The NCBI Genbank). The total RNA isolation was performed by Total RNA Minikit (Blood/Cultured Cell) (Geneaid, RB100), the cDNA was synthesized by ReverTra Ace® qPCR RT Master Mix with gDNA Remover (TOYOBO, FSQ30-301) and the qPCR was conducted by PowerUp™ SYBR™ Green Master Mix (Applied Biosystem, 4391178) in Applied Biosystem® 7300 Real-Time PCR systems. Statistical analysis was performed using IBM SPSS Statistic 21 to compare differences between treatment and control groups with significance $P<0.05$.

Results and Discussion

The result of these siRNA-NPs on the infection rate of the H5N1 virus (HA unit) in the MDCK cells is presented in Table 2 and Figure 1. All of three siRNA-NPs (siRNA-NP672, 1433 & 1469) reduce virus titer significantly ($P<0.05$) comparing with virus control. However, the siRNA-NP672 provides the greatest reduction of virus titer in 24, 48 and 72 hpi. Meanwhile, the siRNA-NP1433 and siRNA-NP1469 give more likely similar results on the virus infection in the MDCK cells, except in the 72 hpi where the siRNA-NP1433 has lower virus titer than siRNA-NP1469. Subsequently, at the cellular level the siRNA-NP672 causes the highest reduction of the expression level of mRNA transcript of NP gene comparing with other siRNA-NPs as well as control virus in 24, 48 and 72 hpi (Figure 2). The better performance of siRNA-NP672 on the reduction of the H5N1 infection level as an in vitro challenge in the MDCK cells could be occurred because of its target gene of silencing is located in the middle of gene segment with the highly conserved region. Moreover, the efficacy of the siRNA in the gene silencing could be also influenced by several factors, such as GC-content and secondary structure of mRNA [9-11].

Table 2. Reduction of virus titer (HA unit) by the siRNA-NPs

Treatment	**Virus Production (HA unit) at:**		
	24 h	**48 h**	**72 h**
siRNA-NP672 (n=3)	0.00	2.67	3.33
siRNA-NP1433 (n=3)	0.00	4.55	6.67
siRNA-NP1469 (n=3)	0.00	4.55	9.10
Control virus (n=3)	2.27	10.67	43.96

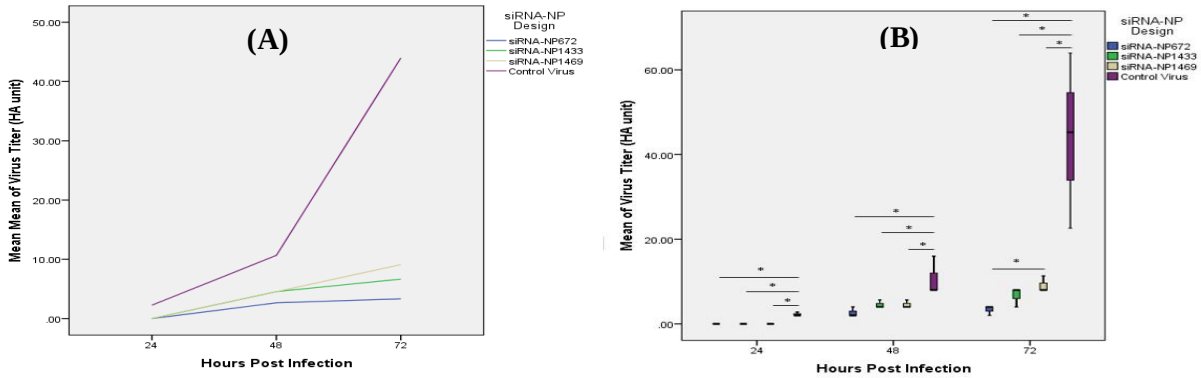

Figure 1. Reduction of the H5N1 production (HA unit) by the siRNA-NP 672, 1433 & 1469. (A) Graphical diagram (B) Boxplot diagram. The difference between the treatment and control groups was tested by the Kruskal-Wallis post hoc Mann Whitney U (*P<0.05)

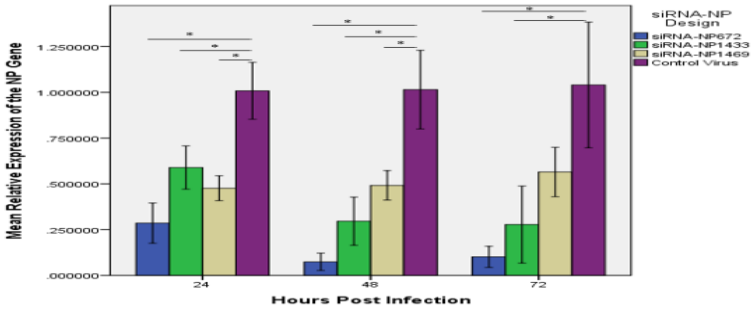

Figure 2. Inhibition of relative expression level of the NP gene by the by the siRNA-NP 672, 1433 & 1469. The difference between the treatment and control groups was tested by One way ANOVA post hoc Bonferroni (*P<0.05)

Conclusion

The study demonstrated promising therapeutic application of siRNA based NP gene in treating the Indonesian H5N1 virus clade 2.3.2, especially the siRNA-NP672 that exhibited the highest inhibition comparing with siRNA-NP1433 and 1469 as an in vitro study in the MDCK cells.

Acknowledgement: This study was funded by the research project of Indonesian Research Center for Veterinary Science 2018-2019. The authors acknowledge all people who support the successfulness of this study.

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IAEA-CN-281-305

CLIMATE CHANGE AND EMERGENCE OF RIFT VALLEY FEVER (RVF) VIRUS IN LOW RISK AREAS; PHYLOGENY, EPIDEMIOLOGY, KNOWLEDGE, ATTITUDES, AND PRACTICES IN THE POPULATION AT RISK

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RVF virus a *Phlebovirus* of the family *Phenuiviridae* and the order *Bunyavirales* is extremely pathogenic haemorrhagic virus. It's a mosquito vector-borne "disease" infecting human and animals, first isolated in 1931 in Kenya (Daubney et al., 1931) and causes outbreaks. The disease has a characteristic occurrence in inter-epidemic of 8-10 years as explosive epidemics (Breiman, 2010) and rampant after heavy "rainfall" especially in hotspots where past cases have been reported. RVF is of public health and economic importance hence necessity of carrying molecular epidemiology of the virus in recent reported cases occurring in areas considered low risk. Previous work on RVF has been in areas considered as high risks with reported outbreaks in the past (Ondiba et al., 2017, Ondiba et al., 2015). The distribution of RVF virus to new geographical areas results from abundance or availability of both primary and secondary vectors (Sang et al., 2017). Persistent heavy rainfall is considered a risk factor to RVF cases due to upsurge of the mosquito vectors; *Aedes spp.* and *Culex spp.*, slaughter of infected livestock, consumption of infected animal products.

RVF virus causes death in sheep and goats, mortality rate is high for the young lambs less than one month compared with the adult, which is 90% to 100% and 10% to 30% respectively. The cases of abortion in adult sheep is also high (Bird et al., 2009).

There is need to study the new emergence of RVF virus in these areas once considered to be of low risk and understand the phenomena behind this cases. Therefore, screening of trapped mosquito vector and suspect animals is required to check those harboring the RVF virus. Moreover, characterization of the mosquito vector and the virus strain using various molecular tools. The communities in areas currently reporting emerging RVF virus for the first time require sensitization and information on the disease (Sow et al., 2016). Scientific data is required to understand the strains of RVF virus present in order to develop proper prevention and mitigation measures. The disease epidemiology, community education, and multidiscipline collaboration is important. The steps required to investigate the RVF virus; performing descriptive epidemiology using time, place and person is important in characterizing the epidemic. Person characterization by age, sex, and occupation help in mitigating risks of exposure to RVF virus (LaBeaud et al., 2015).

The research questions answer the impact of rainfall patterns on distribution of RVF virus to new geographical areas and the strains emerging in low-risk areas and similarity to those found in high-risk areas. The aim is finding the incidence of RVF in mosquito vectors and suspect livestock in this areas after rainfall seasons, identify the species transmitting RVF virus, the epidemiology and characterize the strains (Millstone et al., 2016). Also, Understand the Knowledge, Attitude, and Practices in the population at risk.

Study areas with recent reported cases of RVF virus involving Livestock surveillance for Clinical case detection to detect RVF in susceptible livestock in case of reported abortion. Vector surveillance to establish RVF virus infections in mosquito in breeding areas and around animal sheds. The vector density, infection rates, diversity, and species will be analysed and profiled. Climatic/environmental surveillance of different meteorological data in this areas for longitudinal RVF infection studies and establish the relationships.

Laboratory confirmation helps with the right figures of the cases infected by RVF virus from the time the first suspect case is reported in the population. The epidemiology of RVF is compared and

reconciled with the laboratory findings and the environmental parameters (Bett et al., 2019). Also, bioinformatics analysis of the gene of interest done and sequences compared with those in the GeneBanks for the phylogeny analysis of the RVF virus.
The outputs are **i**nfection rates of RVF after the rainy seasons is available for animal and vector. The number of suspect, probable and confirmed cases of clinical RVF in livestock also available. Development of approximate vector population density, composition, and diversity, infection rates data. The meteorological data on rainfall, humidity, temperature, in this areas enrich future RVF mitigation plans. Generation of geo-referenced maps on areas considered to be RVF low risks in Kenya enriched with the climatic/environment weather forecasts during the surveillance period.
Key: Climate change, Rift Valley Fever (RVF) virus, low risk areas, Phylogeny, Epidemiology**,** KAP

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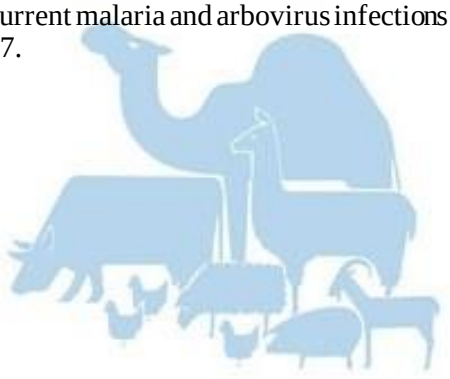

IAEA-CN-281-319

IDENTIFICATION OF H5 AVIAN INFLUENZA VIRUS FROM POULTRY FARM AND LIVE BIRD MARKET IN BANTEN, WEST AND CENTRAL JAVA, INDONESIA, 2018-2019

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Introduction

In Indonesia, the first outbreak by avian influenza virus was found to infect commercial laying hens, broilers, quails, ostriches, and domestic poultry in 200. The causative agent of the outbreak was confirmed as HPAI subtype H5 through field examination, clinical symptoms, pathological, immunohistochemistry, and RT-PCR. The spread of the AI virus took place so quickly, it was found in majority of Indonesia's provinces. Then in 2012, an HPAI H5 outbreak was found in ducks with high mortality rate. Apart from causing economic losses, AI outbreaks also caused human fatalities cases in Indonesia, with the highest mortality rate in the world. Live Bird Market (LBM) is also considered to be a place that plays a role in the transmission of AI viruses between poultry and humans as the discovery of AI viruses in several poultry markets in Indonesia . The lack of proofreading activity make AI viruses have the ability to evolute easily. The segmented genome of influenza viruses also allows genetic reassortment to occur when two influenza viruses infect the same cell. Due to the discovery of antigenic drift and antigenic shift in AI viruses in Indonesia, monitoring of Avian influenza virus circulation is necessary to find out the current situation of AI viruses in Indonesia This study aims to identify AI virus subtype H5 in Banten, West Java and Central Java in 2018-2019 so that it can be used as a reference for further research in controlling AI cases in Indonesia.

Methods and materials

Sterile cotton-tipped swabs were used for sampling and were subsequently stored in viral transport medium. The transport medium consisted of Dulbecco's Modified Eagle Medium (DMEM) with 1000 IU penicillin and streptomycin. The samples were immediately transported to the laboratory after collection and were stored at -70°C. Sample collection was taken from Banten, West Java and Central Java in 2018-2019. Sample representation was shown in Table 2.
The cloaca/environmental swab sample in the Dulbecco's Modified Eagle's Medium (DMEM) transport medium was extracted using QIAmp RNA mini Kit (Qiagen) according to the manufacturer's instructions. The extracted RNA was tested for H5 subtype by RT-PCR using H5-ID and H5-NLPI primers. The amplification results were visualized with UV trans-illuminator.
The cloaca swab, organ and environment swab samples that showed positive results of RT-PCR on the matrix primer, were then inoculated in embryonated specific pathogen free (SPF) eggs of 9-11 old days. Allantoic fluid is harvested and tested for rapid agglutination of 5% red blood cells (RBC), then confirmed with RT-PCR using H5 primer.

Results and discussion

We did a surveillance and collected specimen from poultry farms and live bird market, especially in Banten, West Java and Central Java in 2018-2019. Out of 146 samples from Brebes district, 52 were tested for RT-PCR influenza A viruses, 15 (28.8%) were positive for influenza A viruses, including 8 (15.4%) that were influenza A/H5 positive. In Tegal district, from 112 samples 38 were tested for

influenza A viruses, 2 (5.2%) were positive for influenza A viruses, including 1 (2.63%) that was influenza A/H5 positive. Out of 89 samples from Sukabumi district, 23 were positive for influenza A viruses (25.8%), including 1 (1.1%) that was H5 positive. In Cianjur District, out of 82 samples, 3 were positive for influenza A viruses (3.6%), with no positive samples for H5. In other side, out of 10 samples from Serang, Banten, 7 were positive for H5 (70%), without influenza A viruses test.

The results showed that the poultry farm and live bird market (LBM) in Banten, West Java and Central Java in this study were contaminated by the Flu A virus (Table 1), although not all samples could be confirmed as influenza A/H5. Unsuccessful detection of influenza A/H5 virus is likely due to circulating of AI virus with gene mutation, particularly the mutation that coincide with the primer design so that the H5 primer used in this study cannot amplify the H5 gene in the sample. Furthermore, failure to identify the influenza A/H5 virus can be caused by the differences in virus subtypes, so virus subtyping seems necessary to do.

In table 1, it can be seen that various types of poultry are found on LBM. The LBM is considered as an ideal environment for genetic mixing and transmission of AI virus because it reservoirs, waterfowl, are sold together with other poultry. In Asia, several subtypes of influenza A viruses are simultaneously found on LBM. This allows poultry to be infected by several subtypes or clades (co-infection) and produce virus progeny with antigenic shift (*reassortant*). In poultry farms, mutations can occurred by immunological pressures from the vaccination process [10].

HPAI H5N1 virus in Indonesia has experienced antigenic drift since 2006 with estimated 1% of amino acids changing every year [10]. The mutation of the AI virus causes many unsuccessful identification of influenza A/H5 virus in the field. Because of the ability AI virus mutation, It is important to evaluate the appropriate methods used for the detection and diagnosis of AI virus in the field such as designing new H5 primer based on current circulating AI virus, subtyping viruses and genome sequencing. For this reason, further investigation is needed to determine mutations that occur in AI viruses.

Conclusion

The study concludes that influenza A/H5 virus is still circulating in Poultry Farm and Live Bird Market in Serang, West Java and Central Java, except in the Cianjur District and cloacal swab sample from Tegal District. The reason for unsuccessful influenza A/H5 detection in samples can be caused by mutation or circulation of various subtypes of AI viruses. Designing new H5 primer based on current circulating AI virus, subtyping AI viruses, and genome sequencing of AI viruses are needed to get a better diagnostic test result

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Table 1. RT-PCR Flu A and H5 test results

No	Location	Sample	Number of Samples	Number of sample for Flu A Test	RT-PCR Positive Result	
					Flu A	H5
1	Brebes District	Cloacal Swab (Pool)	**111**	**36**	**10**	**4**
		Organ (Individual)	**6**	**3**	**3**	**2**
		Environmental Swab (Individual)	**29**	**13**	**2**	**2**
2	Tegal District	Cloacal Swab (Pool)	**96**	**32**	**1**	**0**
		Environmental Swab (Individual)	**16**	**6**	**1**	**1**
3	Sukabumi District	Cloacal Swab (Pool)	**89**	**89**	**23**	**1**
4	Cianjur District	Cloacal Swab (Pool)	**82**	**82**	**3**	**0**
5	Serang, Banten	Cloacal Swab (Pool)	**8**	-	-	**6**
		Organ (Pool)	**2**	-	-	**1**

Table 2. Sample Representation

NO.	CITY	LOCATION	SPECIES
1.	Tegal District	Farm1	Layers
		Farm 2	Layers
		Farm 3	Ducks
		Farm 4	Ducks
		LBM	Domestic Chickens, Geese, Muscovy Duck, Broilers, Environment
2.	Brebes District	Farm 1	Layers
		Farm 2	Layers
		Farm 3	Ducks
		Farm 4	Ducks
		LBM	Domestic chickens, Layers, Duck, Muscovy Duck, Environment
3.	Sukabumi District	Farm 1	Domestic Chickens, Layers
		Farm 2	Layers
		Farm 3	Ducks
		Farm 4	Ducks, Muscovy Ducks
		LBM	Domestic Chickens, Ducks, Muscovy Ducks,
4.	Cianjur District	Farm 1	Domestic Chickens, Ducks, Muscovy Ducks
		Farm 2	Domestic Chickens
		Farm 3	Domestic Chickens
		Farm 4	Layers
		LBM	Domestic Chickens, Ducks, Muscovy Ducks, Layers
5.	Serang, Banten	Farm 1	Domestic Chicken

IAEA-CN-281-320

ASSESSMENT OF INTERVENTIONS EFFECTIVENESS AND MOLECULAR EPIDEMIOLOGY OF RABIES TOWARDS ELIMINATION BY 2030 IN ENDEMIC COUNTIES IN KENYA-DIRECTORATE OF VETERINARY SERVICES

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Abstract

Rabies is one of the neglected tropical zoonotic diseases. The disease is estimated to cause 60 000 human deaths annually in over 150 countries, with 95% of cases occurring in Africa and Asia. Ninety nine percent (99%) of rabies cases are dog-mediated and the burden of disease is disproportionally borne by rural poor populations. (Rabies Fact Sheet 2017) In Africa an estimated 21,476 human deaths occur each year due to dog-mediated rabies. In Kenya it is estimated that 2000 human deaths occurs each year due to rabies, Kiambi S (2013), Thumbi M, *et al* 2016). Human rabies cases in Kenya are laboratory confirmed, but mostly diagnosed on clinical grounds only, Kariuki D.P *et al.* (1983), Kiambi S (2013). Due to widespread underreporting and uncertain estimates, it is likely that this number is a gross underestimate of the true burden of disease. Kenya has ranked dog- mediated rabies as one of the top five priority zoonotic disease to be eliminated by 2030. Other countries have demonstrated success in elimination of the disease. Mass vaccination of dogs is one of the most cost effective interventions in control, reduction and elimination of dog rabies and eventually human rabies, Deborah J et *al*, (2018). Kenya has strategy to eliminate human dog mediated rabies by 2030. The strategy has three main interventions; sustained mass dog vaccination, public awareness and pre and post-exposure prophylaxis (PEP) until the country is free of human dog-mediated rabies, Kathleen C et al, (2014), Scott Preiss et al, (2018) and Maganga S et al (2014). The Central Veterinary Laboratories-Molecular Laboratory Intends to assess the effectiveness of the interventions being employed and document the targets met. The findings will be of benefit to the country and will be instrumental in further informed decision making and implementation of the rabies strategic plan in the country. Historical and current rabies samples at the Directorate of Veterinary Services will be screened by PCR and positive samples sequenced to generate more information and understanding on the circulating rabies virus in endemic areas in Kenya

Current and the Way Forward

Rabies cases in Kenya are laboratory confirmed by FAT, but mostly diagnosed on clinical grounds, the negative samples are not farther interrogated, vaccinated animals are not screened for antibody levels and the rabies virus in Kenya has not been sequenced The Central Veterinary Laboratories-Molecular Laboratory plans to screen about 1000 current and historical samples (2012 to date) rabies sample by PCR and Sequence the positives. Implementation of rabies elimination strategic plan has started in two pilot areas of Makueni and Siaya County, Kathleen C et al, (2014). The Central Veterinary Laboratories-Molecular Laboratory plans to screen 400x2 dog serum samples for antibody levels against rabies in the vaccinated population. This will monitor the attainment of 70% immunity with at least 0.5IU/ml of blood, Rabies Fact Sheet (2017). Questionnaires and check list will be used to assess the level of rabies awareness in the dog owners and availability and the uptake of the PEP in the designated local health facilities.

The Central Veterinary Laboratories-Molecular Laboratory will analyzed the data to indicate the level of antibodies in the mass vaccinated populations, rabies awareness and the PEPS uptake The Central

Veterinary Laboratories-Molecular Laboratory findings will be of benefit to the country and will be instrumental in further informed decision making, adoption of PCR as an alternative test after FAT especially the negative samples to ascertain true negatives and implementation of the rabies elimination strategic plan in the country. Historical and current rabies PCR positive samples at the Central Veterinary Laboratories of the Directorate of Veterinary Services will be sequenced to generate more information and understanding on the circulating rabies virus in endemic areas in Kenya. Current scientific knowledge indicates that rabies is preventable and controllable. Rabies qualifies WHO criteria for priority disease for control. Other regions have eliminated rabies by applying the interventions being investigated by the Central Veterinary Laboratories-Molecular Laboratory in the region. Dogs are vaccinated and no post vaccination Sero-monitoring is carried out in Kenya. The Sequence of the rabies virus circulating in the endemic areas of Kenya is not determined. The Central Veterinary Laboratories-Molecular Laboratory will address the genomic character of the virus. To-date rabies is endemic in Kenya; the magnitude of the disease is masked by the poor surveillance system for rabies disease.

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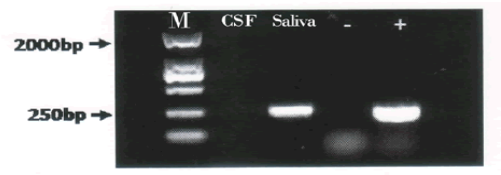

Figure 3. Results of RT-PCR showed positive for rabies virus in saliva sample M, DL2000 marker (Takara Inc.); CSF: Cerebrospinal Fluid sample (negative); Saliva, saliva sample (positive); -, negative control; +, positive control.

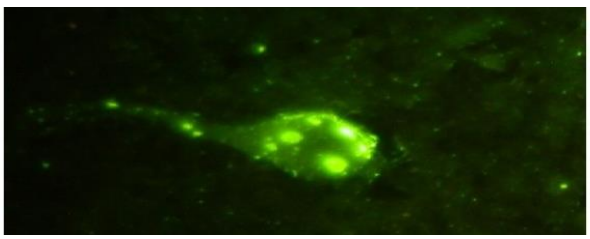

Figure 2: FAT Rabies Positive Slide

Figure 3: Rabid Laboratory Mice

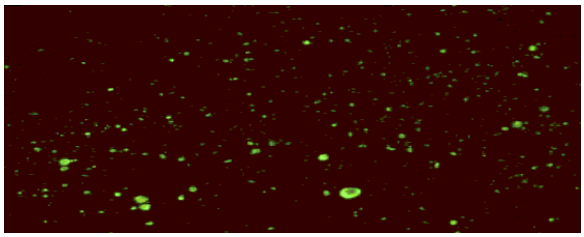

Figure 5: Rabies Positive Slide

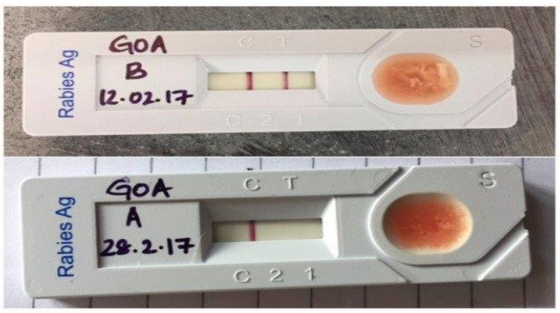

Figure 7: Rabies Antigen Rapid Test

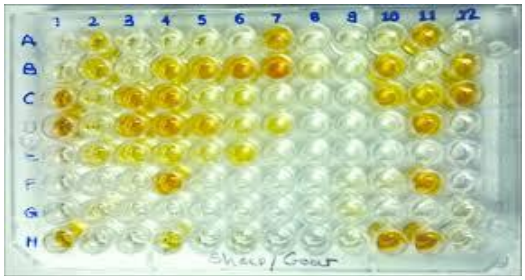

Figure 9: Rabies ELISA Test

Figure 4: Normal Laboratory Mice

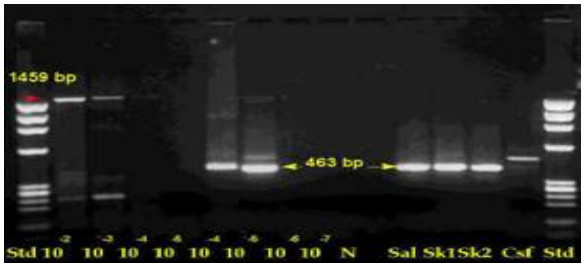

Figure6: Rabies Positive Titration Conventional PCR

Figure 8: Rabies Antigen Rapid Test Kit

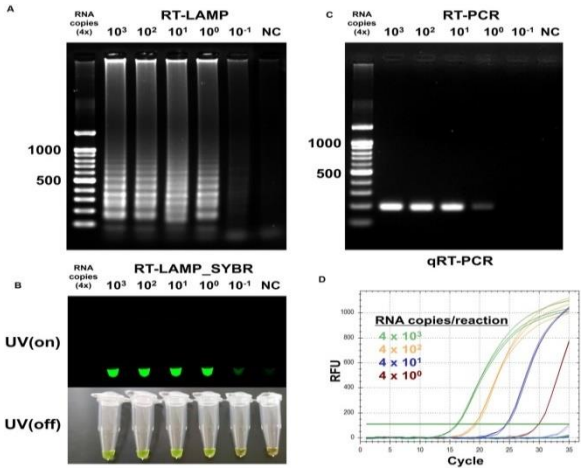

Figure 10: Rabies RT-LAMP Real-time PCR

IAEA-CN-281-327

WEST NILE DISEASE IN TUNISIA: INTEGRATED ANALYSIS OF HUMAN-ANIMAL-VECTOR SURVEILLANCE APPROACH SINCE 2010

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Keywords: West Nile Virus, zoonoses, Tunisia, Risk areas, sentinel chickens, entomological surveillance, integrated approach.

West Nile virus (WNV) is a mosquito transmitted *Flavivirus* belonging to the Japanese encephalitis antigenic complex of the Family *Flaviviridae*. West Nile Disease (WND) is a zoonotic infection with a complicated transmission cycle. In the last decades an increasing number of cases of West Nile infection in humans have been notified in Tunisia and in the Mediterranean basin. Since 2010, Tunisian Veterinary institutions (CNVZ, IRVT) and Human Public Health Authorities (DSSB) with the cooperation of IZS Abruzzo e Molise, Italy, enforced an integrated surveillance plan aiming to early detect the WNV spread and to activate control measures to reduce the risk of human transmission in Tunisia. Based on the analysis of climatic and environmental conditions found in the locations where human cases have been reported, suitable areas for WNV circulation in Tunisia are identified. Remotely sensed climatic and environmental variables together with migratory bird settlements and water bodies distribution have been analysed to investigate potential risk factor associated to the WND spread in Tunisia. In the risk areas, chickens were used as sentinel animals for early detection of viral circulation, whereas mosquitoes were tested for the presence of the virus by molecular methods. A serological surveillance in horses is conducted as part of an active surveillance programme. The integrated approach of WND surveillance in Tunisia has demonstrated a good efficacy to characterize the high risk areas and to understand the dynamic of the spatial and temporal epidemiological situation. The current and the future distribution of WND in Tunisia is determined. The use of sentinel chicken also demonstrated its capacity for early detection of WNV circulation prior to the occurrence in humans and for the phylogenetic characterization of the virus. Results of integrated human-animal-vector surveillance presented provide a comprehensive description of WNV activity in Tunisia and in the Mediterranean basin and will facilitate proactive public health measures to prevent or mitigate potential outbreaks. This cooperative work conducted during about 10 years underlines the importance of the cooperation North-South involving two or more countries around the Mediterranean basin allowing to share knowledge, expertise and resources to meet the goals of controlling disease.

ENHANCING LIVESTOCK'S CONTRIBUTION TO ONE HEALTH AND THE SDGS

IAEA-CN-281-123

GLOBAL TRENDS OF USING ANTIBIOTIC GROWTH PROMOTERS AND ALTERNATIVE STRATEGIES TO COMBAT AMR AND SUSTAINABLE ANIMAL PRODUCTION

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Abstract

A growing global concern of antibiotic use in animal feeds due to its potential adverse effects on animal and human health, food safety and the environment has led to a complete ban or restricted use in some countries, and, at the same time, expanding options for the use of alternative feed additives. Multiple, rather than a single additive may replace antibiotic growth promoters (AGPs) in animal. Blending of feed additives and hygienic farm management, vaccination and biosecurity may help achieve good intestinal health, stabilise enteric ecosystems and result in sustainable and cost effective production performance of birds. Moreover, controlling unsolicited ingredients at the production level must have the support of different markets responsible for the supply of safe and quality animal products for consumers. This requires the further increase and diversification of value added animal products and the expansion of their markets through strategic planning and gradual limitation of live bird markets in the country. More research is warranted in order to explore suitable, reliable and cost effective alternatives to AGPs for commercial use, and strategic animal value chain development.

Keywords: Animal production; AGPs; global trends; alternatives strategies, feed management and food safety

Introduction

Globally antibiotic use in animal feeds has been practicing since middle of the last century. They are used both at therapeutic and sub therapeutic levels to promote growth and feed efficiency. However, judicial use of antibiotics in food animals for growth promotion and disease prevention has been controversial for a long time due to antimicrobial resistance (AMR) in animals and humans, resulting in treatment failure when needed (Marshall and Levy, 2011). This problem has also been increasing day by day due to the misuse of antibiotics in animal feeds. Evidence shows that antibiotic resistant genes can be transmitted from animal to human microbiota (Greko, 2001). As a result, every year there is a huge economic loss due to the medical cost of less effective antibiotics for human health. Recently, a report from the Europian Union (EU) indicated that about twenty five thousand patient died each year from infections by drug resistant bacteria, which is equivalent to €1.5 billion of hospital cost (Ziggers, 2011). This report indicates the seriousness of the problem throughout the world. On the other hand, about 90% of antibiotics given to livestock are excreted into the environment and may be a source of pollution (Marshall and Levy, 2011). It is documented that antibiotic resistance bacteria can transmit directly, and indirectly through the food chains, air, water, and soil. In addition, some antibiotic drugs have carcinogenicity and genotoxicity effects in human health (Cogliani et al., 2011). As a consequence

of public health safety concern, several countries have banned or restricted the use of human health related antibiotics in feed.
Recently, a number of possible alternatives to antibiotic growth promoters (AGPs) are used. Some of the alternatives may include significant changes in husbandry practices or the strategic use of enteric microflora conditions, including acidifiers, probiotics, enzymes, algae and herbal products, microflora enhancers, and immuno-modulators. However, every alternative have some limitations and no one fully act as AGP replacement for sustainable growth and production of animals. As a result, the animal industry is now facing a great challenge to maintain production performance of animals due to increased feed costs for using alternative supplements and the restriction of antimicrobial use in feeds. Therefore, the aim of this paper is to review restrictions on the use of AGPs in animal feeds accrued over time globally, their residual impacts, and efficacy of feeding of their potential alternatives on animal production.

Current status of restriction on antibiotic uses in animal feed

The restriction of antibiotic uses in animal feed is a controversial global issue. A recent survey of 128 countries conducted by Alltech reviewed the growing restrictions on the use of antibiotics as growth promoters. This survey focused on the 59 countries where restrictions exist or are likely to be implemented including 28 from the EU and the top seven countries in terms of livestock production. Sweden is the first country in the world and South Korea is the first country in Asia where antibiotic use in feeds was completely restricted.
In addition, .the US, Canada, Mexico, Japan, Hong Kong, China and India have limited the use of antimicrobials in feed. In October 2010, Bangladesh also imposed a complete ban of AGPs in animal feed through the Fish and Animal Feed Act- 2010 and Animals Feed Rules-2013. Some other countries have limited requirements to obtain veterinary prescriptions for using antibiotics in food animals. Among these countries, Australia, Brazil and Ukraine do not have any formal national restrictions on antimicrobial use for the purposes of growth promotion. Therefore, it is noted that restriction on the use of AGPs in food animal production is expanding and some countries still have been observing the situation and looking for alternatives of AGPs. So, more research is needed to find reliable and cost effective alternatives in animal agriculture. However, a strong monitoring, supervision and quality control system are required to be imposed at industry, the market and at different desks between field and market to ensure the AGP free animal feeds in Bangladesh. Moreover, up gradation and diversification of value added animal products and their market required to be expanded considering biosecurity and food safety measures in the country.

Feeding antibiotic and its mode of action in animal

Dietary antibiotic in animal

The use of antibiotic in animal feeds has many benefits. It improves animal welfare and food safety by maintaining animal health and reducing certain pathogens. It reduces animal production costs and economic benefits are distributed along the food chain, including the feed industry, production of animal agriculture, food processors, retailers, and consumers. Most of the cost savings of antibiotics attributed to improved feed conversion, and this response is the highest in fast-growing genetically improved animals reared in intensive production systems. Other cost savings come from faster growth rate, reduced mortality, high resistance to disease challenge, improved reproductive performance, and improved manure and litter quality.
Rosen (1995) reviewed a total of 12,153 feeding trials conducted on animals by using AGPs and concluded that 72% AGPs gave positive response on animal performance. The magnitude of responses was dependent upon the type of animal management, disinfection procedures, age of the farm buildings, and quality of the feed. Finally, the use of antibiotic growth promoters has a positive impact on two important issues of animal agriculture, such as animal welfare and environmental stewardship. Animal welfare is definitely improved in animals that are healthier due to the disease-suppressing effects of

antibiotics. The improved utilization of dietary nutrients by supplemental antibiotics results in significant reduction in nitrogen, phosphorus, and other nutrients excreted into the environment.

The mode of actions of antibiotics

Antibiotics are natural metabolites of fungi that inhibit the growth of bacteria. They work by altering certain properties of bacterial cellular metabolism resulting in impaired growth or death. Some antibiotics interfere with the building and maintenance of the cell wall of the organism, while others interrupt proper protein translation at the ribosomal level. Because of their elevated rate of growth and proliferation, bacteria are vulnerable to antibiotics that target active cellular metabolism. Limiting the growth and proliferation of certain bacteria and inhibiting the production of various toxins restricts the influence that the microbe has upon the host organism. This enables the host to grow and perform better than grown under normally challenged conditions.
Antibiotics limit the growth of detrimental microbes, such as Clostridium perfringens. They also limit the growth and colonization of numerous non-pathogenic species of bacteria in the gut, including lactobacilli, bifidobacteria, bacteroides, and enterococci (Tannock, 1997). Antibiotics reduce the production of antagonistic microbial metabolites, such as ammonia, which adversely affect the physiology of the host animal. However, sub therapeutic levels of antibiotics in the diet also reduce the weight and length of the intestines. It is documented that, a thinner intestinal epithelium in antibiotic-fed animals may enhance nutrient absorption and reduce the metabolic demands of the gastrointestinal system. The minimization of gastrointestinal bacteria may also ease the competition for vital nutrients between the bird and the microbes (Ferket, 1991). Finally, antibiotics may reduce the adverse effects of immunological stress on growth performance by lowering the enteric microbial load.

Alternative strategies to AGPS in animal diets

Numerous feed additives have been proposed as viable alternatives to AGPs in animal diets (Table 1). The use of compounds that have antimicrobial effects is one way to improve intestinal health, immune response, and animal performance in the absence of AGP. Antibiotics works for decreasing the microbial load in the intestinal tract, resulting in a reduction of energy and protein required maintaining and nourishing the intestinal tissues; thus, more nutrients are partitioning for growth and production. By contrast, most natural feed additives do not reduce overall microbial loads, but, they alter the intestinal microflora profile by limiting the colonization of unfavorable bacteria and promote the activity or growth of more favorable bacteria. Antibiotic growth promoters alternatives modulate the gut health by several possible mechanisms; altering intestinal pH, maintaining protective intestinal mucins, selection for beneficial intestinal organisms or against pathogens, enhancing the fermentation volatile short-chain fatty acids; enhancing nutrient uptake, and increasing the humeral immune response (Ferket, 2003).
The principal mode of action of these supplements can be divided into four basic strategies includes; a) direct reduction of pathogens b) stimulation or introduction of beneficial bacteria c) improvement of nutrient utilization by the host and d) stimulation or modulation of the immune system of bird. Within these general categories there are about hundreds of commercial products available claiming to be as effective to improve growth performance, animal welfare and health. However, an alternative strategy must need comparable economic return and sustainable production efficiency if it is to be accepted for commercial use.

Table 1. Summary impacts of different alternatives of AGPs and their effects on animal production

Alternatives	Effects on animal	References
Organic acids and acidifiers	Lowering the pH of intestine; improves weight gain and feed efficiency and meat quality; increases the immune characteristics and number of lactic acid	Malheiros and Ferket, 2010; Emami et al., 2013; Kim et. al, 2014

	bacteria in the intestine; decreases the number of *coliform* bacteria.	
DFM or Probiotics	Increases the plasma immunoglobulin levels, decreases *E. coli*, and improves gut health; increase beneficial micro organism and decrease pathogenic organism; Improve feed intake & efficiency and weight gain; improves animal production; reduce mortality and stimulates immunity	Schwab et al, 1980; Jin et al., 1997; Dhama et al, 2011; Liu et al., 2012; Salim et al., 2013
Prebiotics	Stimulate the growth of non pathogenic bacteria; provides substrates for the bacterial fermentation in lower guts for host; stimulate immunity and neutralize toxins; inhibit colonization of pathogenic bacteria; provide energy and other limiting nutrients for intestinal mucosa; enhance growth and feed efficiency of animal	Roberfroid, 2007; Konstantinov et al., 2003; Rastall et al., 2005; Lan, 2004; Matteuzzi et al., 2004
Enzymes	Improve fiber, P and NSP digestion and utilization; increase intestinal viscosity; increase endogenous nitrogen flow and bacterial fermentation in the GI tract; improve growth and feed efficiency	Lyons, 1993; Choct et al., 1996; Choct et al., 1999;Yin et al., 2004; Ao et al., 2009
Essential oils and Plant extracts	Improves weight gain, immune characteristics, and the colonization of *Lactobacillus* in the intestines; improves digestive tract health and growth performance; reduce concentration of *C. perfringens* in the intestine; inhibit growth of pathogenic microorganism like *E. coli, Salmonella Spp.* and/or *Clostridium spp.*	Lee and Ahn,1998; Ross et al, 2001; Kang et al., 2013; Salim et al., 2018
Vitamins, minerals, electrolytes, and other feed supplements	Improves feed utilization and immune response, reduce the stress, acts as antioxidant and influence on intestinal microflora, improves growth and carcass quality of animal	Visca et al., 2013; Ahmad et al., 2005; Salim et al., 2008; Salim et al., 2011; Salim et al., 2012

DFM, Direct fed microbials; AGPs, Antibiotic growth promoters; NSP, Non starch polysaccharides

Dietary Organic acids and acidifiers

Several organic acids have strong bacteriostatic effects and they have been proposed as Salmonella-control agents in feed and water supplies for livestock and animal (Ricke, 2003). The most common organic acids in animal nutrition are citric acid, propionic acid, butyric acid, fumaric acid, formic acid, phenyllactic acid, benzoic acid and lactic acid. Generally, lactic acid bacteria are able to grow at relatively low pH, which means that they are more resistant to organic acids than more pathogenic species. However, the use of organic acids has not gained as much attention in animal production, partly because limited positive responses in weight gain and feed efficiency (Langhout, 2000). By contrast, Vogt et al. (1982) reported a positive influence on either feed efficiency or growth performance by dietary supplementation of fumaric acid, propionic acid, sorbic acid and tartaric acid in broiler diets. In addition, organic acids may improve animal performances by increasing the absorption of available nutrients, and reducing the toxic bacterial metabolites, the incidence of subclinical infections and secretion of immune mediators.

On the other hand, organic acids have been used as food additives and preservatives to prevent the growth of microorganisms and extend the shelf life of processed food. In addition to preservation, various organic acids are used for different technical purposes in the animal feed and human food industries such as; acidifiers, antifungal agents, antioxidants, flavor and pH modifiers. However, some organisms are becoming increasingly resistant to some organic acids similar to antibiotic resistance.

Therefore, in the animal diets, it is wise to use new organic acids which have antimicrobial and immunomodulation activity as well as being safe for human health. Very recently one of our study, found that dietary supplementation of phenyllactic acid (PLA) improved growth and feed efficiency of broiler chickens. We also found that PLA supplementation increased the immune characteristics and the number of lactic acid bacteria, decreased the number of coliform bacteria, and improved the meat quality attributes of broiler chickens. Finally, it was concluded that dietary PLA could be a viable alternative to antibiotics in broiler diets (Kim et al., 2014).

Supplemental direct-fed microbials (DFM)

Direct-fed microbial (probiotic) is a source of live beneficial microorganisms, has been used as an effective alternative to antibiotics in the animal feed industry over the last few decades due to its diversified function on animal health and productivity. In general, *Lactobacillus*, *Bifidobacterium*, *Bacillus*, *Enterococcus*, *Lactococcus*, *Streptococcus* and *Saccharomyces cerevisae* are frequently used as DFM in the animal feed industry. These microorganisms may influence the intestinal microbiota as well as host health and welfare in different ways, such as competitive exclusion of pathogenic bacteria, lowering the pH through acid fermentation, competing for mucosal attachment and nutrients, producing bacteriocins, stimulating the immune system associated with the gut, increasing production of short-chain fatty acids, increasing epithelial integrity, reducing epithelial cell apoptosis, and stimulating the intraepithelial lymphocytes (Ng et al., 2009).
Recently, DFM has received special attention from the aniaml industry to promote the balance and quality of the intestinal microflora for the host, but the efficacy of these products varies according to their production procedure and practical application. Several researchers reported that feeding DFM had improved the growth performance of broiler chickens (Mohan et al., 1996; Yeo and Kim, 1997) and egg production of laying hens (Nahashon et al., 1994). By contrast, other researchers didn't find any positive effects of using dietary DFM on growth performance of broiler chickens (Lee et al., 2010) and pigs (Shon et al., 2005). The inconsistent results might be explained by limited species of microorganism added to animal diets as DFM. It is hypothesized that the potential benefit of DFM depends upon the microbial species, strain, concentration, production techniques, and storage condition. There is evidence to show that better performance has been achieved by the use of a mixture of microorganisms with different species rather than a single microbial species or strain (Mead and Impey, 1986). In a recent study in our laboratory found that dietary supplementation of DFM increased the growth performance of birds at an early age, stimulated the immune response, decreased the number of *E. coli*, and improved the ileal morphology of broiler chickens. We concluded that DFM that contained a mixture of several beneficial microorganisms could be a viable alternative to antibiotics in the broiler diets (Salim et al., 2013).

Dietary prebiotics

Supplemental prebiotics components are not digested by the host, but they benefit the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the gut, predominantly those are producing short-chain fatty acids. Prebiotics has been shown to stimulate enteric colonization of unculturable bacteria (Konstantinov et al., 2003; Rastall et al., 2005) that discourage the colonization of enteric pathogens, and they have the advantage of being more stable to the heat and pressure incurred during feed processing. Any feed ingredient that enters the large intestine is a potential prebiotic, but it must be fermented by microorganisms that benefit the host to be effective (Lan, 2004). Most current attention and successes have been derived using non-digestible oligosaccharides; especially those contain fructose, xylose, galactose, glucose and mannose (Gibson, 1998).
Some structural carbohydrate components of non starch polysaccharides (NSP) have been used and studied as potential prebiotics in animal diets. Galactooligosaccharides can modify the colonic microflora by lowering some Gram-negative bacteria, such as coliforms, and increasing potentially health-promoting bacteria, such as Bifidobacteria and Lactobacillus (Matteuzzi et al., 2004). Galactomannan from partially hydrolyzed guar gum has been reported to reduce diarrhea in mice

(Takahashi et al., 1993) and improve intestinal microflora in human (Okubo et al., 1994). Galactomannans also can suppress the colonization of *Salmonella Typhimurium* in vitro (Oyofo et al., 1989) and *Salmonella enteritidis* in animal (Ishihara et al., 2000). Moreover its effect on microbial fermentation, arabinoxylan has also been shown to activate a macrophage cell line in the broiler intestine and thus decrease the enteric pathogen colonization (Zhang et al., 2004).

Enzyme supplementation

Dietary enzyme supplementation has become a standard practice in animal industry for reducing feed costs and good sources of dietary phosphorus, energy, and protein. Supplemental enzymes in the feed are used to achieve the following objectives: a) increase the animal's own supply of enzymes (Schaible, 1970); b) alleviate the adverse effects of anti nutritional factors, such as arabinoxylans, b-glucans, etc; c) render certain nutrients more available for absorption and enhance the energy value of feed ingredients (Lyons, 1993), and d) modulate intestinal microflora to a healthier state (Engberg *et al.*, 2004).The major enzymes used in animal feeds are hydrolytic protease, amylase, lipase, phytase, NSP-degrading enzymes, and cellulase. The enzymes with proven efficacies for animal husbandry include xylanase, arabinoxylanase, b-glucanase, cellulase, phytase, and mannanase (Ferket, 1993; Choct and Kocher, 2000). The amylase and lipase are commonly used in corn-soybean meal based diet to supplement endogenous enzymes of the animal, thus improving nutrient digestibility and growth performance of birds (Ferket, 1993).
On the other hand, Fischer and Classen (2000) reported that bacterial count from the small intestine of animal fed wheat-based diets was lower in xylanase-supplemented birds than the un supplemented ones. Because enzymes supplementation reduces the microbial population in the small intestine that can change the entire intestinal ecosystem of animal (Choct et al., 1995), resulting the decrease in the adverse effects of microbial fermentation. However, in a comprehensive review, Rosen (2001) concluded that the effect of enzymes was nearly equivalent to the effects of antibiotics on growth and feed efficiency of chickens. Therefore, enzymes supplementation in animal diets seems to be capable of limiting the performance losses associated with removal of AGPs.

Essential oils and plant extracts

The use of a wide range of plant extracts, sea algae, essentials oils and other natural substances to enhance animal health and performance has been documented for a long time due to their anti-inflammatory, immunomodulatory, antioxidant and anti-bacterial activities. Among these products, essential oils have long been recognized for their anti-microbial activity, and they have gained much attention for their potential as alternatives to antibiotics (Lee et al., 2004a). In an early study, Lee and Ahn (1998) found that cinnamaldehyde strongly inhibit *Clostridium perfringens* and *Bacteroides fragilis* and moderately inhibit *Bifidobacterium longum* and *Lactobacillus acidophilus* isolated from human. Although the exact anti-microbial mechanism of essential oils is not clear but it may be associated with their lipophilic property and chemical structure (Lee et al., 2004b). In addition, essential oils from oregano are showing the greatest potential as an alternative to AGPs in animal feed.
On the other hand, Yoshizawa (1993) reported that algae extract activated the macrophages and increased the pro-inflammatory cytokine production of laboratory animals. It has been reported that supplementation of Chlorella in human and animal diets performed a numerous biochemical and physiological function, such as growth promoter, antioxidant, and immunomodulation. In addition, antimicrobial properties of Chlorella are considered to be an effective alternative to AGP in the diets to maintain optimum health and productivity of the animal. One of our recent study, Kang et al. (2013) concluded that dietary supplementation of fresh liquied chlorella improved BW gain, immune characteristics, and the production of *Lactobacillus* bacteria in the intestinal microflora of broiler chickens. To be as effective as growth promoters, these herbal antimicrobial compounds must be supplemented to the feed in a more concentrated form than found in their natural state, which will increase usage costs. Additionally, most of these plants extract need further processing before use in animal diets which may also increase the usages costs (Salim et al., 2018).

Farm management practices

Although alternative feed supplements may compensate the reduction or elimination of AGPs in feeds, however; some changes in animal husbandry practices may also be important. Significant evidence shows that application of AGPs or alternatives in feeds are most effective when given to animals raised in unsanitary environmental conditions. Good barn sanitation, proper vaccination, pest control, biosecurity practices, and manure management are necessary to reduce pathogen load and exposure and minimize the need for antimicrobial therapy. Appropriate environmental temperatures and lighting, and maintenance of appropriate ventilation rate in the animal house are important since pathogens may be spread through the air and the environment. Water must be clean and drinkers, feeders and other equipments must be properly maintained to minimize spoilage and prevent a bloom of pathogens in the manure and environment of the animal. Automated watering and feeding systems are associated with a decrease risk of infection with *Salmonella* as compared to trough feeding. In addition, stocking density in the barn should need to maintain according to standard management practices of breed, strain or species of the birds. However, it is documented that implementation of a good sanitation program is usually much less costly than any disease treatment (Ferket, 2003).

Conclusion

The continued availability of effective antibiotic is critically important for combating infectious disease in both humans and animals. However, global ban on AGP use in animal feeds varies across the countries. Therefore, we should take a more proactive approach to considering how antimicrobial drugs are being used, and take necessary steps to assure that such uses are reliable for maintaining the health of humans and animals to combat AMR. A variable number of feed additives are used or has been proposed as viable alternatives to AGPs in animal diets, their efficacy dependent on understanding of their mode of actions, and their influence on gut health and growth performance. Combination of strategic feeding and types of feed additives may achieve good intestinal health, stable enteric ecosystem and sustainable production performance of birds rather than a single option. However, good hygienic farm practices and strict biosecurity are necessary in addition to strategic use of feeds and feed alternatives. Further research is needed to explore suitable, reliable and cost effective alternatives to AGP for animal feed industry. Moreover, upgradation and development of value chain is required in animal agriculture with ensuring quality and safe value added animal products for the consumers in the country.

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ANTIBIOTICS RESISTANCE PATTERNS OF ESCHERICHIA COLI IN FOOD-PRODUCING ANIMALS IN THE NORTHERN REGION OF GHANA

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Abstract

The overuse of antibiotics in food animals is leading to increased risk of human illness and increased healthcare costs, with little to no agricultural economic benefit. Animals can serve as mediators, reservoirs and disseminators of resistant bacterial strains and/or antimicrobial resistance (AMR) genes. Consequently, imprudent use of antibiotics in animals can eventually result in increased human morbidity, increased human mortality, reduced efficacy of related antibiotics used for human medicine and increased healthcare costs.

This project was undertaken in two stages (1) A survey by the administration of structured questionnaires in 5 districts (Tolon and Kumbungu Districts, Savelugu and Yendi Municipalities and the Tamale Metropolis) of the Northern region of Ghana and (2) A clinical study on the resistance of E. coli isolated from faeces of poultry, cattle, goats, sheep and pigs in the same 5 districts of the Northern region of Ghana.

For the survey work, structured questionnaires were administered directly to 120 farmers keeping animals for food in each of the 5 districts, making the total sample size 600 farmers interviewed. Results were presented in percentages using tables and descriptive statistics. For the clinical research, Cary-Blair transport media were used to pick faeces from animals and Escherichia coli were isolated using Chromagar E. coli agar from the faecal samples of poultry, goats, sheep, pigs and cattle from the same 5 districts of the Northern Region of Ghana. Their antibiotic drug-resistance patterns were determined using the EUCAST guidelines. A total of 580 feacal samples were taken from poultry (160/580), goats (105/580), sheep (105/580), pigs (105/580) and cattle (105/580). Antibiotics used for the susceptibility testing were tetracycline; doxycycline, ciprofloxacin, gentamicin, ceftazidime and the results were interpreted using both the EUCAST and CLSI breakpoints.

All the respondents (120/120 - 100%) in the Tamale metropolis use antibiotics, 103 (85.8%) in the Kumbungu district use antibiotics, 118 (98.3%) of the respondents in Savelugu use of antibiotics, 110 (91.6%) of the respondents in Yendi district use antibiotics whilst 104 (86.6%) of them use antibiotics in Tolon district. respondents use the antibiotics for the treatment of various infections resulting into pneumonia, enteritis, mastitis, gastrointestinal, respiratory and any urinary infections in diseased animals. The overall usage of antibiotics in food producing animals among the population samples was 92.5% (555/600). The antibiotics most commonly used were penicillin, amoxicillin, tetracycline and ampicillin. The percentage of farmers who administer antibiotics when animals are sick are; Kumbungu (33.3%), Yendi (45%) Savelugu (47.5%), Tolon (35%) and Tamale (70.8%), of which most are self-prescribed.

A total of 565 (97.4%) *E. coil* isolates were confirmed from the 580 feacal samples taken from all the animals with poultry recording 100% (160/160) recovery of *E. coli*, goats 96% (101/105), sheep 95% (100/105), pigs 97% (102/105) and cattle 97% (102/105). The percentages of the isolates resistant to the various antibiotics used can be found in Table 1 below.

Table 1: Antimicrobial resistance pattern of the isolated E. coli from the various domestic animals

Antibiotics	Antibiotic resistance pattern of *E. coli* in various domestic animals (%)					
	Poultry (n=160) R (%)	Cattle (n=102) R (%)	Goats (n=101) R (%)	Sheep (n=100) R (%)	Pigs (n=102) R (%)	Overall (n=565) R (%)
Tetracycline	93.7	16.6	22.7	42	74.5	54.5
Doxycycline	66.8	8.8	3.9	15	22.5	27.9
Ciprofloxacin	25.6	9.8	3.9	6	1.9	11.2
Gentamicin	15.6	13.7	0.9	1	0.9	7.4
Ceftazidime	2.5	14.7	4.9	3	4.9	5.6

The highest resistance percentages of antibiotics were observed in poultry followed by pigs, sheep, cattle and the smallest were in goats. Among all the isolates, 13 *E. coli* samples were noted as Multi-drug resistant (MDR: resistance to ≥3 antibiotics) with poultry recording the highest multi-drug resistant strains. The high resistance in poultry could be attributed to the regular vaccination and drug administration regimes in the production of commercial poultry.

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EVALUATION OF ANTIMICROBIAL RESIDUES IN MEAT AND MILK IN TUNISIA

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In order to produce enough meat to meet the growing demand of the world population, antimicrobials are increasingly used in livestock. They can be used to treat diseases, but prophylactic and metaprophylactic treatments are employed to prevent the spread of infections from sick animals to healthy animals in the same production unit. In addition, antimicrobials are sometimes used to promote growth of livestock and poultry. However, the two possible consequences of antibiotic use in livestock and poultry are the chemical danger and the biological danger occurring in meat. In fact, veterinary drug residues are among the chemical dangers. They can be found in milk, meat and meat products, especially if the dosage and/or necessary withdrawal periods are not respected. Consumption of meat containing excessive amounts of antimicrobial residues poses several risks to human health including immediate toxicities such as allergic reactions or longer-term health problems such as cancer or disturbance of the human microbiota [1]. Antibiotic residues in meat and milk may also lead to the selection of bacteria with resistance characteristics. In fact, it was reported that antimicrobial resistant bacteria can represent a reservoir of resistance genes transferable to pathogenic or commensal bacteria in the digestive tract and therefore could be a serious threat to disease treatment in humans and animals [2]. The contribution of food, in particular meat and milk, as a vector or source in humans of antibiotic resistant bacteria is a major problem for the 21st century medicine.

The monitoring and control of compliance with the admissible thresholds in foodstuffs for antimicrobial substances, present in the form of residues, are defined by regulations and ensured by official monitoring/surveillance plans. Several tests to detect residues in meat and milk have therefore been developed [3-4]. Nationally, there are no validated screening methods included in the residue monitoring plan. In this context, the CNSTN uses the Charm II technique, which is a radioimmunoassay based on the use of radioactive markers, for detection of antimicrobial residues in milk as well as bovine, ovine and poultry meat. It warrants consideration as a sensitive method for the detection of residues in animal origin foods at the European Union recommended limits.

The Charm II test uses an antibody (binder) with specific receptor sites to bind all the target antibiotics. The sample extract is incubated with the binder to allow the antibiotic to combine with the receptor sites followed by addition of ^{3}H or ^{14}C labeled antibiotic (tracer). After the competition reaction is complete, centrifugation and filtration steps allow recovery the remaining pellet, which is a tracer-binder complex. Finally, a liquid scintillation is added and the amount of bound labeled antibiotic is measured in counts per minute (cpm) with a scintillation counter. Figure 1 shows the principle of the Charm II test.

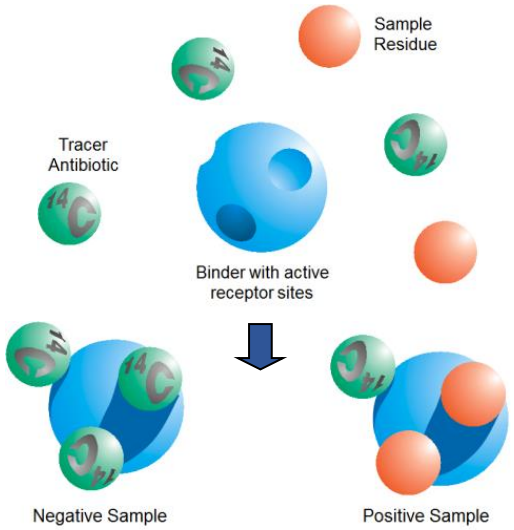

Figure 1. Charm II test principle.

We performed the analysis of 49 muscle meat samples; some were in the framework of the National Residue Control Plan and others from Tunisian markets. The experiment included 15 bovine meat samples, 20 poultry meat samples and 14 ovine meat samples. Figure 2 illustrates the results of the screening for antimicrobial residues.

The data in Figure 2 indicate that 2 out 49 samples (4.1%) were suspected positive for tetracycline residues, 6 out of 49 samples (12.25%) were suspected positive for sulfa-drug residues and 8.16% (4 out 49 samples) were contaminated with macrolides. All samples were compliant for beta-lactams and streptomycin residues. The samples suspected of containing tetracyclines and sulfonamides were poultry meat, which suggests that these veterinary drugs are misused in poultry to prevent and combat diseases related to intensive farming conditions. We should mention that only samples of ovine meat were contaminated with macrolides.

Concerning milk samples, we analyzed 34 samples in the framework of the national monitoring plan and purchased from 28 raw milk samples to detect tetracyclines, sulfonamides, macrolides, beta-lactams and aminosides. The obtained results are summarized in Table 1.

Table 1. Results of veterinary drug residues analysis in Tunisian milk.

Antimicrobials	Number of analyzed samples	Number of non-compliant samples	Non-compliance rate
Teracyclines	34	0	0
Sulfa-drugs	34	0	0
Macrolides	34	0	0
Beta-lactams	34	2	7.15%
Aminoglycosides	34	0	0

We remark that all samples were compliant for tetracyclines, sulfa-drugs, macrolides and aminoglycosides and only 2 out 28 (7.15%) were suspect positive for beta-lactams. This suggests that beta-lactams are frequently used in dairy farms and often the withdrawal periods are not respected.

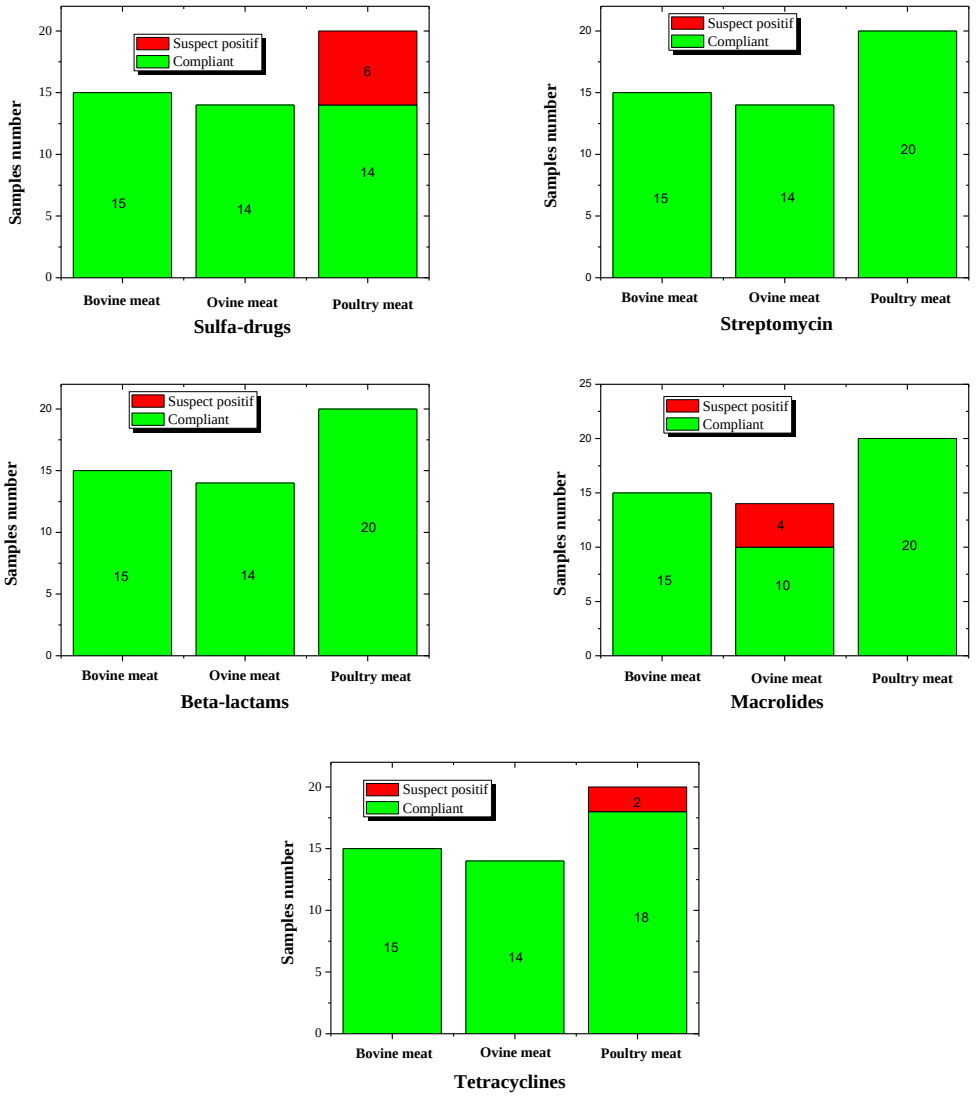

Figure 2. Results of veterinary drug residues analysis in Tunisian Meat.

This work provides a basis for establishing a database on Tunisian milk and meat contamination with antimicrobials by using the Charm II test as a reliable and sensitive method. Routine use of these procedures would help food agencies and the government to better regulate the use of antibiotics in poultry and livestock. The described procedures could also be used to correlate the presence of residues in milk and meat with antimicrobial-resistant bacteria in food-producing animals. The Charm II test is a quick, reliable and simple method that could help to consolidate the efforts of the national meat monitoring network and establish a national database on families of antibiotics.

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THREAT OF FOOD SECURITY AND OUTBREAK OF AFRICAN SWINE FEVER VIRUS IN AMBILOBE DISTRICT, NORTH OF MADAGASCAR

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Countries of the Southern African Development Community (SADC), particularly Mozambique and South Africa, are recognized to have beenthe origin of the emergence of African Swine Fever (ASF) and are still today the region with the highest variability of ASFV strains in the world (i.e., 23 out of the 24 ASFV genotypes are present)[1-4]. The high variability of ASFV in those countries can be explained by the complex host-virus-environment interactions and the diverse ecoepidemiology, particularly in areas where both the sylvatic cycle and domestic cycle coexist (e.g., South Africa hosts 7 genotypes, which is the highest number of genotypes recorded for one country). Moreover, the presence of around 1.5 million pigs in each of these countries together with their worldwide commercial exchanges (legal and illegal) reflects the importance of this region in ASFV local and global emergence,[1,5]. For example, Mozambique has been hypothesized as the likely source of the first (i.e., 1998) ASFV strains described in Madagascar[6] by genotype II. Also the ASFV strain that was introduced into Georgia in 2007 and now is extensively spreading through Russian Federation and Eastern Europe is assumed to have originated in Mozambique/Madagascar[5]. Similarly, a recent survey of smallholder pig farmers in the South African ASF control area revealed regular illegal movements of pigs for sale into the ASF free area[6].

In Madagascar, ASF has become endemic since its first diagnosis in 1998[7,8]. Today, the disease is prevalent across the island with serious economic impacts on pig farming. Bushpigs are present mainly in northern and central areas and are intensively commercialised as bush meat in some areas of the country. Our previous research suggests a minor role for bushpigs in ASFV transmission, although investigations have been limited in sample size and area[9,10]. Further studies will help us better understand how ASFV has been maintained for years in Madagascar. The lack of effective control in Malagasy pig farming prevents any permanent recovery of this sector, and furthermore constitutes a permanent threat for close trading partners such as Mauritius and other regional and global partners.

Last year, four districts in north western Madagascar were infected by ASFV and selected for this study: Nosybe, Ambanja,Ambilobe and Sambava. During this outbreak, our results revealed that 9 pigs were purchased and transferred from Nosybe district to a butcher's farm in Ambanja district in September 2019. Six (6) samples (4 sera and 2 tissues) were taken by the veterinarian in the Ambanja district and 2 tissues were positive in ELISA antigen (1.1 K2 Ingezim PPA Das) and the 4 sera were negative in Elisa Antibody (1.1 K3 Ingezim PPA Compac).

ASF disease wasspread and identified in Ambilobe in November 2019.Surveys carried out in the Ambanja and Ambilobe districts affected by the epizootic made it possible to carry out an initial estimate of the impact (in Ambilobe alone, approximately 66% of the 900 pigs died) and to trace the dissemination route of the ASF virus.

The transport of live pigs out of the Ambilobe district is prohibited until further notice. Diseased is banned from sale. In any case, the majority of consumers, frightened and doubting the quality of all the pork in this area, prefer for the moment to abstain. Consumer concerns extend to products made from pork, such as deli meats. In the restaurant industry, pork is scarce.
Shortly after, cases of ASF were found along the route to Sambava in eastern Madagascar following the introduction of live pig from Ambilobe.
Often, several collectors buy pigs from this area and transport them to slaughterhouses in Antananarivo, where the outbreak will have an impact on the consumption of pork in the capital.
It would be interesting to confirm ASF disease in these areas, to understand if the situation is controlled or if it spreads, how many farms and animals are infected. This area could constitute a site with a pig-bushpig interface and viral circulation. Therefore, we believe that this outbreak and the associated epidemiology in these regions need to be further investigated. Further studies are planned to elucidate the role of bushpigs and domestic pigs in ASF epidemiology in Madagascar. Analysis of the fragment of the gene B646L showed 100% homology among isolates, despite over 20 years of ASF presence in Madagascar, suggesting a single source of contamination[11]. More study is needed to confirm this hypothesis.

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VALIDATION OF DIAGNOSTIC TESTS FOR INFECTIOUS DISEASES: CHALLENGES AND OPPORTUNITIES

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The Word Organization for Animal Health (OIE) has developed a science-based procedure for purpose-oriented validation, certification and registration of diagnostic tests for infectious diseases. Parameters such as analytical and diagnostic sensitivity (DSe) and specificity (DSe), selection of an appropriate cut-off, repeatability and reproducibility inform about core performance characteristics and provide an objective assessment of the assay's fitness for purpose. In addition predictive values and prevalence-independent likelihood ratios provide useful information about interpretation of results.
Bayesian Latent Class Modelling (BLCM) is a powerful tool to overcome constraints faced by traditional diagnostic test evaluation methods, which require both a perfect reference standard test and ample numbers of appropriate reference samples.
Fundamentally different assays, e.g. antigen, antibody or molecular tests as well as "new technologies" such as point-of-care tests and multiplex assays need to be validated for the species, target population and specific sample type that they will be applied for.
It is important that source and target population in which a diagnostic test will be applied have similar characteristics, e.g. if individuals from the source population in which the test was validated had all been in an advanced stage of infection, e.g. with clinical symptoms and the target population where the test is applied is mainly in an early stage of infection the test's sensitivity may be compromised. Similarly, specificity may be affected when the test has been validated in an artificially "clean" population e.g. SPF animals (source population) and is then applied in a population in the field (target population).
A clear and consistent case definition about what constitutes an infected and not-infected individual is important to obtain realistic estimates for DSe and DSp. A frequent limitation is the lack of statistically robust numbers of samples from infected or non-infected animals. "Provisional recognition" provides an opportunity to use a promising test with limited validation and to further assess and corroborate test performance when more samples have been tested. Interlaboratory comparison panels provide information about accuracy and precision of a test when applied on the same samples in different laboratories.
It can not be assumed that changes in the population, species or specimen do not affect test performance. Verification and comparability studies are needed when a validated test is used in a new laboratory or when a change has been made in a test protocol. Template based approaches have been developed to facilitate complete, transparent and objective assessment of a new assay.

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THE GLOBAL LABORATORY LEADERSHIP PROGRAMME: STRONG LEADERS FOR HEALTH SECURITY.

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To help ensure that laboratories can effectively play a critical role in the prevention, detection, and control of diseases, current and emerging laboratory leaders worldwide need specialized training in leadership and management. Leading organizations partnered to develop the Global Laboratory Leadership Programme (GLLP) targeting human and animal health laboratories, as well as laboratories with public health impact (environmental, agricultural, food, or chemical laboratories). The partners are the Association of Public Health Laboratories (APHL), Centers for Disease Control and Prevention (CDC), European Centre for Disease Prevention and Control (ECDC), Food and Agriculture Organization of the United Nations (FAO), World Organisation for Animal Health (OIE) and the World Health Organization (WHO). The GLLP partners are committed to the programme's vision of laboratory leaders empowering national laboratory systems across the globe using a One Health approach to strengthen health security.

International experts agree that laboratory leaders need certain core competencies to meet national, regional, and global disease prevention and control objectives. The GLLP encapsulates the following nine core competencies outlined in the Laboratory Leadership Competency Framework: Laboratory System; Biosafety and Biosecurity; Communication; Disease Surveillance and Outbreak Investigation; Leadership; Quality Management System; Emergency Preparedness, Response, and Recovery; Management; Research.

The programme, available for virtual or in-person implementation, is flexible in length, format, and content and may be adapted to meet country-specific workforce needs. The GLLP Learning Package provides the materials necessary to implement programmes in any country or educational institution in the world and includes: the GLLP Planning and Implementation Guide and the GLLP Mentorship Guide. The virtual and in-person course materials include: PowerPoint presentations, instructor guides and participant guides. The GLLP course materials include four sections (Introduction, Laboratory Management, Laboratory Leadership, and Laboratory Systems) comprising 13 units and 43 modules, with over 200 contact hours' worth of materials, all with a strong One Health focus. The GLLP Learning Package will be available on the WHO Health Security Learning Platform in the second half of 2021.

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A COMMON APPROACH TO BUILD VETERINARY LABORATORIES DIAGNOSIS CAPACITIES, NATIONAL AND REGIONAL LABORATORY NETWORKS IN AFRICA.

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Background

The ability of National animal health laboratories to detect and characterize infectious agents plays a crucial role in prevention and management of zoonoses and transboundary animal diseases (TADs) and related threats relevant to animals like antimicrobial resistance (AMR). Deficiencies in the capacities and capabilities of laboratories may lead to delayed and inadequate responses to disease emergencies at the animal–human interface. As part of efforts to address these deficiencies, the FAO is assisting member countries in the sustainable development of Veterinary laboratory capacities under the One Health approach. Support is provided to national veterinary services of beneficiary countries by: 1) Improving the capacity of laboratories (central and regional) to rapidly, accurately and sustainably diagnose zoonoses, TADs and AMR under quality assurance and within a biosafe and biosecured environment; 2) Strengthening laboratory networks at global, regional and national levels to enhance information, expertise and resource sharing; and 3) Supporting linkages between laboratory and epidemiology networks for rapid information sharing to enable faster responses to animal disease outbreaks, prevent further spread and support evidence-based disease control policies.
Under a programme funded by the United States Agency for International Development (USAID), this is implemented by the Laboratory Unit team of the Emergency Prevention System for Animal Health (EMPRES-AH) and the laboratory experts based at the FAO's Emergency Centre for Transboundary Animal Diseases (ECTAD) regional and country offices together with the Veterinary Public Health Institute of Abruzzo and Molise Regions (Teramo) in Italy, in close collaboration with international partners, in particular FAO Reference Centres, the World Organisation for Animal Health (OIE), the Joint FAO/IAEA (International Atomic Energy Agency) Division of Nuclear Techniques in Food and Agriculture, EU-FMD and the World Health Organization (WHO).

Method

In order to assist national veterinary services of beneficiary countries, FAO has supported countries in the implementation of a harmonized initiative including the implementation of a common laboratory Information Management System (LIMS) in veterinary laboratories in Africa and its linkage with several other tools from FAO and partners, to allow for faster and better diagnosis and appropriate national responses to disease emergencies at the animal– human interface.
A Laboratory Information Management System (LIMS) is a powerful tool for improving the standardization of laboratory diagnostic processes and sample tracking as well as the management of laboratory data and external reporting to enable early detection and targeted, cost-effective response to zoonotic disease outbreaks. Since 2013, FAO in partnership with Teramo is installing a LIMS system called "SILAB for Africa" (SILABFA) in the national and sub-national veterinary laboratories. SILABFA is highly customizable with modular options of different features which can be selected to install and/ expand based on each laboratory needs. SILABFA modules include for example the sample

storage management (biobanking), reagents management, AMR, epidemiological, early warning system and One Health modules.

Further to the implementation of SILABFA in veterinary laboratories, linkages with other animal health, and disease control related systems as well as those using One Health approach have been developed over the years, as described below:

-While installing SILABFA at country level, the system has also been linked to the local animal identification, registration, and traceability systems and any other relevant national epidemiological reporting systems, when applicable and depending on each country request.

- Interoperability is being developed since 2019, between SILAB-FA and the FAO Event Mobile Application (EMA-i). The EMA-i has been developed by FAO since 2012 to enhance veterinary services' disease reporting system from the field and to generate a real time report on animal diseases, including zoonoses to the FAO Global Animal Disease Information System (EMPRES-i) database. The linkage between SILABFA and EMA-i allows for rapid and fluid geo-referenced epidemiological data transfer from the field to the laboratories, and from the laboratories back to the field. Through this process, the test result available in the laboratory will be sent back to the field in a timely manner, ensuring faster and appropriate response to any outbreak.

- Interoperability is being developed since 2020 between SILABFA and the Laboratory Infrastructure Network Analytics system (LINA) tool from Sandia National laboratories (from the U.S. Department of Energy's National Nuclear Security Administration) to provide solutions to enable decision makers to improve decision making during outbreaks. LINA is a One Health laboratory network optimization system designed to improve the planning and efficiency of national laboratory networks. It provides modelling and data analytical functionality for interactive laboratory capacity maps, sample transport routing, and capacity development analysis. The real time information on individual laboratory testing activities stored under SILABFA will greatly improve the quality, accuracy and utility of LINA analyses. In the near future, data on laboratories capacities and capabilities (as assessed using the FAO Laboratory Mapping Tool) will also be included in the model. EMA-i has a mapping component that shows the location and epidemiological details of a disease event and this information will also be connected.

Results

Decreased time in animal disease diagnostic tests by the National veterinary laboratories network due to faster management of samples

The SILABFA has been installed in a total of 8 national and 23 sub-national laboratories in 8 countries including the United Republic of Tanzania, Ethiopia, Uganda, Kenya, Cote d'Ivoire, Cameroon, Senegal and Ghana, and will be extended to other 18 sub-national laboratories in 2021. SILABFA has also been installed by Teramo in Namibia, Botswana, Zimbabwe and Zambia under another project. Veterinary laboratories in African countries using SILABFA have showed improvement in laboratory management and in technical activities (increasing data quality and consistency, increased quality of test reports and a decrease in time needed for test result) leading to a considerable growth in service delivery. The SILABFA compliance with ISO/IEC 17025 standards also facilitated the accreditation process in central laboratories from three countries. To ensure sustainability to this approach, extensive training has been provided to two laboratory staff from each country to be able to maintain and update SILABFA in their own country as well as serve as resource persons for their region. While success has been shown at individual laboratory level, the use of the common SILABFA system in Central and district laboratories have also shown to improve communication among the national veterinary laboratory network in five countries.

Increased data sharing at national level, between field and laboratories and between laboratories and national epidemiological units to enhance surveillance and early warning

The interoperability between SILAB-FA and the FAO EMA-i is being piloted since 2020 in Tanzania and showed an increase in data sharing between the veterinary laboratory and the field. This will be expanded to other countries in the future. The links between SILABFA and national epidemiological units allowed to increase harmonized and reliable data sharing for decision making.

Way forward

FAO will continue assisting countries in implementing SILABFA and linking it with any relevant national database, thus ensuring reliable results from disease diagnostic tests conducted by the National veterinary laboratories network will be shared in a timely manner for decision making. The approach developed to build and strengthen the National One Health Laboratory Network and support the outbreak response decision process by using the system allowing to link information between SILABFA/ LINA/ FAO-LMT and EMA-I will be piloted in Tanzania. The implementation of a common LIMS system in many countries from a same region may in the future facilitate discussion and sharing of specific information between countries for a regional harmonized response to disease emergencies at the animal– human interface. Finally, FAO will assist countries in increasing their data sharing with regional and global animal health networks (such as the West and Central Africa Veterinary Laboratory Network for avian influenza and other transboundary diseases (RESOLAB-WA and RESOLAB-CA), the Regional Support Laboratories in Africa or the Veterinary Diagnostic Laboratory (VETLAB) network globally), by extending the SILABFA-National Databases linkages to any relevant regional or global database.

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THREATS TO FOOD SECURITY, PUBLIC HEALTH AND INTERNATIONAL TRADE DUE TO EMERGING AND RE-EMERGING TRANS-BOUNDARY AND ZOONOTIC ANIMAL DISEASES

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As the world's population continuous to grow, there is an increased need for food, trade activities, as well as public health risks. Food security, public health and international trade are under threats due to emerging and re-emerging Transboundary animal (TADs), as well as zoonotic diseases. According to the World Organisation for Animal Health (OIE),TADs are diseases that can affect the economy and trade and have potential to spread to other countries. OIE defines Emerging disease as an occurrence of a new disease, infection or infestation in an animal and negatively affect animal/s or public health. Emerging diseases are usually a result of either a change of a known pathogen or its presence in new geographical areas or species; or a previously unrecognised pathogenic agent or disease detected for the first time. Meanwhile a re-emerging disease is one already known, that either shifts its geographical setting or expands its host range, or significantly increases its prevalence. Finally, Zoonotic diseases are those that can spread from animals to humans or vice versa.

The entire world is at risk of disease outbreaks and spread, considering factors such as international trade and supply chains, travel and tourism, domestic animals-human-wildlife-ecosystem interface, as well as climate change. Currently, there are several disease alerts on the OIE-WAHIS accessed on the 12th February 2020, Highly pathogenic avian influenza China (People's Rep. of), African swine fever in Sierra Leone, Highly pathogenic avian influenza in Germany, Leishmaniosis in Iceland and Peste des Petits Ruminants, Morocco. On the other hand, the World Health Organisation website accessed on the same day had the following headlines or alerts: Ebola virus in Democratic republic of Congo, Novel Coronavirus in different countries (China, Japan, Korea and Thailand.)

Some of the negative effects of disease outbreaks on the world's economy and food security are:

- Disruption of export and import of animals and animal products, Trade is affected, countries cannot trade with one another, and there is no import and export of food and other commodities, leading to compromised food security.

Figure 1: Example for closure of importing live birds due to outbreak of H5N1, *https://assets.theedgemarkets.com/chicken-poultry_20200203233758_reuters.jpg?null*

- Diseases causes losses and damages, leading to Poor or low productivity in the agricultural sector, the biggest contributor to food security and poverty reduction. Subsequently, people's livelihoods are affected, especially those that depend on agriculture for income and food security.

Figure 2: Loss of livestock due to disease outbreak, *https://ocdn.eu/pulscmstransforms/1/qskk9kqTURBXy8wOTk1YjVmZWIwOTk5M2VlMDMwMWI4ZjQwZDNkOTBmZS5qcGVnkZMFzQMUzQG8gaEwAQ*

- Economic losses to sectors such as tourism and transport, as measures such as movement control are implemented to contain and control disease/s

On the other hand**, Zoonotic diseases** such as High pathogenic Avian Influenza, Anthrax, Rabies, Rift Valley Fever Brucellosis, Ebola etc. have a negative impact on animals and public health. Approximately 60% of infectious diseases are zoonotic and 75% of these originate from wildlife, some of their effects are;

- Tragic loss of lives, some diseases can cause mortalities
- Ill health and disability due to infections
- Unproductive work force due to ill health, hospitalisation or death
- Government expenditures on immunisations, acquiring high containment and treatment facilities and other control measures.
- Loss of investors due to fear of impact of disease

Figure 3: Example of Public health threat - Head line of Ebola outbreak – a public threat reported, *https://allegralaboratory.net/wp-content/uploads/2014/12/ebola-virus.jpg*

What should be done with regard to all these threats and risks due to emerging and re-emerging Transboundary animals and zoonotic diseases?

Success to prevent the loss of livelihoods, enhance food security and nutrition, reduce poverty and promote economic growth for each country, highly depends on:

- The Level of **Preparedness**, in terms of allocated and available resources to handle disease outbreak

- Capacity to **Detect** disease rapidly and accurately
- Capacity to **Respond** and contain disease outbreak
- Capacity to **Prevent** occurrences of future outbreaks

FAO for example, supports member countries by developing strategies and policies on how to prepare better for disease outbreaks, to improve their detection or diagnostic capacity as well as strengthen their response and control measures.

Conclusion

The entire world is at risk, threats due to emerging and re-emerging Transboundary animal and zoonotic diseases are on the increase. These threats can spread rapidly affecting animal and public health, they can disrupt international trade and compromise food security. In order to minimise their impact or prevent their occurrences, countries should adopt the **One Health concept**, whereby different sectors should be involved in the fight against emerging and re-emerging TADs and zoonotic diseases. According to Centre for Disease Control and Prevention (CDC), it is a collaborative, multisectoral, and trans-disciplinary approach—working at the local, regional, national, and global levels—with the goal of achieving optimal health outcomes recognizing the interconnection between people, animals, plants, and their shared environment.

Figure 4: Illustration of One health Concept - the interdependence of human, animal and environmental health, **https://www.uaf.edu/onehealth/**

Furthermore, strategies and policies on how to prepare, detect, respond and prevent disease outbreaks should be developed or strengthened for all countries and organisations.

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CHALLENGES FOR BETTER LIVESTOCK PRODUCTION IN DEVELOPING WORLD

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EMPHASIS ON RICE MILLING BY-PRODUCTS INSTEAD OF STRAW FOR FEEDING LIVESTOCK

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Abstract

Rice straw is the sole feed for livestock in some rice-producing countries. Although it has low nutritive value, some mechanical and biochemical means have been adopted to improve its quality for low-producing native livestock. Nowadays the situation has changed due to increase the production of high yielding varieties of rice because the plant has become shorter and stronger, which indicates further lignification. Cultivation of high-yielding varieties of rice thus makes the straw meaningless for feeding. Alternatively, high-yielding varieties increase production of rice milling by-products. Rice milling by-products would be a potential source of animal feed after improving their quality following some biochemical methods.

Keywords: Rice milling, animal feed, straw, rice bran

Rice is the cheapest and most energy-densestaple food around 50% of the global population. In Asia and South Asia, the figure is around 70% (Bishwajit *et al.*, 2013). Bangladesh is the fourth-largest rice producer. In spite of the decline in the country's arable land since its independence in 1971, the area harvested increased from almost 10 million ha in 1995 to nearly 12 million ha in 2010. Rice yield per hectare has also increased in the last decades, from a low of 2.85 t/ha in 1995 to almost 4.42 t/ha in 2014 (http://ricepedia.org/bangladesh). These increases in rice yield and total harvested area contributed to higher rice production, which nearly doubled from over 26 million tons in 1995 to 52 million tons in 2014 (http://ricepedia.org/bangladesh). Rice production trends in the world and in Bangladesh are shown in Figure 1 and 2, respectively. Higher production of paddy rice increases the production of straw and milling by-products. Now it's a question of either continuing to use the straw as feed for livestock or giving emphasis to the milling by-products.

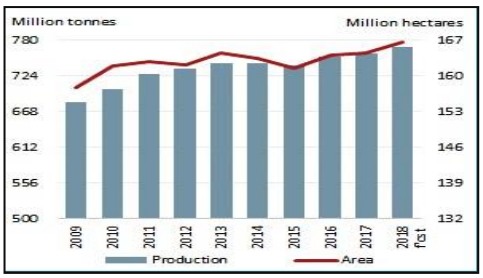

Fig. 1. Global paddy production and area trend (http://www.fao.org/economic/RMM

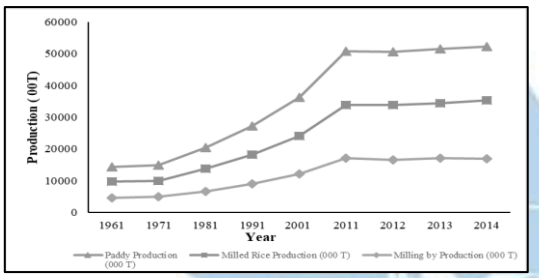

Fig 2. Paddy, milled rice and rice milling production trends in Bangladesh (http://ricepedia.org/bangladesh

ue to presence of lignin, fibrer and silica (mainly in leaves) in rice, several researchers suggested chemical, physical and microbiological treatments to improve the quality of straw for feeding livestock. In some cases, urea was considered as a non proteinnitrogenous substances for treatment of straw for the synthesis of protein by ruminants. Molasses was also added as a source of readily available carbohydrate to enhance microbial growth. Treatment by ammonia and alkali were some strategies to improve its nutritive value.But nowadaysthe straw from high yielding varieties of rice are shorther andstronger with erect leaves, which is related to lower biomass yield and further lignification.Those factors are also related to higher grain to straw ratio (Figure 3 and 4). Straw production has thus beenreduced and quality has further decrease for feeding livestock.

The relationship between quality of straw from high yielding varieties with palatability as livestock feed was studied. Several case studies reflected that most of the cattle were pasturing where very short length grasses were available (Figure 5). Cattle have no interest for eating straw when they have the choice to graze, even when grass is scarce (Figure 6).

Fig. 3. Conventional (left), high-yielding and low tillering ideotype (right)

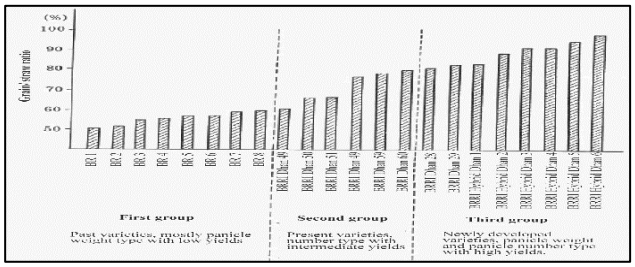

Fig. 4. Trend to increase the grain and straw ratio (http://ricepedia.org/bangladesh)

Figure 5. Grazing in a poor pastureland

Figure 6. Cattle does not like fresh straw

In rice producing countries, straw has long beenfed to livestock, butthese havetypically been low-producing breedsrand recent development of high yielding varietieshas made the situation crucial. The low production, high lignification andsilica content of straw from high yielding varieties is not suitable feeding modern dairy cows (or even for low producing local breeds). So, this is the time to give emphasis on fodder production for livestock feeding. Fortunately, high yielding varieties of rice produce large amounts of by-products from the rice milling industry, which would be a good source of feed for livestock.

Most rice varieties are composed of roughly 20% rice hull, 11% bran layers, and 69% starchy endosperm, but the categories and the number of by-products depends upon the type of rice and milling

industry. The rice mills are generally classified as traditional rice mill, husking rice mills, semi-automatic rice mills and automatic rice mills.

a) Traditional rice millsare those rice mills, which are operated at village level using simple traditional technology and local made devices. The process involves cleaning of paddy, soaking, parboiling (traditional), sun drying, and milling with Engelburg huller, aerating and bagging.
b) The production process of a husking mill also follows different stages like automatic ricemill and most of the works of a husking mill is conducted manually.
c) The process of semi auto rice mill involves cleaning of paddy, steaming, mechanical drying, milling with rubber-roll huller, rubber polishing, aerating, bagging and weighing. Rice produced in semi-automatic rice mill is well polished and less broken. Husk and bran are obtained separately and have better use in briquetting and edible oil extraction.
d) Automatic rice mill has four basic stages-dryer, husking, whitening-polishing and finishing (grading, blending, and packaging). During milling process different types of by products has proceduced along with the rice.

Table1 gives a clear picture of capacity, separation method, product and by-product of different categories of rice mills. There is a clear picture that around 15% of by-products are available after milling the grain, which would be usefull as feed for livestock. Each of the by-products has special features for chemical composition and nutritive value. Utilization of bran as feed, especially for monogastric animals, is limited due to its high fiber content and antinutritional factors such as phytic acid. Inclusion of deoiled rice bran at not more than 10.0% in poultry rations has recommended. Further processing would improve the quality of by-products.

Table 1. Categorization of rice mills and their products

	Traditional	Husking	Semiauto	Autometic
Bran separation	Manually	Manually	Mechanically	Fullymechanically
Totalsteps	Seven (7)	Eight (8)	Ten (10)	Sixteen (16)
Min.Capacity(t/h)	0.3-1.0	0.6-1.0	2-5	5-20
Product (%)				
Milled Rice (edible)	70.00	62.50	62.50	65.00
Husk	-	23.25	23.25	20.00
Rice Bran	30.00	8.75	8.75	7.30
Rice Polish	-	-	-	2.70
Broken Rice	-	5.50	5.50	1.25
Black Rice	-	-	-	2.10
Paddy Dust	-	-	-	1.65
Total	100.0%	100.0%	100.0%	100.0%

The previous researchers had attempted to follow different techniques to increase inclusion level of rice bran in poultry rations, such as fermentation (Wizna*et al.*, 2012) and enzyme supplementation (Tirajoh*et al.*, 2010).Supriyati*et al.*, (2015) also found significant ($p<0.05$) effects of fermentation on the fiber content of rice bran. Yanke (1998) observed that phytate phosphorus decreased from rice bran by using rumen liquor. Fermentation of rice bran with *Bacillus amyloliquefaciens* increased digestibility of crude protein, calcium and phosphorus (Abbas*et al.*, 2012). Fermentation by yeast increases single cell protein in rice bran,which is a quality protein having perfect amino acid profile as animal protein.It also would reduce phytate-P and fiber content and in some cases uses non protein nitrogenous substances as a source of nitrogen for body protein of microorganism. Consideringthe above factors, rice mill by-products should be emphasized for further development as a promising animal feed.

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FODDER PRODUCTION, PRESERVATION AND SUPPLEMENTARY FEEDS FOR SUSTAINABLE DAIRYING IN SRI LANKA

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Introduction

In Sri Lanka, the percentages of cattle and buffaloes are in milking was 28% in 2017, which was relatively low compared to the standards, mainly due to poor nutrition of the animals. As a result, currently, Sri Lanka produced only about 40 % its milk requirement.
The main feed resources for ruminants in the country are natural pasture and crop residues, which are generally low in quality and quantity. Due to this, nutritional status of most high producing dairy cows is not at optimal. For example, serum NEFA and BHBA contents of high producing cross bred cows at post-partum transition, early-lactation, mid-lactation and non-pregnant stages exceeded the upper critical limit of the reference range, indicating that they suffer from negative energy balance (Herath *et al*., 2018). Another major drawback is seasonal availability of forages. Two seasons; wet and dry are prevailed according to two monsoonal rainfall patterns. During the wet season, about 0.86 million MT of excess dry matter are available but in the dry season, about 1.02 million MT of dry matter is deficient, highlighting the importance of preservation of forages to be used when they are scarce (Weerasinghe, 2019).
To minimize negative energy balance in early lactation, supplementary feeds such as by pass fat are popular in other parts of the world, but not in Sri Lanka. Methane is emitted by ruminants during feed fermentation in the rumen and dietary adaptations to minimize these emissions and thus global warming are a high priority in many countries, but little is known about methane production during fermentation of improved fodder varieties grown under Sri Lankan conditions.
Therefore, the objectives of this paper is to highlight recent findings of several experiments conducted by the Veterinary Research Institute of Sri Lanka with other collaborative institutes on agronomy of some improved fodder varieties, silage production, methane emission of improved fodder varieties harvested at different intervals and the use of bypass fat as an energy supplement to high producing dairy cows.

Improved fodder

An agronomy study followed by *in-vitro* digestibility and assessment of methane production of three recently introduced improved fodder varieties (Hybrid Napier CO_3 and CO_4: *Pennisetum purpureum x P. americanum* and fodder Sorghum var. Sugar graze) have been conducted.
In general, CP, ME and OMD decreased whilst DM and Methane production increased with increasing harvesting interval (Table 1). Therefore, except for DM content, CO_3 and CO_4 varieties were better harvested at 4th week intervals in terms of higher CP, ME, OMD and lower methane production. With regards to forage types, sorghum was superior to CO_3 and CO_4 at the 4th week of harvest, but due to the risk of high cyanide content of immature sorghum plant, it may be best harvested at 6th weeks. Overall, there was no greater difference between CO_3 and CO_4 on nutritive composition at different harvesting

intervals and sorghum was always superior to other two. It can be concluded that CO3 and CO4 harvested at 4th week and sorghum at 6th week will provide maximum nutrient contents to dairy cows.

Table 1. Nutritive analysis of Hybrid Napier CO3, CO4 and fodder sorghum harvested at different intervals

	Variety	Harvesting interval: HI (Weeks)		
		4	6	8
Crude protein (%)	CO_3	20.16 ±0.27^{b}	14.27 ±0.09^{e}	13.24 ±0.06^{f}
	CO_4	20.10 ±0.05^{b}	14.38 ±0.10^{e}	13.41±0.15^{f}
	Sorghum	20.79 ±0.09^{a}	18.93 ±0.07^{c}	14.93 ±0.11^{d}
Dry matter (%)	CO_3	12.92 ±0.41^{cd}	13.83 ±0.04^{cd}	15.99±0.92^{abc}
	CO_4	13.01±0.36^{cd}	14.67 ±0.71^{bcd}	17.76±2.03^{ab}
	Sorghum	12.06 ±0.09^{d}	15.85±0.35^{abc}	19.29±0.07^{a}
ME (MJ/kg/DM)	CO_3	8.30 ±0.08^{c}	7.76 ±0.17^{d}	7.35±0.01^{e}
	CO_4	8.73 ±0.05^{b}	8.18 ±0.09^{c}	7.41±0.14^{e}
	Sorghum	9.16±0.07^{a}	8.68 ±0.03^{b}	8.16 ±0.05^{c}
OMD (%)	CO_3	57.19 ±0.53^{cd}	52.85±1.12^{e}	50.06±0.48^{f}
	CO_4	59.70±0.33^{b}	55.60 ±0.62^{d}	50.45±0.94^{f}
	Sorghum	62.88 ±0.45^{a}	59.27±0.24^{bc}	55.37±0.30^{d}
Methane (mg/g of digested DM)	CO_3	12.24 ±0.50^{bc}	17.65±2.04^{a}	19.43±0.19^{a}
	CO_4	11.75±0.34^{bc}	13.90±1.38^{b}	19.80 ±1.05^{a}
	Sorghum	10.07±0.74^{c}	12.06±0.90^{bc}	14.22±0.55^{b}

^{abcdefg} Values with different superscripts within a row are different ($p< 0.05$)

Silage production

Silage production and quality testing study was conducted to evaluate the best type of fodder or combination (50:50 basis); of three fodder varieties (CO_3, CO_4 and Sorghum) harvested at different intervals.
DM contents of all silages were low compared to standard values indicating that wilting before ensiling is necessary (Table 2). Compared to CO_3, CO_4 and combinations of them with Sorghum, silage of sorghum harvested at 6th week had highest pH, lactic acid and soluble carbohydrates. Therefore, it can be concluded that among newly introduced improved fodder types, Sorghum harvested at 6th week is best for silage making.

Supplementation of bypass fat

As previous studies (Herath *et al*., 2018; Ranaweera *et al*., 2018) confirmed that most high producing dairy cows in Sri Lanka are at negative energy balance at early lactation, an experiment was conducted to investigate the influence of rumen bypass fat supplementation on milk production. The control group (n=6) was fed with a basal TMR whilst the treatment group received 200 g/ day of rumen bypass fat.
Dairy cows supplemented with bypass fat had higher milk production than the control group until the 11th week of the lactation (Table 3). As such, bypass fat supplementation resulted 132.38 L/cow higher cumulative milk production at 11 weeks compared to their counterparts, which equals about 60 USD extra net income per cow for first 11 weeks of lactation. Therefore, it was concluded that bypass fat can be successfully used as a supplement, which improve the production and profitability.

Table 2. Quality parameters of silages of different improve fodders and their combinations

Parameter	HI	Silage type

		CO_3	CO_4	Sorghum	CO_3 + Sorghum	CO_4 + Sorghum
DM (%)	4	14.14 ±0.12^{b}	13.28±0.65^{b}	14.88±0.37^{b}	15.44±0.78^{b}	14.63±0.77^{b}
	6	15.58±0.25^{b}	16.73±1.05^{ab}	17.88±1.66^{a}	14.24±1.57^{b}	16.31±2.81^{ab}
	8	14.95±0.28^{b}	15.83±1.16^{ab}	18.77±0.42^{a}	16.49±1.75^{ab}	15.63±0.30^{b}
pH	4	5.28±0.16^{ab}	5.45±0.25^{a}	4.82±0.26^{b}	5.04±0.07^{ab}	5.00±0.22^{ab}
	6	5.52±0.26^{a}	5.47±0.17^{a}	4.92±0.07^{bc}	5.41±0.19^{a}	5.22±0.21^{a}
	8	5.16±0.08^{a}	5.35±0.14^{a}	4.54±0.05^{c}	5.28±0.23^{a}	5.15±0.26^{ab}
Lactic acid (%)	4	0.68±0.48^{b}	1.26±0.77^{b}	2.08±0.77^{b}	1.62±0.56^{b}	2.53±0.97^{ab}
	6	1.03±0.45^{b}	1.25±0.14^{b}	4.14±0.77^{a}	2.65±1.4^{ab}	2.71±0.06^{ab}
	8	0.44±0.04^{b}	1.15±0.76^{b}	2.35±1.05^{b}	0.64±0.59^{b}	0.66±0.59^{b}
SCHO (%)	4	0.88±0.15^{d}	1.07±0.18^{bcd}	2.12±0.88^{abc}	1.86±0.13^{b}	1.84±0.28^{b}
	6	1.52±0.19^{b}	1.36±0.15^{acdb}	2.54±0.12^{a}	2.63±0.57^{a}	1.59±0.41^{b}
	8	0.88±0.11^{c}	0.46±0.15^{d}	0.49±0.45^{cd}	0.28±0.80^{d}	0.83±0.11^{c}

^{abc}Values with different superscripts within a row are different ($p<0.05$)

Table 3. Influence of rumen bypass fat supplementation on milk yield[1]

Weeks in milk	Milk yield (L/cow/day)[2]	
	Basal mixed ration	Basal mixed ration with bypass fat supplement (200g/cow/day)
2	11.48 ± 0.38a*	15.11 ± 0.97^{b}*
4	12.39 ± 0.39^{a}	14.39 ± 0.87^{b}
6	12.01 ± 0.59^{a}	14.42 ± 0.70^{b}
8	12.16 ± 0.29^{a}	14.78 ± 0.67^{b}
10	12.06 ± 0.32^{a}	15.05 ± 0.62^{b}
12	12.64 ± 0.41^{a}	13.88 ± 0.72^{a}
14	12.82 ± 0.47^{a}	13.29 ± 0.74^{a}

[1] Fat corrected (3.5%) milk yield,
[2] Mean ± SE
^{a,b} Means followed by different superscripts are significantly different ($p<0.05$)

Conclusions

The combination of improved fodder varieties harvested at the correct time, preservation as silage to be used in lean periods and supplements such as by pass fat can be successfully use to improve milk production in Sri Lanka, although further studies on methane production by ruminants in the country are warranted.

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USING NUCLEAR AND RELATED TECHNIQUES IN ANIMAL NUTRITION FACING CLIMATE CHANGES AND SUSTAINABILITY IN BRAZIL

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Abstract

Grasslands are among the largest ecosystems in the world and are important as a feed source for livestock and for providing secure livelihoods and economic opportunities. Nowadays, the wise use of land is a challenge and concern for more sustainable production and consumption of animal source food and forages play key role in ruminant production systems. Optimization models for ruminant production and emissions of greenhouse gas have shown that increasing production through improving pasture quality increases efficiency. This requires accurate knowledge of quantity and quality of nutrients consumed and natural tracers and the isotopic composition of forages animal faeces reflects the contributions of forage intake. We report the results of using n-alkanes, the isotopic signatures of ^{13}C and ^{15}N in forages and the undigested neutral detergent fiber (uNDF) for estimating dry matter intake and identifying the forages eaten by dairy cows fed tropical forages.

Introduction

Grasslands are among the largest ecosystems in the world and are important as a feed source for livestock. They have direct impacts on animal productivity, environmental protection, sustainable food security, satisfying livelihoods and economic opportunity (Mottet *et al*. 2018). The wise use of land is a challenge for sustainable production and consumption of animal source foods and achieving the sustainable development goals (Ryschawy *et al*. 2017, Vandermeulen *et al*. 2018, Congio *et al*. 2018). Forages play a key role in ruminant production systems of ruminants (Mottet *et al*. 2018), however, study of converting forage to high quality protein and energy has shown that increasing production through improving pasture quality leads to more efficient systems, with increased soil carbon stocks decreased emissions per kg of production (de Figueiredo *et al*. 2017; Makkar 2018; Savian *et al*. 2018). Solutions to reduce emissions in existing grazing systems involve the reduction of deforestation and intensification of the extensive systems (Cohn *et al*. 2014; Strassburg *et al*. 2014).
Evaluating feed consumption by animals is a valuable tool for grazing management, but the evaluation of green forage and nutrient intake on pasture can be costly, time consuming, labor intensive and not suitable over long periods (Decruyenaere *et al*. 2009a). Conventional technologies for objectively estimating intake of grass are limited. Natural tracers and the isotopic composition of forages animal faeces can be used to estimate forage intake. Near infrared reflectance spectroscopy (NIRS) of faeces is a rapid and easy analytical method that could be an interesting tool for managing grazing ruminants and optimizing production systems (Decruyenaere *et al*. 2009b; Decruyenaere *et al*. 2012), applying. Dove and Mayes (1996); Ali *et al*. (2005); and Bezabih *et al*. (2011) have suggested to use plant cuticular n-alkanes and stable carbon isotopic (^{13}C) composition as markers to estimate diet intake and composition by grazing herbivores.

This synopsis presents partial results of ongoing experiments on estimating intake and composition of tropical forages fed to dairy cattle.

Materials and methods

Assay 1 was run at a traditional dairy cattle experimental farm in the wet season, November 2017. Crossbred cows (n = 8) with 40-48 months of age, non-lactating and non-pregnant (347 ± 23.4 kg) were allocated to 8 individual pens (feed and water separately).
The rangeland supplied 5 plants, *Brachiaria* spp (BRAQ), *Cynodon nlemfuensis* (CYNO), *Pennisetum purpureum* (NAPI), chopped *Saccharum officinarum* (CANA) and *Tithonia diversifolia* (TITH) which were cut fresh daily to prepare a mixed diet. Forages were sampled individually on a daily basis prior to the preparation of the experimental diet at the proportions of BRAQ = 0.31, CYNO = 0.15, NAPI = 0.14, CANA = 0.21 and TITH = 0.18.
For five days, all cows were fed a mixture of the plants for adaptation to the diet. On d0 animals were randomly divided in two groups (n = 4) and individually offered two feeding regimes (LW = 0.8 and HG = 1.2 maintenance). On d1, all cows were dosed with two "bugs" of N-Alkanes at 09:00 and 17:00h and then with just one bug throughout d13. On d9 to d13 daily forages (05) and diet (LW and HG) offered and individual refusals were controlled, sampled and then kept under freezing conditions. From d11 to d13, six spot faeces samples (rectum) per cow were collected, twice daily: Day 11 (1st sample = 0h) then subsequent sampling times at 12, 26, 40, 54 and 68h. Individual total feacal collection were done at 24h intervals, weighted and sub-sampled.
Assay 2 was run on December 2018at the same traditional dairy cattle experimental farm. It used eight different cows (36 to 40 months of age, non-lactating and non-pregnant, 293 ± 19.9 kg) and it followed the same protocol as Assay 1 described above.
Forages were sampled individually on daily basis prior to the preparation of the experimental diet at the proportion of BRAQ = 0.17, CYNO = 0.18, NAPI = 0.26, CANA = 0.16 and TITH = 0.22. All the procedures with the animals, feed and feacal handling, N-Alkane dosing and sample preparation were done as for described for Assay 1.
All samples were split into 3 replicates, two of which were sent to APHL-IAEA Laboratories – Seibersdorf for determination of n-alkanes and the third replicate underwent wet chemistry at LANA-CENA/USP.
Through the analyses of feacal and diet C32 and C33 alkanes we estimated the intake from the C32 alkane dosed to each cow daily: Intake = (C32 dose ate / (F_{C32} : F_{C33} x (D_{C33} – D_{C32})) where F_{C32} : F_{C33} is C32:C33 feacal concentration ratio and D_{C32} and D_{C33} are respective C32 and C33 concentrations in diet offered.
The internal biological tracers, the isotopic signature of carbon and nitrogen, expressed in $\delta^{13}C$ and $\delta^{15}N$, the C:N ratio, the acid detergent fiber (ADF) content, ^{13}C and ^{15}N and uNDF in forages and feaces, were used for identifying the forages consumed by the cows. The isotopic ratio of $\delta^{13}C$ and $\delta^{15}N$ was determined in an elemental analyzer coupled to a mass spectrometer and the uNDF was determined through in situ incubation.

Results

Dry matter intake and organic matter digestibility determination in vivo

The diet intake and apparent digestibility determined through the calculations using the controlled data for offered and refused diet and feacal output are shown in Table 1.

Table 1. Intake and apparent digestibility of multispecies diet composed of *Brachiaria* spp, *Cynodon nlemfuensis*, *Pennisetum purpureum*, *Saccharum officinarum* and *Tithonia diversifolia* fed to dairy cattle at two feeding regime according to the treatments (LW = 0.8 and HG = 1.2 maintenance)

Group	ID	LW (kg)	Offered DM (kg/d)	Refusal DM (kg)	Faecal DM (kg/d)	Dry matter intake		Digestibility coefficient (%)			
						DMI (kg/d)	DMI (g/MW[1])	DMD	OMD	CPD	NDFD
2017	LW	337	6.4	0.05	2.5	6.3	81.0	0.59	0.60	0.62	0,54
	HG	352	9.6	1.75	3.3	7.8	96.5	0.45	0.45	0.47	0,35
2018	LW	295	4.8	0.01	2.1	4.8	67.3	0.56	0.57	0.60	0,53
	HG	289	6.3	0.21	2.5	6.1	87.7	0.57	0.57	0.64	0,53
Prob P		ns	***	***	ns	ns	ns	***	**	**	***
SE		10,50	0.83	0.75	0.14	0.08	2.49	0.075	0.074	0.098	0.098

[1] MW = metabolic weight (Kg $^{-0,75}$); ** = P < 0.01; *** = P < 0.001

Estimating diet intake using n-alkanes

The assay used a C32 dose rate of 934 mg/d and a feacal C32:C33 ratio of 2.5 ± 0.45 (n=8). The regression for DMI (kg/d) determined vs estimated through n-alkane dosing is show in **Figure 1**.

Estimating forages in the excreted feaces

The different forages consumed by the animals was estimated using the MixSIAR, a Bayesian mixing model that uses internal biological tracers to estimate the proportion of determined ingredients (sources) in products (mixtures) (Ward *et al.*, 2010).
For this analysis, the sources were the different forages, and the mixtures were the excreted feaces. The following tracers were used in this study: δ15N and δ13C as mass basis, as well as the content of ADF, uNDF and the ratio C:N in the uNDF residue. The Markov Chain Monte Carlo in MixSIAR was set as follows: chain length: 300000; burn: 200000; thin:100, and number of chains: 3. With these settings both Gelman-Rubin and Geweke diagnostics attested the chains convergence.
According to the MixSIAR output results, based on median values the main forage in the excreted feces was CYNO (67%) followed by TITH (20%). On the other hand, BRAQ (1%), CANA (2%) and NAPI (7%) constituted a small proportion of the excreted feces (Figure 2).

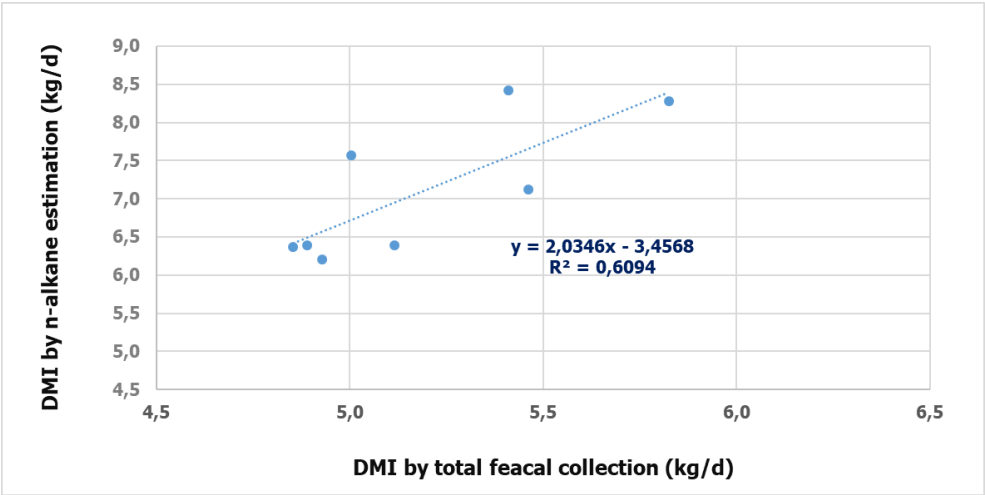

Figure 1. Observed vs estimated DMI (kg/d) for dairy cattle using n-alkanes as tracer

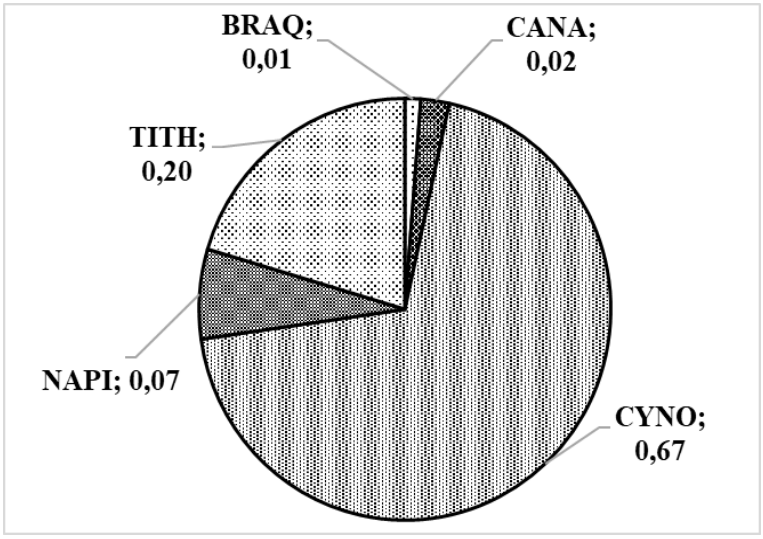

Figure 2. Proportion (%) of forages in faeces estimated by MixSiar using the internal tracers ADF content and $\delta^{15}N$ in dry matter, uNDF and $\delta^{13}C$ and C to N ratio in the uNDF residue

Conclusions

Using n-alkanes and the isotopic signatures of ^{13}C and ^{15}N in forages and faeces were able on estimating dry matter intake and on identifying the forages eaten by dairy cows fed tropical forages

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CHARACTERISATION OF THE OVARIAN CYCLE IN SYRIAN AWASSI EWES USING PROGESTERONE AND OESTRADIOL RADIOIMMUNOASSAY

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Abstract

Eighteen cycling Awassi ewes, aged about 3 years and weighing an average of 57.6 kg, were used during the normal breeding season for two consecutive oestrous cycles. Results showed a cyclic pattern in the serum progesterone concentration. The average length of 36 oestrous cycles was 17.0 days, divided into: 3.7 days, where progesterone concentration was low, averaging 1.03 nmol L^{-1}; followed by a sharp increase, which lasted an average of 9.8 days, with a mean progesterone concentration of 8.98 nmol L^{-1} (luteal phase); followed by a sharp decline in progesterone concentration, (follicular phase), that lasted an average of 3.5 days, with a mean progesterone concentration of 0.56 nmol L^{-1}. Serum oestradiol concentration, despite fluctuations, also showed a cyclic pattern. It was slightly high during the first 3 days of the oestrous cycle (22.91, 26.97, 35.27 pmol L^{-1}), followed by a concentration of around 20 pmol L^{-1} (18.73-21.37 pmol L^{-1}) for a duration of 10 days, and then, followed by an increase in the concentration that reached a maximum of 58.5 pmol L^{-1}, one day before the end of the cycle. A negative and significant correlation ($r = -0.56$, $P < 0.05$) was found between serum progesterone and oestradiol concentrations.

Introduction

The female oestrous cycle in most mammals is regulated by a variety of hormones of hypothalamic, pituitary and ovarian origin. Progesterone and oestradiol are steroid ovarian hormones that have several practical biological functions in the management of reproduction. In ewes, the oestrous cycle has been hormonally characterised in several breeds, such as Swakara and Damara (Kandiwa et al., 2019).
Awassi, a fat-tail triple-purpose sheep, is the local breed in Syria. The oestrous cycle of the Awassi breed has never been characterised. Therefore, the main objectives of the current experiment were: 1) to characterise precisely the different phases of the cycle, 2) to determine the normal serum progesterone and oestradiol patterns using radioimmunoassay; and 3) to study the relationship between the above two hormones throughout the oestrous cycle of a group of Syrian Awassi ewes.

Materials and methods

Eighteen cycling Syrian Awassi ewes aged about 3 years and weighing (Mean ± SD) 57.6 ± 4.5 kg were used during the breeding season. To determine the duration and hormonal characteristics of the different phases of the oestrous cycle in the Awassi ewes, ovulation was synchronised using intravaginal sponges containing 40 mg of flugestone acetate (FGA, Intervet, the Netherlands) for 14 days.
Blood samples (10 ml) were taken daily from the jugular vein for 45 days, covering the duration of two consecutive oestrous cycles, starting one day after the removal of the intravaginal sponges. Serum was harvested and stored at -20 °C until assayed. Validated progesterone and oestradiol radioimmunoassay (RIA) kits (COAT-A-COUNT, DPC, USA) were used. Progesterone levels equal to or exceeding 3.18 nmol L^{1} were considered indicative of normal luteal function, and levels under 3.18 nmol L^{-1} were

considered indicative of anoestrous, follicular, or early luteal phase of the oestrous cycle (Zarkawi, 1997).

Means of the studied parameters were subjected to an analysis of variance (ANOVA) test using the Statview-IV programme (Abacus Concepts, Berkeley, CA, USA) at the 0.05 probability level. In addition, the coefficient of correlation between progesterone and oestradiol concentrations throughout the oestrous cycle was calculated.

Results

Serum progesterone concentrations

The pattern of progesterone concentration was cyclic: low, followed by a sharp then a low concentration corresponding with the different phases of the oestrous cycle (Figure 1).

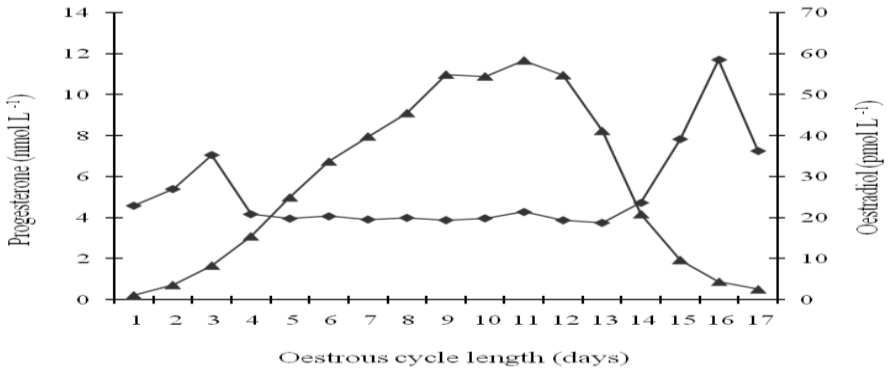

Figure 1. Patterns and serum concentrations of progesterone (▲) and oestradiol ( ) during the oestrous cycle of Syrian Awassi ewes (n=36)

Length of oestrous cycle

The mean length of 36 oestrous cycles was found to be 17.0 ± 0.7 days, ranging between 16-18 days. The majority of ewes (58.3%) had an oestrous cycle that lasted 17 days, whereas 19.5% and 22.2% had cycles lasting for 16 and 18 days, respectively. Overall mean serum progesterone concentration during the oestrous cycle was 5.61±2.64 nmol L^{-1}. Hormonally, and based on the activity of the corpus luteum, the oestrous cycle was divided into three phases:

- *Early luteal phase*
  The average length of this phase was found to be 3.7 ± 0.8 days, ranging between 2 and 5 days, with a mean progesterone concentration of 1.03 ± 0.93 nmol L^{-1}, ranging between 0.01 and 3.14 nmol L^{-1}.
- *Luteal phase*
  The length of this phase averaged 9.8 ± 1.0 days (range: 8-12 days), with a mean progesterone concentration of 8.98 ± 3.65 nmol L^{-1} (range: 3.24 -38.89 nmol L^{-1}). Maximum concentration of progesterone during this phase was 11.66 nmol L^{-1}, occurring on day 11 of the oestrous cycle.
- *Follicular phase*
  This phase had an average length of 3.5 ± 0.8 days (range: 2-5 days), with a mean progesterone concentration of 0.56 ± 0.60 nmol L^{-1} (range: 0.10-2.77 nmol L^{-1}).
  Thus, concentrations were below 3.18 nmol L^{-1} for an average of 7.2 ± 0.9 days, with a mean progesterone concentration of 0.80 nmol L^{-1}, and progesterone concentrations were above 3.18

nmol L^{-1} for an average of 9.8 ± 1.0 days, with a mean progesterone concentration of 8.98 nmol L^{-1}.

Serum oestradiol concentrations

A cyclic pattern in serum oestradiol concentration during the oestrous cycle, was found: slightly high for the first 3 days (22.91, 26.97, 35.27 pmol L^{-1}), followed by a concentration of around 20 pmol L^{-1} (18.73-21.37 pmol L^{-1}) that lasted for 10 days, and a sharp increase thereafter a day before the end of the oestrous cycle (58.5 pmol L^{-1}), with a sharp decline on the last day. Minimum and maximum serum concentrations of oestradiol during the oestrous cycle were 7.2 and 150.67 pmol L^{-1}, respectively, whereas, the overall mean concentration was 30.67 ± 13.42 pmol L^{-1}.

Relationship between serum concentrations of progesterone and oestradiol

During the oestrous cycle, the mean progesterone concentration (5.610 nmol L^{-1}) was more than 180 fold higher than that of oestradiol (30.67 pmol L^{-1}). A negative and significant correlation ($r = -0.56$, $P<0.05$) was found between serum progesterone and oestradiol concentrations during the different phases of the oestrous cycle. Figure 1 illustrates serum concentrations of both progesterone and oestradiol during the oestrous cycle of the Syrian Awassi ewes.

Discussion

The oestrus cycle length reported here (17.0 days) is similar to Swakara and Damara sheep, which also averaged 17 days (Kandiwa et al., 2019), and the pattern of progesterone concentration during the oestrous cycle is similar to that of Ghezel sheep (Najafi et al., 2014).
In the current study, overall mean serum progesterone concentration during the oestrous cycle was 5.61 ± 2.64 nmol L^{-1}, ranging from 0.01 to 11.66 nmol L^{-1}. In white Karaman ewes, Arsoy and Sağmanlıgil (2018) reported that the progesterone concentration during the oestrous cycle ranged from 0.01 to 9.0 nmol L^{-1}.
The serum oestradiol concentration pattern was also cyclic, but with an opposite shape to that found for the progesterone. This was confirmed by the negative and significant correlation between the concentrations of the two hormones during the oestrous cycle. Serum progesterone and oestradiol pattern in the Syrian Awassi ewe followed the luteal and follicular phases of the oestrous cycle; however, the progesterone pattern was more reliable in assessing the different phases of the oestrous cycle. Arsoy and Sağmanlıgil (2018) reported mean plasma oestradiol concentration of 31.67 pmol L^{-1} during the oestrous cycle in Karaman ewes, which decreased towards day 8 of the cycle and increased on day 11: 22.36 pmol L^{-1}. It decreased to its minimum level at the end of the cycle (day 15; 12.85 pmol L^{-1}).
Progesterone concentrations pattern confirms Cahill et al.'s (1981) suggestion that the corpus luteum constitutes the principal source of progesterone in ewes. Despite the cyclic pattern, the oestradiol concentration showed fluctuations until day 14 and then gradually increased from 23.62 to reach a peak of 58.5 pmol L^{-1} one day before the end of the oestrous cycle. Zieba et al. (2002) reported similar results in the serum concentrations of oestradiol in Olkuska ewes reaching 20.08 and 48.25 pmol L^{-1} on days 14 and on the day before ovulation during the oestrous cycle, respectively.

Conclusions

It was possible here, for the first time, to characterise the oestrous cycle of the local Syrian Awassi ewes. Assessing the physiology of the oestrous cycle of the Syrian Awassi ewes will be useful for future reproductive, genetic and/or breeding studies, pertaining to or dealing with this particular breed of sheep.

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BRACHIARIA RUZIZIENSIS AND *DOLICHOS LABLAB* MUTANT LINES FOR TMR FEED BLOCKS FOR SUSTAINABLE DAIRY CATTLE PRODUCTIVITY

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Introduction

Appropriate mutation induction is highly valuable in pasture species to increase genetic variability and improve performance. This study used mutant lines (M7) of *Brachiaria ruziziensis* and *Dolichos lablab* that had been developed through exposure of local seeds to gamma irradiation using ^{60}cobalt.

Materials and Methods

The mutant seeds were established at Lanet Research Centre (Nakuru, Kenya) and had been selected for 3 years based on yield, and nutritional content. Agronomic performance of the mutant lines was recorded and formed the basis for selection. At maturity the mutant lines were cut, dried and used along with mineral premix, binder and preservative for total mixed ration (TMR) block formulation. The TMR blocks were produced by using simple, cheap and available brick molds. Nutrient composition of the blocks was analyzed by wet chemistry. Nutritive value of the blocks was determined by evaluating the performance of ranched postpartum dairy cows. Thirty in-calf (7 months) Friesian dairy cows (20 treatment and 10 control) were used to study the effect of supplementation on metabolic status, ovarian function and postpartum reproductive performance. Feed blocks contained 16.8±1.1% crude protein and 45.7±9.5 % neutral detergent fibre. The treatment group was supplemented with 1kg TMR feed block per cow twice a day for an average of 30 days prepartum. (Supplementation was started 30 days prior to each cow's expected date of calving. Postpartum supplementation was at 2 kg feed block per cow twice a day for 60 days. Control cows grazed only on established pastures. Nutritional values of the pastures and feed blocks were determined. Water and minerals were given ad lib.

Results

Irradiation increased herbage yield of Brachiaria grass from 25 ton/ha (control) to 60 ton/ha (mutants line exposed to 40 Gy). Supplemented cows had lower NEFA of 0.33 compared to 0.35 nmol/l for control cows, lower Betahydroxybutyrate of 0.35 mmol/l compared to 0.36 mmol/l of control and higher glucose concentrations of 45.5±0.5 compared to 35.5±0.9 mg/dL for control. Milk progesterone was significantly higher ($p<0.05$) for the treatment group (3.48 nmol/l) compared to control (2.94 nmo/l) at 45 days postpartum. This result indicated resumption of cyclicity with 40% of treatment cows exhibiting standing heat by day 45 postpartum. IGF-1, insulin, leptin, P4, glucose and protein levels decreased as the cows approached calving and steadily increased. In contrast, the NEFA and BHB increased as the cows approached calving and during the first 15 days postpartum. Insulin levels were positively correlated with the leptin, glucose and P4 levels. There was a significant negative correlation between the NEFA and the P4 levels ($P=0.001$, $r = -0.628$). After calving, the BCS was negatively correlated with the NEFA and P4 levels. IGF-1 was negatively correlated with the NEFA and BHB levels.

Supplementation enhanced involution of uterus, early resumption of cyclicity and thereby early rebreeding.

Conclusions

We concluded that mutant *Brachiaria ruziziensis* and *Dolichos lablab* TMR feed blocks can be safely used as transition diet to modulate reproductive performance of dairy cattle.

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USE OF UNCONVENTIONAL AGRO-INDUSTRIAL BY-PRODUCT AS SUPPLEMENTATION OF GRAZING DAIRY CATTLE IN THE AMAZONIAN REGION OF PERU.

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Abstract

Use of unconventional agro-industrial by-products in diets of extensively raised dairy cattle and effect on productivity and economy of stakeholders was evaluated in the Amazonian region of Peru. Ten lactating cows were tudied in a simple cross-overesign with two periods of 21 days each; and two treatments, conventional supplementation (rice polishing) and a mix of unconventional agro-industrial by-product supplementation- MUABP (including rice polishing, rice middling, cocoa hull and coconut meal) compared. Significant differences in milk production ($p < 0.001$) between the MUABP (10.2 kg /cow/day) and conventional supplementation (8.8 kg /cow/day) were observed. Milk composition and body condition did not differ between the two treatments. TSupplementation with the MUABP was superior to the conventional supplementation by improving milk production and presumably the profitability associated with supplementation.

Keywords: cocoa hull, coconut meal, dairy cattle, Rice by-products, Tropic.

I. Introduction

Cattle husbandry in the Amazonian region of Peru is one of the most important activities for its inhabitants. However, the low nutritional quality and seasonal low availability of pastures [1], among other factors, negatively affect productive and reproductive performance, causing a negative economic impact for farmers. Faced with this problem, supplementation strategies have been proposed in order to meet the animals' nutritional requirements. In the Peruvian Amazon, the production of various crops such as rice, cocoa, coffee, orange, banana, *sacha inchi*, coconut, sugar cane, among others, generate-products that could be used for cattle feeding [2]. However, it is important to evaluate the incorporation of these by-products in animal diets and analyze their effect on livestock productivity. Therefore, the objective of this study was to evaluate the use of unconventional by-products as a supplementation in dairy cattle and its effect on animal production and the economic profitability of stakeholders in the Amazonian region of Peru.

II. Methods

Study area

The experiment was carried out in a farm located in San Martín region (200 mosl), with temperatures that vary between 21°C and 35 °C according to the season, being considered tropical rain forest-premontane [3].

Animals

Ten Holstein x Gyr crossbred lactating multiparous cows with average body condition of 3.2±0.14, 135±19.3 days in lactation and average live weight of 403.3±43 kg were used. The animals grazed about 16 hours/day in fields where *Brachiaria brizantha* predominated.

Treatments and experimental design

Cows were subject to a simple cross-over design with two periods of 21 days each (11 days of adaptation and 10 days of evaluation); and two treatments: conventional supplementation (rice polishing) and a mix of unconventional agro-industrial by-product supplementation - MUABP (including rice polishing, rice middling, cocoa hull and coconut meal) (Table 1). During the first period, "group A" received the conventional supplement (2 kg) and "group B", the supplement based on MUABP (3.5 kg). In the second period, the supplementation was reversed.

Table 1. Formula and nutritional composition of conventional and unconventional supplement based on agro-industrial by-products.

Ingredients	Conventional supplement	MUABP
		% (as fed)
Coconut meal	---	36.6
Rice polishing	100	33.4
Rice middling	---	13.1
Cocoa hull	---	11.7
Urea	---	2.3
Dicalcium phosphate	---	1.1
Salt	---	1.2
Minerals	---	0.6
Total	100	100
Nutritional content (dry matter basis)		
Crude protein (%)	12.3	21.8
Net Energy of Lactation (Mcal/kg)	1.5	1.7
In vitro dry matter digestibility (%)	50.2	36.7

Data collection

The variables evaluated were daily milk production, milk composition (fat, protein and lactose), initial and final live weights of the cows. Body condition data were collected along with the weighing.

Statistical analysis

The analysis of variance for simple change design was carried out on all data collected using SAS 9.4 [4]. The comparison of means between both treatments was done by the test of T (LSD) and Duncan at a level of significance of 5%.

III. Results and Discussion

Milk production and composition

Significant increases in milk production ($p < 0.001$) were observed with MUABP (10.2 kg /cow/day) in comparison to conventional supplementation (8.8 kg /cow/day) (Table 2). Regarding milk composition no significant differences were observed. The increased milk production could be explained by the better energy-protein balance of the MUABP and the best use of the supplement by animals [5]. The MUABP

supplement generate a benefit of $ 0.2 dollars per Kg of milk than conventional supplement considering intake and cost of supplement, average of total milk production and gross income per average of total milk production. As a consequence, these results showed an improvement in the economic profitability for the farmers.

Table 2. Average milk production (Kg/day), milk composition (%) and body condition of dairy cattle supplemented with agro-industrial by-products.

	Rice polishing	MUABP	p
Milk production (kg/day)	8.8[a]	10.2[b]	<0.001
Milk composition			
- Fat (%)	3.48[a]	3.50[a]	0.899
- Protein (%)	3.17[a]	3.17[a]	0.923
- Lactose (%)	4.54[a]	4.55[a]	0.828
Body condition (1-5 scale)	3.2 [a]	3.2 [a]	0.74

IV. Conclusions

The use of unconventional agro-industrial by-products of the San Martin region, such as coconut meal, rice middling and cocoa hull, as supplements in dairy cattle grazing pasture improved milk production and economic profitability of farmers.

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THE EFFECT OF HAY SUPPLEMENTATION ON PERFORMANCE OF GRAZING ALPACA IN THE PERUVIAN ANDES

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Abstract

Productivity of alpaca grazing rangelands in the Andes is often limited by the low availability and quality of those pastures during the dry season. The use as supplements of forages cultivated during the rainy season in appropriate areas in the Andes may be a strategy to improve performance of alpacas. Sixty-three female alpacas (15 months of age, 34 ± 1.0 kg BW), divided in three groups, grazing range pastures (6.1% crude protein and 61.3% NDF) during dry season in Puno region of the Peruvian Andes, were used for the study. Supplementation of oat-vetch pellets or oat hay was compared to a non-supplemented control group. The supplement was offered daily after grazing (400 g/alpaca/day). The study lasted for 84 days with evaluations of weight gain and intake every 28 days. Weight gain was greater for animals supplemented with oat-vetch pellet (3.1 ± 0.27 kg) compared to oat hay (0.98 ± 0.33) ($P \leq 0.05$) while the control group lost BW (0.64 ± 0.22). The total dry matter intakes (pasture and supplement) were 502, 575 and 579 g / alpaca/ day for the control, oat-vetch pellet and oat hay groups, respectively, indicating partial replacement of pasture by the supplement. Under the study conditions, the supplementation of oat-vetch pellets improved performance more than oats hay relative to no supplementation for alpacas grazing range pastures during the dry season.

Keywords: Supplementation; Alpaca; oat; vetch; pasture.

I. Introduction

Alpaca (*Vicugna pacos*) is the most important species for fiber production among South American camelids and is adapted to Andean environments with a high capacity to efficiently use dietary energy [1]. This animal feeds on pastures of low nutritional quality and is adapted to environments with poor food availability [2]. However, several studies predict that the adverse effects of climate change will worsen and will be more recurring in the coming years that will affect the availability of pastures and production of alpacas [3]. The use of nutritional supplements in critical periods may be an appropriate strategy to alleviate some nutritional deficiencies [4]. Therefore, the present study aims to evaluate the effect of supplementation of oat-vetch pellets or oat hay on weight gain and consumption of alpacas in range pastures during the dry season.

II. Methods

Study area

The study was carried out in the Instituto Nacional de Innovacion Agraria (INIA) experimental unit "Quimsachata" (4200 masl), Puno Region, Peru, which is characterized by having an average annual temperature of 7 °C, being considered Cool temperate moist forest [5].

Treatments

Sixty-three 15-month-old female alpacas of Huacaya breed were used. The animals were assigned to one of three treatments: grazing range pasture (T1), supplementation with oat-vetch pellets + grazing range pasture (T2) or supplementation with oat hay + grazing range pasture (T3). The animals after grazing received 400 g of oat-vetch pellets or alpaca oat hay/day. The variables evaluated were dry matter intake (grass and supplement) and weight gain every 28 days for 84 days during the dry season (July-October) of 2017. Total dry matter intake was calculated considering an intake of NDF equivalent to 0.9 percent of live weight [4]. As supplement intake was registered daily the difference was considered pasture intake. The chemical composition of range pasture, oat-vetch pellets and oat hay is shown in Table 1. Dry matter, crude protein, calcium and phosphorous content were determined according to the method of the Association Official Analytical Chemist [6]. The determination of Neutral Detergent Fiber (NDF) was carried out by the method of ANKOM Neutral Detergent Fiber in feed - Filter bags technique [7]. *In vitro* Digestibility of Organic Matter was determined by ANKOM Daisy Incubator. Metabolizable energy was estimated using the formula proposed by [8].

Table 1. Chemical composition (100% dry matter) of range pasture, oat-vetch pellets and oat hay

	Range pasture	Oat-vetch pellets	Oat hay
Dry matter, %	91.7	89.7	89.8
Crude protein, %	6.1	8.9	6.7
Neutral detergent fiber, %	61.3	55.7	46.6
Calcium, %	0.3	0.5	0.3
Phosphorous, %	0.2	0.2	0.1
In vitro organic matter digestibility, %	44.4	60.1	60.4
Metabolizable energy, Mcal/kg	1.7	2.31	2.32

Statistical analysis

The variables were evaluated through an analysis of variance, using a completely randomized design with 3 treatments and 21 repetitions with 3 subsamples of 7 animals per pen. The comparison of means was carried out by Tukey test with a level of significance of 5%. The statistical software used was SAS 9.4 [9].

III. Results

Dry matter intake

The average dry matter intakes of oat-vetch pellets and oat hay in dry matter were 327.4 and 280.5 g / alpaca / day, covering 57 and 48% of the ration, respectively. While the consumption of range pasture was high at the beginning of the experiment and decreased over time, this is probably due to shortage of pasture and higher intake of supplement.

Table 2. Dry matter intake and nutrients of animals

Period of evaluation	Treatment 1	Treatment 2			Treatment 3		
	Range pasture	Oat-vetch pellets	Range pasture	Total	Oat hay	Range pasture	Total
	Dry matter intake (g)						

28 d	507	269.1	289.9	*559*	256.8	312.1	*568.9*
56 d	509	356.1	226.2	*582.3*	293.6	292.3	*585.9*
84 d	489	357.0	228.6	*585.6*	291.0	292.7	*583.7*
Average	**502**	327.4	248	**575.6**	280.5	299	**579.5**
			Average nutrient intake				
Crude protein, g	30.6	29.1	15.1	44.3	18.8	18.2	**37**
Ca, g	1.5	1.6	0.7	2.4	0.8	0.9	**1.7**
P, g	1	0.7	0.5	1.2	0.3	0.6	**0.9**
Metabolizable energy, Mcal	0.85	0.76	0.42	1.2	0.65	0.51	**1.2**

Weight gain

The treatment supplemented with Oat-vetch pellets had a weight gain of 36.9 g/d, followed by the treatment supplemented with oat hay (17.9 g/d). These values are lower than the 95 g/d reported in female alpacas fed with 750 g of alfalfa pellets plus 1 kg of alfalfa hay [10]. The lower weight gain of alpacas in this experiment is due to the fact that they were grazed in the dry season with low quality pastures. While the treatment without supplementation lost weight (-0.01 g/d), this weight loss was due to the low supply of nutrients from range pasture and low *in vitro* organic matter digestibility (44.4%).

Table 3. Total and daily weight gain of animals

Period of evaluation	Treatment 1	Treatment 2	Treatment 3
01-28 d	0.57^{b} ± 0.24	1.81^{a} ± 0.33	0.57^{b} ± 0.25
28-56 d	0.19^{a} ± 0.24	1.05^{a} ± 0.31	0.67^{a} ± 0.20
56-84 d	-1.14^{b} ± 0.19	0.24^{a} ± 0.18	0.24^{a} ± 0.05
Total weight gain (kg)	-0.64^{c} ± 0.22	3.10^{a} ± 0.27	0.98^{b} ± 0.33
Daily weight gain (g/animal)	-0.01	36.9	17.9

IV. Conclusion

Alpacas supplemented with oat-vetch pellets improved performance better than oats hay or no supplementation for alpacas grazing range pastures during the dry season.

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PERFORMANCE AND RUMEN FERMENTATION OF WEST AFRICAN DWARF GOATS RAISED ON *PANICUM- BRACHIARIA* PASTURE SUPPLEMENTED WITH CASSAVA-BASED CONCENTRATE DIETS

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Introduction

The production of small ruminants in Nigeria has been influenced by inadequate nutrition resulting from low nutritional value of native pasture and natural grassland (Obua, 2005). Scarcity and prohibitive cost of conventional grains and legumes, aggravated by stiff competition between man and livestock for these feeds, as well as insufficient emphasis on production (Purcell, 1998), have prompted the search for alternative and cheap agro-industrial products as sources of feed. Crop residues have considerable potential as feed and reduce the cost of animal production without decreasing productivity. Many agricultural crop residues have great potential for feeding ruminants. Among such residues are cassava peels and leaves. (Oni *et al.*, 2017). Cassava peel is rich in metabolizable energy and very well degraded in the rumen (Onwuka, and Akinsoyinu, 1989). Cassava peel could be fed to ruminants either as a sole source of energy or as an energy supplement to foliage or poor-quality feed. Cassava leaf has high protein content (16.7-39.90%) with almost 85% of the crude protein as true protein (Ravindran, 1991). This study aimed at evaluating the performance of West African dwarf (WAD) goats under semi-intensive management fed *Panicum-Brachiaria* pasture supplemented with cassava-based concentrate diets.

Materials and Methods

The experiment was carried out at the Small Ruminant Unit of the College of Animal Science and Livestock Production (COLANIM) farm, Federal University of Agriculture, Abeokuta, Nigeria (Longitude 7°13'49.66''N, and latitude 3°26'11.98''E and 73m above sea level (Google Earth, 2019). Twenty WAD buck goats with an average weight of 6.50±1.50kg were used for the study and managed under the University's ethical guidelines for research studies. The animals could graze on Panicum-Brachiaria pasture between 10.00 hrs and 14.00 hrs and were later confined into individual pens. In confinement, cassava-based concentrate diets (Table 1) were offered to the animals at 5% body weight while water was made available for ad libitum intake. Data were collected on chemical composition, concentrate intake, weight changes, rumen fermentation parameters in the feeding trial that lasted for 84 days. Data collected were subjected to one-way analysis of variance (ANOVA) in completely randomized design using IBM SPSS v23 (2015).

Results and Discussion

The DM values (Table 1) for concentrate diets in this study were similar to 91.30%– 92.45% reported by Ogundu et al. (2018). The NDF values decreased with inclusion of cassava leaves while ADF values were lower than values (48.45 – 50.74 %) reported by Olamilusi et al. (2019). The inclusion of cassava

leaves increased the tannin and cyanide contents of diets, but these values were lower than those reported by Oni et al. (2014) and Fasae et al. (2016). Though all the goats were raised with the same pasture and supplemented with cassava-based concentrate diets, greater weight gain (Table 2) was recorded from those that consumed cassava leaves, which is attributable to better nutrient content, as seen from the chemical composition (Table 1).

Rumen pH values (Table 3) were higher than reported by Oni et al. (2014) and Wachirapakorn et al. (2016) but were within the normal range of 6.7–6.9 reported for optimal microbial digestion of fibre and protein (Wanapat, 1999). Increased rumen NH3-N was obtained as levels of cassava leaves increased in the diets, which could be due to the protein content of diets. This was close to optimal rumen NH3-N (15-30 mg/dL (Perdok and Leng, 1990) for increasing microbial protein synthesis, feed digestibility, voluntary feed intake and efficient rumen fermentation. The values for VFA were greater than recorded by Abegunde et al. (2019) with 1.97 mMol/dL, 0.26 mMol/dL, 0.18 mMol/dL and 4.44 mMol/dL reported for acetate, propionate, butyrate and total volatile fatty acid, respectively. Volatile fatty acid content (3.43 – 5.54 mMol/dL) was within the range (4-7 mMol/dL) reported by Ward (2011). The butyrate obtained in this study was greater than values (0.18–0.58 %) reported by Oni et al. (2014).

Table 1: Ingredient composition of the experimental concentrate diets

Ingredients	**Levels of inclusion of cassava leaves (g/kg)**			
Cassava leaves	**0**	**150**	**250**	**350**
Cassava Peel	350	350	350	350
Dried brewers' grains	200	100	50	-
Wheat offal	150	100	50	0
PKC	220	220	220	220
Oyster Shell	40	40	40	40
Salt	20	20	20	20
Sulphur	20	20	20	20
Total	**1000**	**1000**	**1000**	**1000**

chemical composition of concentrate diets (%)

Parameters	**Levels of inclusion of cassava leaves (g/kg)**					
	0	**150**	**250**	**350**	**SEM**	**P-value**
Dry matter	90.65^{c}	90.55^{c}	92.90^{a}	92.44^{a}	0.32	0.01
Crude protein	15.26^{d}	16.39^{c}	17.78^{b}	19.67^{a}	0.50	0.00
Crude fibre	11.13^{b}	10.17^{a}	11.23^{b}	13.78^{a}	0.40	0.02
Ether extract	6.14^{a}	5.49^{d}	5.63^{c}	5.95^{b}	0.08	0.01
Ash	15.95^{b}	12.55^{d}	14.82^{c}	18.64^{a}	0.66	0.00
Neutral detergent fibre	63.17^{a}	60.34^{b}	58.34^{c}	56.78^{d}	0.73	0.00
Acid detergent fibre	30.55^{c}	33.33^{b}	34.25^{a}	34.71^{a}	0.49	0.00
Acid detergent lignin	10.00^{a}	9.06^{ab}	8.28^{b}	10.01^{a}	0.25	0.01
Nitrogen free extract	77.12^{c}	82.02^{a}	80.89^{b}	73.74^{d}	0.98	0.01
Cellulose	20.55^{c}	24.27^{b}	25.97^{a}	24.71^{b}	0.63	0.00
Hemicellulose	32.62^{a}	27.01^{b}	24.09^{c}	22.07^{d}	1.20	0.00
Tannin (g/100g)	10.92^{b}	16.53^{a}	14.24^{ab}	14.05^{ab}	0.78	0.03
Phytate (mg/100g)	1.66^{bc}	1.44^{c}	2.72^{a}	1.90^{b}	0.16	0.00
Cyanide (mg/kg)	0.23	0.28	0.33	1.65	0.17	0.00

^{abc} *Means on the same row having different superscripts are significantly different ($p<0.05$)*

Table 2: Performance characteristics of the West African dwarf goats

	Levels of inclusion of cassava leaves (g/kg)					
Parameters	**0**	**150**	**250**	**350**	**SEM**	**P-value**

Total concentrate intake (kg)	17.18[a]	16.08[ab]	13.60[c]	14.52[bc]	0.47	0.03
Initial weight (kg)	5.33	6.67	6.33	6.50	0.46	0.79
Final weight (kg)	7.42[b]	8.71[ab]	10.00[a]	8.50[ab]	0.41	0.04
Weight gain(kg)	1.90[b]	2.04[b]	3.67[a]	2.00[b]	0.28	0.04

[abc] *Means on the same row having different superscripts are significantly different (p<0.05)*

Table 3: Rumen fermentation parameters of the West African dwarf goats

	Levels of inclusion of cassava leaves (g/kg)					
Parameters	**0**	**150**	**250**	**350**	**SEM**	**P value**
Rumen pH	6.87	6.80	6.88	6.94	0.80	0.46
Rumen temperature (˚C)	30.70	29.67	30.30	29.50	0.34	0.63
NH_3-N (mg/dL)	28.07[a]	23.70[b]	24.66[ab]	23.25[a]	0.74	0.00
Total VFA mMol/dL	5.54[a]	4.88[b]	3.66[c]	3.43[c]	0.27	0.00
Acetate (C_2) mMol/dL	3.69[a]	3.26[b]	2.44[c]	2.28[c]	0.18	0.00
Propionate (C_3) mMol/dL	2.46[a]	2.17[b]	1.63[c]	1.52[c]	1.19	0.00
Butyrate (C_4) mMol/dL	1.23[a]	1.09[b]	0.81[c]	0.76[c]	0.06	0.00

[abc] *Means on the same row having different superscripts are significantly different (p<0.05)*

Conclusion

Inclusion of cassava leaves in the diets of pastured goats reduced production of NH_3-N and VFAs. Inclusion at 250g/Kg decreased feed intake and optimised weight gain.
Dried cassava leaves should be used as feed for goat. The use of ^{35}S-methionine could be explored to monitor the efficacy of sulphur sources in the improved utilization of cassava leaves while nuclear technology could be used to track feed intake by grazing animals.

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ASSESSMENT ON CURRENT POPULATION SIZE AND RISK STATUS OF INDIGENOUS ENDANGERED SHEKO CATTLE BREED IN BENCH MAJI, SHEKA AND KAFFA ZONE SOUTH WESTERN, ETHIOPIA

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Current population size and risk status of indigenous sheko cattle breed in was conducted in three zone at 14 woreda of Bench Maji, Kaffa and Sheka Zone, Southern Ethiopia from September 2017 to May 2018. Sheko breed is one of the Ethiopian indigenous cattle breeds which represent the last remnants of Africa's original Bos taurus cattle that were probably the first to be domesticated in eastern Africa. The sheko cattle breed is only tryponotolerance breed in Ethiopia. Despite the unique characters and attributes of the breed, there is shrinkage in effective population size of the breed. The population estimate of the breed by the year 1999 was about 31,000, however, another estimates by the year 2007 indicated that the population size declined to 4040 a more recent estimates reported the population of the breed as low as 2400 heads. The current population size of Sheko cattle breed is estimated to be about 7252 this result to indicate more progress than the recent reports. The risk status of sheko cattle breed is the endangered. The mean reasons for the endangered of Sheko cattle breed were mainly attributed to their aggressive behaviour (78%), Interbreeding with zebu breed (6.5%), Lack of sheko cattle bull (6%) and lack of breeding policy for extinct breed (5.5%). To conserve the breed population number should increase through back crossing, monitoring and genetic evaluation of Sheko cattle breed, identification of the pure line of the Sheko breed through molecular evidences, level of genetic admixture of the pure line and inbreeding between relatives, organized and collaborate work among stockholders to conserve the breed.

Keywords: population size, Risk status, Sheko population,

Introduction

Indigenous African cattle can be broadly classified into four categories: humpless *Bos taurus*, humped *Bos indicus*, sanga (African humpless *Bos taurus*×humped *Bos indicus* hybrid), and zenga. African *Bos taurus* includes two groups, humpless shorthorns and longhorns. They mostly inhabit West and Central Africa. Both these groups are small in size and their productivity is lower compared to most of the zebu cattle populations in tropical areas (Rege, 1999). However, they have unique evolutionary adaptation to harsh climate (Hansen, 2004) and various endemic diseases. One of these adaptations is their documented tolerance to trypanosomosis (Roberts and Gray, 1973), a parasitic disease due to infection with *Trypanosoma* sp. whose vector is the tsetse fly.

Sheko is among the recognized cattle breeds in Ethiopia (DAGRIS, 2007) the breed represents the last remnants of Africa's original Bos taurus cattle which were probably the first to be domesticated in eastern Africa (Hanotte et al., 2000). These cattle were first reported in 1929 from South-western Ethiopia, and later in 1982 (Albero and Haile-Mariam 1982), at present some of the Sheko cattle manifest small humps that they inherited from zebu introgression. These cattle are generally smaller in body size and have shorter or no horns than the Humpless Longhorns, which made them much easier to manage. They also appear to have been deliberately developed for milk production (Rege, 1999).

The breed is known for their better performance and ability to survive, produce and reproduce in tsetse infested areas. Early information claims that Sheko breed is believed to have some level of trypanotolerant attributes (Alberro & Haile-Mariam, 1982; Epstein, 1971) and later research has supported this (Lemecha *et al.*, 2006; Taye *et al.*, 2007; Stein, 2011). The current distribution of the breed in the breeding tract coupled with the declining tendency for controlled pure breeding of the animals suggests genetic erosion of the breed at an alarming state of affairs (Elias, 2008).

The loss of cattle genetic diversity has accelerated for several reasons that include unbalanced assessments, genetic introgression, lack of market incentives, new technologies that intensified the use of some sires in detriment of others (Reproductive technologies such as Artificial Insemination—A.I, Embryo Transfer, etc.), political instability and natural disasters, among others (FAO,2007) Salient factors that contribute to the erosion of specific cattle breeds and their magnitudes still remain unclear.

The geographical distribution of Sheko cattle is mainly restricted to Bench Maji Zone and partly in the adjoining parts of Kaffa and Shaka Zones of south west Ethiopia (Taye, 2007). The population estimate of the breed by the year 1999 was about 31,000 (Rege., 1999), However, another estimates indicated that the population size declined to 4040 (Taye, 2007) a recent estimates reported by Dadi, (2009) revealed that the population of the breed become as low as 2400 heads

Current population size is an important factor in determining risk status. In addition to overall population size and growth rates, the risk status of a population is affected by other factors such as the number of herds, and the geographical concentration of the population, which influence exposure to threats (Woolliams, 2004). However, a mere headcount of animals, or even of animals of breeding age, does not give the whole picture with regard to risk status (FAO, 2007).

Reliable and accurate information on the breed census need to be available to make programs and policies that match the needs and requirements of the population. Unfortunately, information available on Ethiopian cattle breeds' population is scanty (Workneh, 2004) and information available on Ethiopian Sheko cattle breed population have been based on sampling survey (DADIS, 2000; DAGRIS, 2004; Takele, 2005). Even though these few works were able to provide information on Sheko breed population and distribution they are less reliable and does not succeeded to cover the entire breeding tract in southwest regions like Menit-Goldia and Menit Shasha. More importantly, conducting specific breed wise census at the time interval of 5 years in cattle, which can be best utilized to monitor the population status and trend in minimum time duration (Rawlynce, 2013).

Furthermore updating of the previous results and/or conducting to produce same data is vital since genetic resources and production systems are not static, routine inventories and on-going monitoring is needed (Sölkner, 1998) and was also important to keep hold of continuity and update users in general (CSA, 2016). Therefore, reliable statistical information is needed to guide the design and implementation of breed development programs that should be carried out in the rebuilding of the Ethiopian Sheko cattle breed.

Thus, this study was aimed at evaluation of current status, population size, and distribution of Sheko cattle breed in the entire breeding tract of south western part of Ethiopia to produce data that could be used for development, planning and policy formulation regarding the breed having the following objectives.

- To assess population size of sheko cattle breed in different concentration areas of south western Ethiopia
- To identify major breeding tract for Sheko cattle breed and their associated risk status in south western Ethiopia

Materials and Methods

Description of the Study Area

The study was carried out in Bench-Maji, Sheka and Kaffa zone of the Southern Nations Nationalities and Peoples Regional State (SNNPRS) in South west Ethiopia as shown in Fig 1.

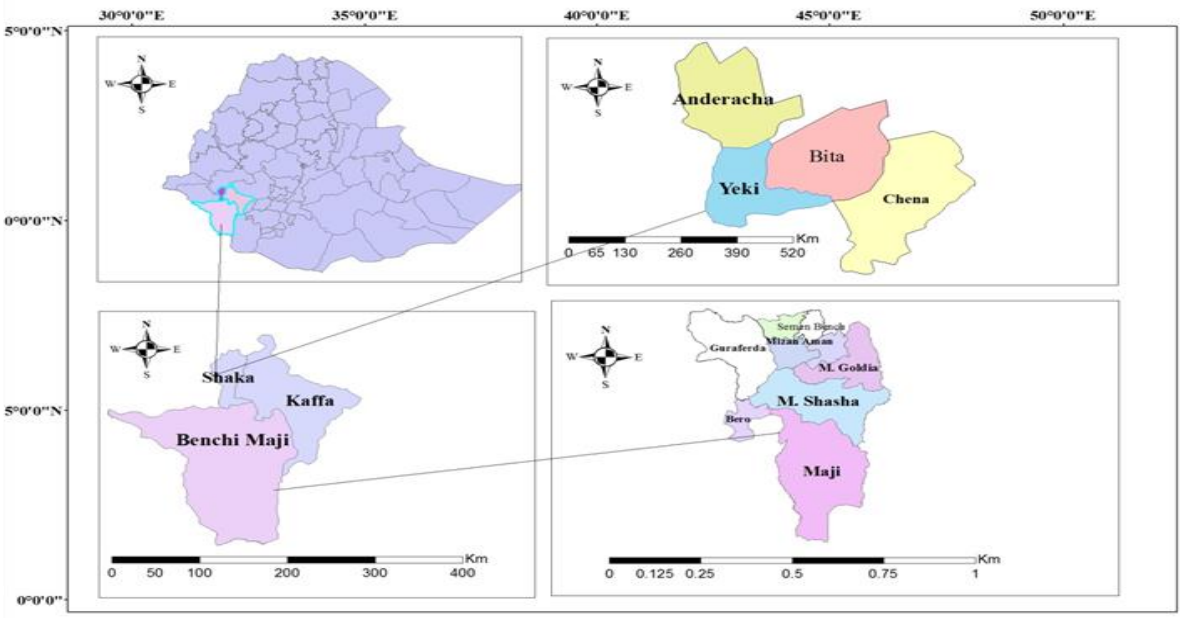

Figure 1. Map of the study area

Sampling techniques and data collection

A rapid field survey was conducted before the main survey. To identifies the geographical distribution and concentration of the breed. In each area focus group discussion was held with farmers and other stakeholders to identify and develop standards for Sheko cattle. Observable morphological, behavioral and performance levels was considered. Complete list of breed characters and photographs were collected from each area in the selected districts. Based on the developed standard guideline for the breed complete census were undertaken implemented in all areas except areas classified as no Sheko cattle concentration.

Morphological characters (qualitative) and body measurements (quantitative) were also taken from 250 Sheko cattle breed (198 female and 52 males) which was randomly selected from each of low, medium and high Sheko cattle concentration area based on sex and age.. In addition, 202 households were selected for interview using structured questionnaire to assess breeding practices, husbandry practices, constraints of production, interest of farmers on Sheko breed, risk status of the breed and so on.

2.3. Data Analysis

Breed characteristics obtained in group discussions and pictures was ranked and compared with respect to the breed descriptor list developed. The data collected through questionnaire and morphological record were analyzed by descriptive statics using SAS (SAS Institute Inc 9.1.3, 2003).In addition ,using similar soft ware association among categorical variables was assessed using Chi square test. General linear model was employed to test the effect of agro-ecology and districts on proportion of Sheko cattle breed.

Results and Discussion

Current status and distribution of Sheko cattle breed

The result of study showed that the population size of Sheko cattle breed was estimated to be about 7252 heads as indicated in Table1. As compared with the previous reports the population number varies greatly from as high as 31,000 (DAD-IS, 1999; Rege, 1999) to 4,040 animals in a field assessment (Taye, 2005) and 2,400 animals according to a recent census (Dadi, 2008) this variation attributed due to time and source of information. But the current result indicated that there was an increment on the number of sheko cattle breed this might be due to the previous data was based on sampling census and

did not cover all Sheko cattle breeding tracks, also there was conservation works stared by disseminate breeding male by natural mating and estrus synchronization and mass animal artificial insemination Sheko cattle with Sheko were undertaken, awareness creation works undertaken for farmers. Difference conservation practice made by regional and federal biodiversity institute and also difference applied by various organizations involved in the conservation program of animal genetics resources. The earliest procedure to identify breeds worthy of support as endangered breed was formulated by (Olivier, 2005).

Figure 2, pure sheko cattle breed

Table1. Current Population Size of Sheko Cattle Breed

Zone	Woreda	Sex						
		Female		Male intact		Male castrated		% Total
		N	%	N	%	N	%	
BMZ	MI/ AMAN	122	82.9	22	14.97	3	2.04	2.39
	N/ BENCH	421	73.3	153	26.6	0	0	9.32
	S/ BENCH	695	77.2	194	21.5	11	1.2	14.61
	SHEKO	618	76.5	119	14.7	70	8.6	13.10
	GURAFRDA	208	90	23	9.9	0	0	3.75
	MAJI	99	75	11	8.3	22	16.6	2.14
	SHI BENCH	1766	72	553	22.5	133	5.4	39.81
	MINT	332	69.1	118	24.5	30	6.2	7.19
	SHASHA MINT	285	75.8	86	22.8	5	1.3	6.10
	GOLDIYA BERO	39	63.9	22	36.07	0	0	0.99
	Sub total	4585		1301		274		6160
KAFFA	CHENA	261	73.5	92	25.9	2	0.5	75.69
	BETA	97	85	4	3.51	13	11.4	24.31
	Sub total	358		96		15		461
SHEKA	YEKI	429	75.4	116	20.3	24	4.2	91.33

	ANDRACHA	42	77.7	12	22.2	0	0	8.67
	Sub total	**471**		**128**		**24**		**623 7252**

The risk status of sheko cattle breed

The assessment of risk status of livestock breeds or populations is an important factor in planning of AnGR management (FAO, 2007, Gandinig, Olivier, 2005) conservation and genetic improvement. Population size, more importantly the number of breeding animals (Number of breeding males and Number of breeding females). According to the risk status criteria were the total number of Sheko cattle population was 7252. The Sheko cattle breed under the endangered categorized according to the criteria set reported by FAO (2000). What so ever the present population census of the Sheko cattle is alarming and calls for urgent steps the future conservation of this breed.
So to increase of this breed and protect from extinct need of the conservation of domestic animal diversity is essential to meet future needs (FAO, 2000). It is important to communicate about risk status of the breed to all the relevant stakeholders at national and state level including, animal and fishery resources, FAO, biodiversity center should also be informed about risk status of a breed at international level.

Table.2 the risk status of sheko cattle breed

Description	**ZONE**			**Over all**	**Risk status (FAO, 2007; Ganding, 2005)**	**Categorize for risk status results**
	BMZ	Kaffa	Sheka			
Breeding female	2893	260	259	3412	>500	Endangered
Breeding male	708	76	43	827	>20	
Total	3601	336	302	4239		

The level of sheko cattle breed

To identify the level of sheko cattle breed is very important for the future conservation practice. I have before identify the level of sheko cattle breed prepare selection criteria for sheko cattle breed based on previously report, farmer group discussion and physical observation then Finally agreed breed standard was used for further breed enumeration. Here we have to see this table.3. The Level of sheko cattle breed based on physical observation Estimated to be about (4073, 2934 and 1245) 1st, 2nd and 3rd level of sheko cattle breed, respectively. Currently to get pure sheko cattle breed is very rare in the home areas because interbreeding with zebu breed. To indicate this result 3rd level of sheko cattle breed is very far from 1st and 2nd level of sheko cattle breed need for the future gradually change of genetic makup of the animal. 1st level of sheko cattle breed physical characteristics is (above 80%blood level) no horn, humpless, long polled, rectangular face and long broad muzzle. 2nd level of sheko cattle breed physical characteristics is (above 60-80%blood level) small horn, small hump, medium polled, rectangular face and medium broad muzzle, 3rd level of sheko cattle breed physical characteristics is (above 40-600%blood level) medium horn, medium hump, small polled, rectangular face and small broad muzzle. To be indicate this result the 2nd and the 3rd level of sheko cattle breed is to need urgently conservation practice in the future because the integration with zebu breed higher than as compared to the 2nd level of sheko cattle breed

Table.3. The level of sheko cattle breed

Sex	Level of sheko cattle breed			
	1st level	2nd level	3rd level	
Female	3100	1614	800	
Male intact	815	307	403	
Male castrated	158	113	42	
Total	4073	2934	1245	7252

The reason extinct of endangerment of Sheko cattle breed

The reasons for declining population status of the Sheko cattle breed was due to their find interview and group desiccation farmers and Kebele extension worker. the reports was unmanageable and aggressive behavior 78.5%, interbreeding with zebu cattle 6.5%, , lack of bull 6% , lack of breeding policy 5.5% and lack of awareness 4%.
The result is agreement perversely report of reason Sheko cattle breed is indiscriminate inter breeding with local zebu, Sparse distribution over a wide geographical area, and a critical shortage of bulls (Takele, 2005; Bayou; 2015; DAGRIS, 2004; DAGRIS, 2007).
Another the mean reason extinct of Sheko cattle breed find by group desiccation is regular Artificial Insemination (AI), Estrus synchronization and Mass artificial insemination service all district more focus on local zebu cattle with Holliston Frisian and jersey breed semen inseminated but not inseminated Sheko cattle with pure Sheko breed semen program and by this case time to time to decrease the number of sheko cattle breed. Detail from Table 4.

Table.4 the reason for endangered of sheko cattle breedNReasons for endangerd

Aggressive behavior	**78**
Interbreeding with other local zebu breed	**6.5**
Lack of sheko bull	**6**
Lack of breeding policy for extinct breed	**5.5**
Lack of awareness	**4**

Conclusion and Recommendation

Sheko cattle breed is only one representative of the Hump less Shorthorn group of cattle in Ethiopia. At present, some of the Sheko manifest small humps that they inherited from zebu introgression. Genetic diversity study showed that Sheko breed is distantly related with other indigenous cattle populations found in Ethiopia. Sheko cattle breed is valued for its milk yield, adaptation and exhibit superior trypanotolerance than any other indigenous cattle populations found in Ethiopia. The total population of true Sheko cattle is at declining rate from time to time and considered as critically endangered. But the current total population size of Sheko cattle is estimated to be around 7252, which is some progress of the recent report. Farmers should be involved and engaged widely in breeding program. Management aspects and nutrition of sheko cattle should be also improved to fully utilize the breed performances.

The conservation strategy should be intensively implemented with the participation of the farmers and stakeholders. Identification of the pure line of the Sheko breed through molecular evidences, level of genetic admixture of the pure line and inbreeding between relatives should be identified. Semen collection work in place by NAGII & Biodiversity should be further strength & the semen already collected from sheko bull should be utilized as part as future conservation management. To pure sheko bull cattle should be disseminated to the farmer and to control free matting system. Changing in sheko breed cooperative society to breeding society. Establishment of the new cooperative society

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DEVELOPMENT OF LOCAL CALIBRATIONS FOR THE NUTRITIONAL EVALUATION OF FEED PROTEIN MEALS BY USING NEAR INFRARED REFLECTANCE SPECTROSCOPY

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Abstract

Commercial poultry farming is one of the fastest growing and most promising industries in Bangladesh. Due to the strong link between feed and food, the quality and safety of feed protein materials is of public concern. Soybean meal, full fat soybean, fish meal and meat and bone meal samples were collected from different locations and feed mills in Bangladesh. Near infrared reflectance (NIR) spectrum of protein meals were obtained and multivariate analyses with Partial Least Squares (PLS) algorithm were performed for the development of local calibration equations. High correlation between laboratory values and NIR values (R^2>90) were shown in predicting nutrient contents in all feed protein meals although the correlation (R^2) is relatively lower in predicting CP (78.34), EE (83.29) and total ash (82.32) contents in soybean meal; EE (88.45) and P (75.41) contents in fish meal and Ca (85.84) and P (82.23) contents in meat and bone meal. The highestroot mean square error cross validation(RMSECV) was observed in CP and total ash determination of fish meal (1.15% and 1.01%) and meat and bone meal (1.27% and 1.32%), However, RMSECV in predicting other nutrients of feed protein meals were in acceptable ranges indicating the potentiality of NIRS for predicting nutrient content in feed protein meals.

Keywords: Nutrient evaluation, Feed quality, NIRS, Soybean meal, Fullfat soybean, Fish meal and Meat and bone meal

Introduction

Commercial poultry farming is one of the fastest growing and most promising industries in Bangladesh. Intensive production systems, however, depend heavily on compound feeds and ingredients, the cost of which represents 65-70% of the total cost of poultry production. Feed protein materials (soybean meal, full fat soybean, fish meal and meat and bone meal) are usually added to poultry feed as protein sources. Driven by a booming need for livestock production in Bangladesh, the consumption of feed protein materials in 2018 has amounted to about 1.5 million metric tons in manufacturing 4 million metric tons commercial feeds (BPICC, 2018). Due to the strong link between feed and food, the quality and safety of feed protein materials is of public concern. The Fish Feed and Animal Feed Act of 2010 was passed by the government of Bangladesh to ensure the safe use of feed protein materials (GoB, 2010). The effective enforcement of these regulations requires efficient and accurate analytical tools. Presently, traditional wet chemical analyses are commonly employed and mainly focus on targeted parameters or properties of feed protein materials, which is time-consuming, laborious, and costly. Near-infrared reflectance (NIR) spectroscopy, while much simpler and more rapid than traditional analytical methods, typically requires grinding a sample to a fine particle size to give a smooth and homogeneous surface

for reflection but requires no chemical reagents, therefore, avoids the problems of organic and other chemical waste disposal. Once calibrations are in place, it takes just minutes to have the result of one or more constituents, which by conventional chemistry may take hours or days. The technique has extensive application for the analysis of constituents of agricultural crops, feeds and foods (Williams and Norris, 2001; Roberts et al., 2004). However, little is known about the potential of NIR spectroscopy for the nutritional evaluation of locally available ingredients in Bangladesh, as well as other parts of Southeast Asia, and quick prediction of nutritional quality of feed ingredients in the feeds is necessary for achieving sustainable poultry production. Therefore, the aim of this study was: a) to develop local calibration procedures using NIRS and validation of calibrations for the evaluation of locally available soybean meal, full fat soybean, fish meal and meat and bone meal accurately and b) to determine the nutritive value of feed protein materials within shortest possible time.

Materials and Methods

Soybean meal, full fat soybean, fish meal, and meat and bone meal samples were collected from different locations and feed mills in Bangladesh. Near infrared reflectance (NIR) spectra were obtained in duplicate (scanning number 32, resolution 8) with a monochromatorsystem (700-2400 nm) using a Quartz cup sampling device. Multivariate analyses were performed for the development of calibration equations of these feed protein materialsaccording to the procedure of Conzen (2003). Data were centered using Partial Least Squares (PLS) algorithm and spectral outliers were identified from each calibration. The standard error of calibration or root mean square error of estimation (RMSEE) was calculated. A comparison of the resulting analysis values with the original raw data allowed the calculation of the predictive error of the complete data system, the root mean square error cross validation (RMSECV).In addition, coefficient of determination (R^2) from the linear regression of measured values of nutrient component determined by analytical laboratory versus predicted values by the NIR calibration was calculated to give the accuracy of the model. During validation, potential outliers could be detected easily and only after all outliers had been removed from the calibration data set, and finally after the optimum parameters had been found, the calibration model was established. Sample moisture, crude protein (CP), crude fat (EE), total ash, calcium (Ca) and phosphorus (P) were determined according to the procedures of AOAC (2000).

Results and Discussion

Table 1 summarizes the results of the analyses. High association between laboratory values and NIR values (R^2>90) were shown in predicting moisture content in soybean meal; all nutrient contents in full fat soybean meal; moisture, CP, total ash and Ca contents in fish meal; and moisture, CP, EE and total ash contents in meat and bone meals. These results demonstrate that the NIR has high potential for determining nutrient content in feed protein meals. The $R^2$was relatively lower in predicting CP (78.34), EE (83.29) and total ash (82.32) contents in soybean meal; EE (88.45) and P (75.41) contents in fish meal and Ca (85.84) and P (82.23) contents in meat and bone meal, however. The samples were collected from different locations of Bangladesh and variety of the samples might be the possible reasons for higher variations of nutrients. Sometimes, if too many factors (rank) are chosen, the model tries to account even the smaller changes in data set, which creates spectral noise ("overfitting"). In this case, spectral information, unspecific for the sample is included in the model and the deterioration of the analysis results is also to be expected from the model (Conzen, 2003).
The highest RMSECV was observed in CP and total ash determination of fish meal (1.15% and 1.01%) and meat and bone meal (1.27% and 1.32%), but the RMSECV in predicting other nutrients of feed protein meals were within acceptable ranges. The results of the present study revealed that NIRS could potentially be used to predict the nutrients in feed protein meals available in Bangladesh. The use of NIRS could save money and time and be more environmentally friendly, be eliminating the use of polluting chemicals.

Table 1. General and calibration (NIR) statistics for the chemical composition of feed proteinmaterials

Nutrients	Sample No	General statistics (%)			Calibration (NIR) statistics		
		Min.-Max.	Mean	SE[1]	Rank	RMSECV[2](%)	R^2
Soybean Meal							
Moisture	552	7.90-14.22	11.77	0.063	09	0.253	92.30
CP	231	44.36-51.47	46.46	0.038	06	0.328	78.34
EE	238	1.00-3.99	1.99	0.053	07	0.221	83.29
Total ash	235	5.18-8.40	6.32	0.102	11	0.267	82.32
Full fat Soybean							
Moisture	209	4.05-10.51	7.57	0.288	10	0.203	99.29
CP	209	33.94-43.24	36.50	0.046	08	0.417	90.24
EE	209	16.66-21.77	19.50	0.047	10	0.460	96.72
Total ash	209	4.05-9.42	5.32	0.156	09	0.178	89.49
Fish Meal							
Moisture	688	3.40-14.90	7.65	0.088	09	0.387	93.25
CP	944	47.0-70.20	61.21	0.213	13	1.150	91.11
EE	595	3.32-19.00	9.05	0.232	09	0.813	88.45
Total ash	683	5.40-32.65	20.13	0.526	09	1.010	94.10
Ca	338	2.65-10.71	6.00	0.085	11	0.404	91.22
P	140	1.96-5.08	2.57	0.065	08	0.230	75.41
Meat and Bone Meal							
Moisture	618	2.30-10.0	4.90	0.055	08	0.365	90.88
CP	675	34.86-66.18	51.63	0.528	08	1.270	93.81
EE	602	5.98-19.30	11.18	0.088	08	0.617	90.09
Total ash	612	12.02-46.70	28.42	0.812	07	1.320	95.67
Ca	346	2.67-15.06	9.04	0.088	07	0.660	85.84
P	177	2.41-6.52	4.47	0.036	08	0.377	82.23

[1]SE = Standard error; [2]RMSECV = Root Mean Square Error Cross Validation; R^2 = correlation coefficient

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DAIRY GOAT PRODUCTION IN INDIA

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In India, development of the goat sector has the potential to impact the livelihoods of 20 million goat rearers (1) belonging to resource poor and socially backward segments of the society living in ecologically vulnerable areas. Goat rearing is a traditional backyard activity supplementing household income, largely considered as a moving ATM to be sold in situation of emergency. It contributes about 10 to 40 % of household income in different regions.

Dairy goat farming is slowly gaining momentum in India. In order to assess the dairy goat industry in India, the published data on dairy goat breeds and their performance over the periods in different agro-climatic conditions were collected, compiled and presented as per the standard methods (2) to give an analytical overview of the dairy sector in India. The basic animal husbandry statistics, viz., livestock population, growth rate and the milk production were collected from the livestock census reports over the decades (i.e., 1951 to 2019) and analysed.

The study revealed a positive population growth rate in cattle, buffaloes, sheep and goats in most of the periods (Table 1). The goat population of India as of 2019 is 148.88 million and an increase of 10.14 % has been noticed (Fig 1) over the previous census period. It is 27.8 % of the total livestock population of the country. About 95 % of the goats are reared in rural areas and contribute greatly to the rural economy.

Table 1. Growthpattern of livestock populations of India from 1951 to 2019

Species	Annual Growth Rate (%)											
	1951-56	1956-61	1961-66	1966-72	1972-77	1977-82	1982-87	1987-92	1992-97	1997-2003	2007-12	2012-19
Cattle	2.19	10.65	0.34	1.19	0.95	6.92	3.76	2.45	-2.79	-6.89	7.50	-4.10	0.8
Buffaloes	3.46	14.03	3.52	8.30	8.01	12.55	8.87	10.85	6.78	8.90	7.58	3.19	1.0
Sheep	0.51	2.29	5.47	-5.66	2.50	18.93	-6.28	11.12	13.21	6.92	16.41	-9,07	14.1
Goats	17.37	9.93	6.08	4.49	12.00	25.99	15.71	4.60	6.45	1.34	13.01	-3.82	10.1

Source: (3; 4; 5)

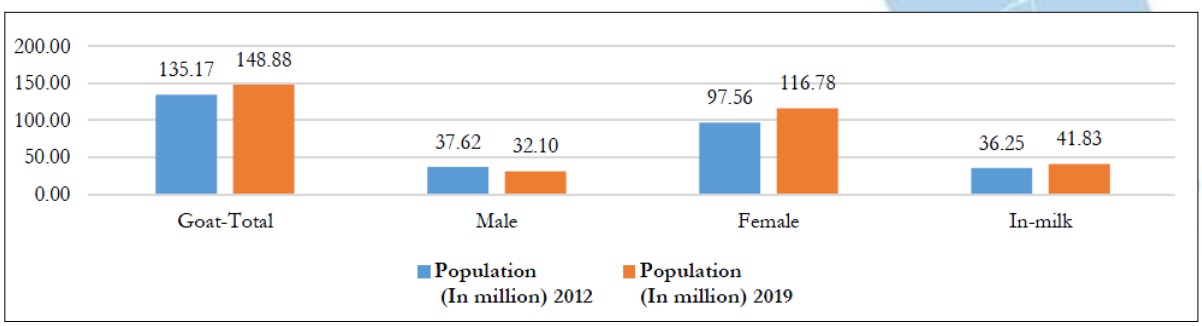

Fig 1. Goat population as on 2012 and 2019 in India

The top ten goat rearing states in India are Rajasthan (14.0%), West Bengal (10.9%), Uttar Pradesh (9.7%), Bihar (8.6%), Madhya Pradesh (7.4%), Maharashtra (7.1%), Tamil Nadu (6.6%), Jharkhand (6.1%), Odisha (4.3%) and Karnataka (4.1%). As per the 2019 livestock census, the number of animals in milk in the country is 181.99 million, which includes cattle (40.8%), buffalo (21.0%) and goats (38.3%).

Goat milk production represents only 2.3 % of the total milk produced around the world. India is the largest goat milk producer in the world with a share of 20.17 %. The milk production of goats has increased from 3.266 million tonnes in 2000-01 to 7.052 million tonnes in 2017-18. When compared to cows and buffaloes, the annual growth of goat milk production is low. The top five states in terms of the goat milk production estimate in India are: Rajasthan, Uttar Pradesh, Madhya Pradesh, Gujarat and Maharashtra.

India has rich repository of goat genetic resources and its genetic resources are reflected by the availability of 34 breeds of goats (6). Among the recognised breeds of goats, four breeds are dairy goats (*viz.*, Beetal, Surti, Jhakrana and Malabari), eight breeds are dual purpose breeds (i.e., Jamunapari, Sirohi, Barbari, Marwari, Kutchi, Mehsana, Zalawadi and Osmanabadi) and produce fairly good amounts of milk and the remaining are of meat type breeds. The body weight and milk yield details of the important dairy and dual-purpose goat breeds are presented in Table 2.

Table 2. Body weight and milk yield details of dairy and dual purpose breeds of goats

Name of Breed	Original State	Body weight Buck (kg)	Doe (kg)	Average daily Milk yield (kg)	Breed wise population (lakh)
Dairy Breeds					
Beetal	Punjab	59.07	34.97	1.16	07.15
Surti	Gujarat	29.50	32.03	2.50	04.06
Jhakrana	Rajasthan	57.80	44.48	3.18	14.46
Malabari	Kerala	38.96	31.12	0.65	05.69
Dual Purpose Breeds					
Jamunapari	Uttar Pradesh	44.66	38.03	1.06	39.13
Sirohi	Rajasthan	50.37	22.54	0.41	30.77
Barbari	Uttar Pradesh	36.70	20.30	0.71	62.82
Marwari	Rajasthan	33.18	25.85	0.53	71.83
Kutchi	Gujarat	46.96	39.91	1.84	04.43
Mehsana	Gujarat	37.00	32,00	1.32	06.11
Zalawadi	Gujarat	38.84	32.99	2.02	05.32
Osmanabadi	Maharashtra	33.66	32.52	0.50	00.14

Source: (7; 8; 9)

Milk production from goats in India is essentially a subsistence enterprise serving the needs of rural people. Of late, there has been an increased awareness about the importance of goat milk in human nutrition and hence there is higher demand for goat milk and it can be sold at higher price than milk from cows and buffaloes. Recent initiatives on commercial goat farming and processing have focused on goat milk to capitalize on the nutritional value and market for high value processed products.

The dairy goat industry in India has slowly started to adopt scientific advances. The main marketing limitation for prospective dairy goat producers is the number of commercial processors to whom raw milk can be shipped. Dairy goat production in India is an alternative livestock enterprise suitable for many small-scale or part-time livestock operations. To very resource-poor peasant farmers in India, rearing of dairy goats is better than cattle because of its faster generation turnover, earlier maturity for milk production and lowest cost per animal.

The goat probably will never replace the cow for commercial production of milk, but there seems to be a great potential for diligent efforts in practice and research to improve production and marketing of goat milk and its products. Developing the sector will require focused efforts on up-grading of dairy breeds, support to commercial farms, encouraging entrepreneurs to invest in the sector through training programs on processing, along with facilitating access to financing.

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DYNAMICS OF SELECTED CYTOKINE CONCENTRATIONS, UTERINE INFLAMMATION AND FERTILITY AFTER PROTEOLYTIC ENZYME TREATMENT OF CYTOLOGICAL ENDOMETRITIS IN ESTRUAL WATER BUFFALO.

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Synopsis

Subclinical/cytological endometritis (SCE) is the inflammation of endometrium without any clinical manifestation. SCE remains mostly undiagnosed and untreated, as its diagnosis necessitates the evaluation of inflammatory status of endometrium (Galvão et al 2011). It is an important cause of subfertility in dairy herds. Moreover, diagnosis and treatment of SCE at the time of breeding, the optimum time to ascertain its effects on imminent fertility, is of utmost importance. Currently employed treatments for SCE including antibiotics, prostaglandins and anti-inflammatory drugs yield inconsistent results. The interplay of pro-inflammatory and regulatory cytokines controls the amplitude, direction and duration of inflammatory response. Our objective was to evaluate the effects of intrauterine administration of proteolytic enzymes on endometrial inflammation and conception rates in water buffaloes diagnosed with SCE at estrus. We hypothesized that endometrial inflammation, reflected by changes in associated cytokine concentrations and polymorphonuclear cells (PMNs), would be reduced by the proteolytic enzymes. A total of 97 buffaloes in spontaneous estrus presented for artificial insemination were screened for SCE by the cytobrush based endometrial cytology technique using ≥5% threshold for PMNs (Melcher et al 2014, Singh et al 2020). Buffaloes (*n*=22) diagnosed with SCE were randomly allocated into two groups. The treatment group (TR; *n*=11) received intrauterine infusion of trypsin, chymotrypsin and papain dissolved in 20 ml of normal saline, whereas the procedural control (PC; *n*=11) received 20 ml of saline, at the same estrus (E1). Buffaloes without SCE (<5% PMNs) and kept untreated acted as the negative control (NC; *n*=14). Uterine ultrasonographic examination and samplings (endometrial cytology, low volume uterine flush, blood, Galvão et al 2011) were carried out before the intrauterine infusions. Similarly, ultrasonographic examination and samplings at the subsequent estrus (E2) were conducted in all the animals, without any intervention. Concentrations of cytokines in uterine flush and serum samples were estimated by bovine specific quantitative ELISA kits. Buffaloes were artificially inseminated (AI) twice at the subsequent estrus (E3) and fertility parameters (until 110 days from the start of experiment) were recorded. Post-treatment, percent PMNs decreased ($P<0.05$) in the TR group only (Fig.1). Endometrial thickness was significantly ($P<0.05$) greater in the SCE positive (PC and TR) compared to healthy buffaloes at E1, and decreased post-treatment ($P<0.05$) in the TR group only (Fig. 2). The uterine concentrations of interleukin (IL)-1β and TNF-α and serum concentration of TNF-α were significantly higher at both E1 and E2 in the buffaloes with SCE (PC and TR) compared to the healthy counterparts. Post-treatment, a significant decrease in IL-1β and increase in IL-6 uterine concentrations was recorded at E2 compared to E1 in the TR group (Fig. 3). Post-treatment, uterine and serum concentrations of TNF-α were significantly lower at E2 than at E1 in the TR group. Uterine and serum IL-8 concentrations were significantly higher at E1 in buffaloes with SCE (PC and TR) than in healthy counterparts. IL-8 concentrations in uterine flush dropped significantly in SCE positive buffaloes irrespective of treatment (Fig. 3). The IL-10 concentrations were uniform in uterine flush as well as serum between the groups at E1 as well as E2. Pregnancy rate at first AI were

similar in PC (27.3%) and NC (42.9%) groups, with a non-significant improvement in the TR group (45.4%) (Table.1). Median days to conception from first service were similar for all the groups (*P* >0.05). Overall pregnancy rate (until 110 days from the start of experiment) was non-significantly greater in TR (72.7%) compared to PC group buffaloes (45.4%). In conclusion, our results recorded the amelioration of endometrial inflammation and associated differential changes in cytokine concentrations. Hence, application of the proteolytic enzymes in a larger cohort of animals is warranted to corroborate the effects.

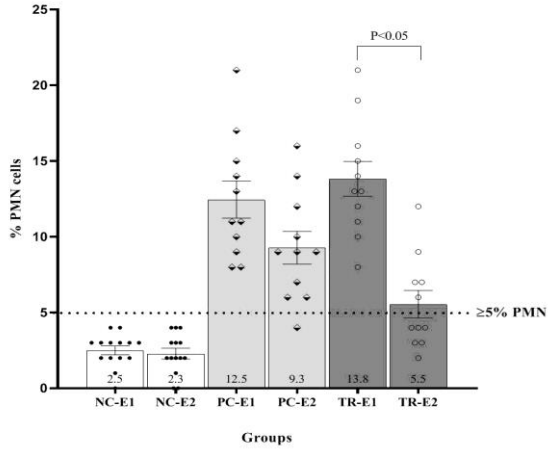

Figure 1. Percent (Mean±SE) PMNs.

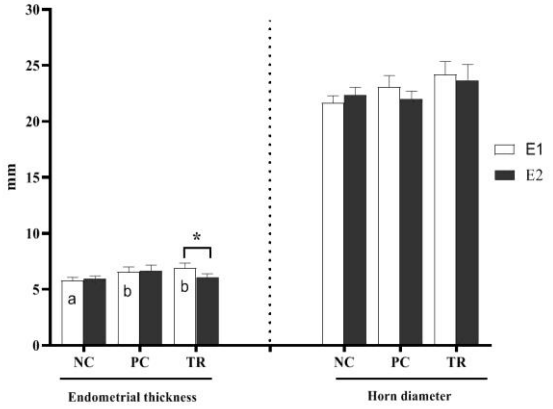

Fig 2. Endometrial thickness and uterine horn diameter.

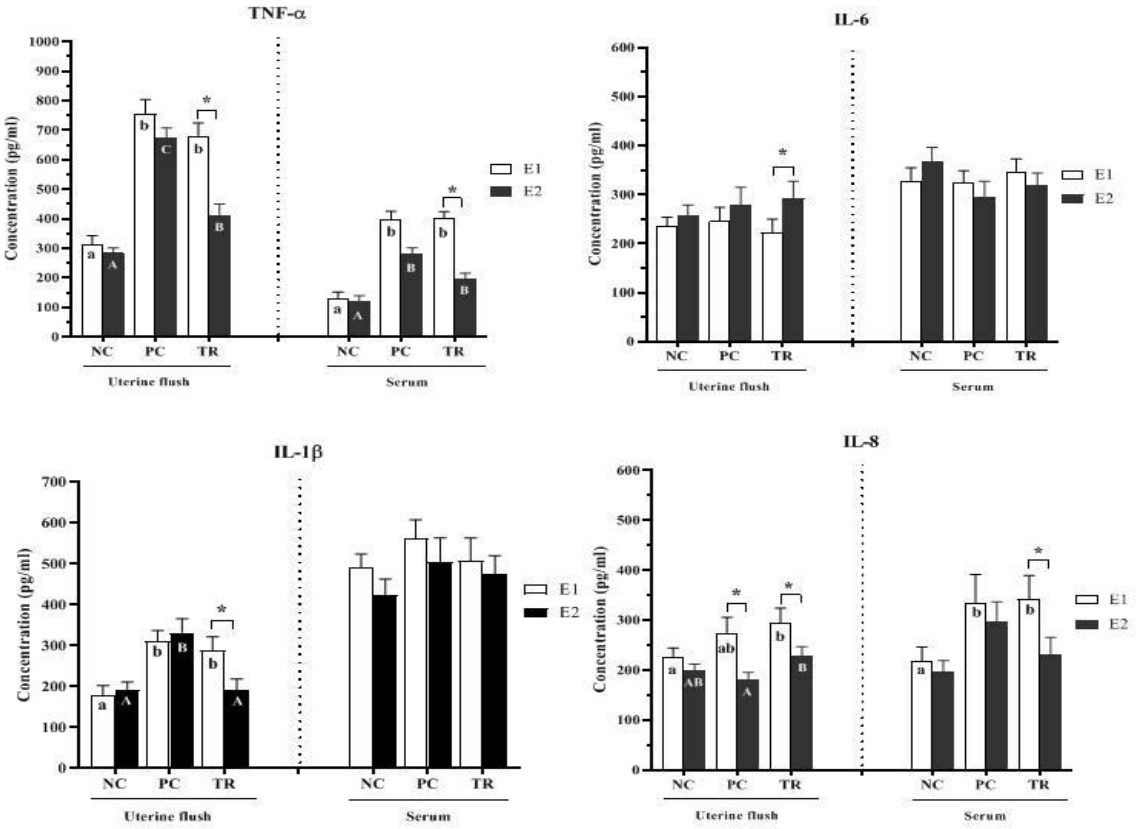

Fig. 3. Cytokine concentrations (pg/ml) in uterine flush and serum.
Asterisk (*) indicates significant (P <0.05) difference

Table 1: Reproductive variables of estrual buffaloes treated for subclinical endometritis (SCE) in comparison to those without SCE.

Observation (n=36)	**Negative Control** (n=14)	**Positive Control** (n= 11)	**Treatment** (n= 11)
Median days to conception from first AI (95% CI)[¥]	34[a] (26-51)	62[b] (42-81)	44[ab] (22-58)
No. of AI per pregnancy (95% CI)[$]	1.8[a] (1.6-2.5)	2.7[b] (2.1-2.9)	2.2[ab] (1.7-2.4)
Pregnancy rate at first AI[#]	42.9% (6/14)	27.3% (3/11)	45.4% (5/11)
Overall pregnancy rate (until 110 days from the start of experiment)[#]	71.4% (10/14)	45.4% (5/11)	72.7% (9/11)

Different superscripts (a,b) indicate significant (P<0.05) difference.
Statistical test applied: [¥]Log-rank (Mantel-Cox) test; [#]Chi square test; [$]Kruskal-Wallis test.
SCE=Subclinical endometritis; NC=buffaloes without SCE kept as untreated control
TR=buffaloes diagnosed with SCE and treated with proteolytic enzymes
PC= buffaloes diagnosed with SCE and treated with vehicle

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EVALUATION OF DIGESTIBILITY DISCREPANCY OF DIFFERENT PARTS OF CORN STOVER VIA A SIMPLE CO-CULTURE OF AN ANAEROBIC FUNGUS AND METHANOGEN

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Objective

The structural components and nutritional value of different crop straws are very different; even the structural components and nutrient utilization rates of different parts of the same straw are significantly different. There are differences in the content and structure of cellulose and hemicellulose in the stem bark, leaf blade and stem pith of corn stover. Therefore, the potential of these components to produce feed is different. The purpose of this experiment was to study the differences in the degradation and utilization of different parts of corn stover by anaerobic fungus and methanogen co-culture, and to provide a scientific basis for the comprehensive utilization of corn stover.

Materials and methods

The corn stover was collected, air-dried, and separated as the leaf blade, stem pith, and stem bark, to serve as substrates in the present study divided into three groups. The co-culture of anaerobic fungus and methanogen (*Pecoramyces* sp. F1 & *Methanobrevibacter thaueri*) was previously isolated from goat rumen. The co-culture was grown at 39°C for 72 h in 90 ml media containing of the different substrates. The gas and methane production were measured every 6h. The pH was determined at the end of fermentation and supernatant was collected for the analysis of fermentation metabolites and carboxymethyl cellulase and xylanase activity. The substrates were collected for the analysis of *in vitro* digestibility of dry matter, neutral detergent fiber, and acid detergent fiber. The water-soluble metabolites were determined using gas chromatography. Data were analyzed using SPSS 20.0 and the significance was declared at $P < 0.05$.

Results

The stem bark of corn stover had the lowest digestibility and methane production, as it had the lowest contents of neutral detergent solution and hemicellulose, and had the highest contents of cellulose and lignin (as shown in Table 1). Stem pith and leaf blade of corn stover had similar gas and methane production, though their digestibility and chemical composition were significantly different. The pH, activity of fiber degrading enzymes, water-soluble metabolites were significantly different among three groups.

Table 1. Gas production, methane production, chemical composition and degradation of the stem bark, leaf blade, and stem pith of corn stover

Items	Corn stover parts			SEM	*P*-value
	SB	LB	SP		
Gas production (ml)	105.8^{c}	184.6^{b}	188.8^{a}	5.56	< 0.001
Methane production (ml)	25.8^{c}	42.3^{a}	40.9^{b}	2.04	< 0.001

NDS (%)	31.6[c]	42.8[b]	45.4[a]	0.73	< 0.001
Cellulose (%)	40.9[a]	26.5[c]	28.0[b]	0.42	< 0.001
Hemicellulose (%)	21.8[b]	27.4[a]	23.3[b]	0.90	0.002
Lignin (%)	4.8[a]	2.4[b]	2.6[b]	0.62	0.013
DMD (%)	38.0[c]	74.8[b]	82.0[a]	2.51	< 0.001
NDSD (%)	62.4[c]	81.4[b]	86.9[a]	0.07	< 0.001
HD (%)	25.7[b]	77.4[a]	78.6[a]	0.30	0.002
CD (%)	24.2[c]	60.1[b]	75.0[a]	0.75	< 0.001

SB, stem bark; LB, leaf blade; SP, stem pith; NDS, neutral detergent soluble solute; DMD, digestibility of dry matter; NDSD, digestibility of neutral detergent solubles; HD, digestibility of hemicellulose; CD, digestibility of cellulose; SEM, standard error of the mean (n = 4).
Values in the same row with different superscript letters are significantly different ($P < 0.05$).

Conclusions

The substrate characteristics significantly affected the fiber degradation and methanogenesis of the co-culture of anaerobic fungus and methanogens. The degradation rate of stem pith was significantly higher than that of the leaf blade, but its methane yield was similar to that of the leaf blade. Therefore, the stem pith with high degradation rate and low methanogenesis might be more suitable for feed utilization of ruminants, but its specific mechanism still needs to be further studied.

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THE EFFECT OF PARTIAL REPLACEMENT OF CONCENTRATE BY BROWSE SPECIES *IN-VITRO* AND ON GROWTH PERFORMANCE OF GROWING GOATS

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Introduction

Smallholder farmers in Mauritius use cut and carry system for collecting fodder intended for feeding their animals (Boodoo *et al*., 1999). Tree and browse species are important components of ruminant diets and are rich in most essential nutrients particularly, proteins and minerals(Saraye *et al*., 2016). Although they are important sources of forage throughout the year for smallholder farms, little information is available on their nutritional value locally. A sustainable alternative to mitigate the high price of concentrate is to produce good quality fodder (grass and leguminous species) to replace part of the concentrate in the diet of ruminants. The utilization of locally available and home-grown high protein forages and browses is therefore being encouraged.
The objective of this study was to investigate the *in-vitro* rumen fermentation kinetics and dry matter intake and growth of goats fed of partial replacement of concentrate by browse species.

Materials and Methods

In-vitro studies

The selected feeds sugarcane tops(*Saccharum officinarum*), *Albizia lebbeck, Litsea monopetala* and concentrate (compounded feed with CP17%) were evaluated using the *in vitro* gas production technique.Dried (60^{o}C) and ground (1 mm) samples were incubated using calibrated glass syringes following the procedure of Menke and Steingass (1988). The samples were incubated alone and in mixture. For the basal diet, sugar cane tops were mixed with concentrate at a ratio of 60: 40. Diets were also formulated replacing 50% of the concentrate witheach of the browse species such that the ratio of sugarcane tops: concentrate: browse species was 60: 20: 20. Cumulative gas production data were fitted to the model of Ørskov, 2000.The chemical composition of the feeds was performed according to AOAC (1990).

B: Feeding Trial

Eighteen goats were distributed in three groups in a completely randomized design and allocated to different treatments as shown in Table 1. The animals were weighed at start of experiment,every fortnight and at the end of the experiment. Daily feed intake (fodder type and concentrates) was recorded and samples taken for chemical and degradability analysis.

Table 1: Diets composition and animals

	Diet	Ratio	**No of animals**
Control	SCT: C*	6: 4	3 males+3 females
Treatment 1	SCT: C: AL	6:2:2	3 males+3 females
Treatment 2	SCT: C: LM	6:2:2	3 males+3 females

*SCT: sugar cane tops, (*Saccharum officinarum*) C: Concentrate, AL: *Albizia lebbeck*, LM: *Litsea monopetala*.

Results and Discussion

The chemical composition of the feeds and mixture of fodder browses and concentrate is shown in Table 2.

Table 2: Chemical composition of the feed

Feeds	Dry matter	Crude Protein	Crude Fibre	Ether Extract	ASH	Phosphorus	Calcium	Acid Detergent Fibre	Acid Detergent Lignin	Neutral Detergent Fibre
SCT	33.5	5.6	34.7	1.7	5.4	0.3	0.2	41.8	5.9	70.6
Litsea monopetala	54.1	15.9	29.2	3.8	5.4	0.3	0.8	35.1	11.2	47.9
Albizia lebbeck	42.0	15.5	28.1	4.0	8.2	0.3	1.7	34.3	11.8	46.1
Concentrate	86.5	17.2	4.2	3.8	6.4	1.4	1.2	-	-	-
SCT+ C(6:4)	54.1	10.5	22.4	2.4	6.6	0.7	0.6	25.1	3.5	42.4
SCT+C+ AL(6:2:2)	45.4	9.20	26.4	2.8	7.1	0.5	0.8	31.0	5.4	50.4
SCT+C+ LM(6:2:2)	42.5	9.90	27.7	2.2	6.3	0.6	0.5	34.9	7.6	53.0

*SCT: sugar cane tops, (*Saccharum officinarum*) C: Concentrate, AL: *Albizia lebbeck*, LM: *Litsea monopetala*.

Saccharum officinarum had a crude protein (CP) of 5.6±3.5 while *A.lebbeck* and *L.monopetala* had CP of 15.48 ±2.5, and 15.85±1.35, respectively.Because of greater CP levels in browse species compared to the sugarcane tops, a mixture of fodder and browse can be used to meet animal requirements.The *in vitro* cumulative gas production (mL/200mg DM) of the sugar cane, concentrate, and different combinations with *A.lebbeck* and *L.monopetala* are shown in the Figure1.

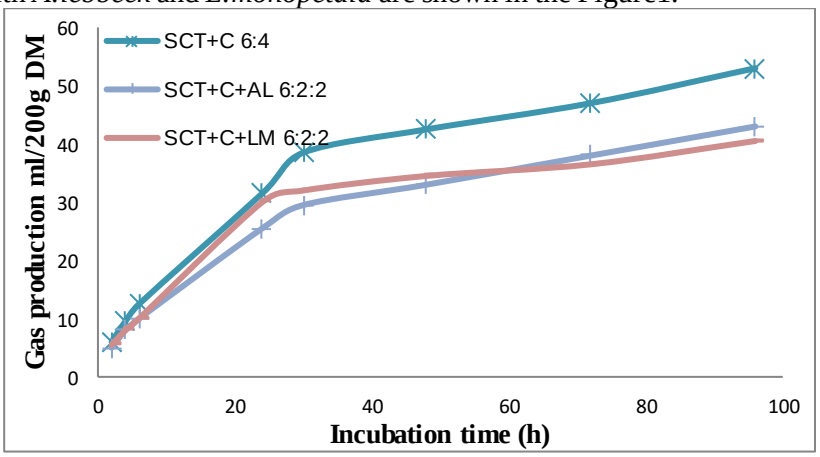

Figure 1: *In-vitro* analysis of fodder and feed mixture

The basal diet (sugarcane tops) mixed with concentrate at 40 % on DM basis resulted in greater gas production (47 ml/200mg DM) after 72 h of incubation. Greater gas production indicates greater DM degradability for basal diet mixed with concentrate dairy trial. However, upon replacing 50% of concentrate in the ratio of 60: 20: 20 (sugarcane tops: concentrate: browse species) gas production was 38, and 36 mL/200mg for *A.lebbeck* and *L. monopetala*, respectively after 72 h of incubation.
The results of the feeding trial are shown in Table 3.

Table 3: Results of feeding trial

	Diet	No of animals	Initial average body weight, kg	Final average body weight, kg	Average daily gain, g/day
Control	SCT: C	3 males	18.2± 2.3	22.3± 3.5	40±3
		3 females	22.5 ±5.2	26.7 ± 4.8	30±5
Treatment 1	SCT: C:AL	3 males	16.7± 3.1	22.0± 4.5	40± 2
		3 females	20.7 ±7.1	23.0 ±7.9	17±5
Treatment 2	SCT: C: LM	3 males	17.8±1.3	20.8±0.1	22±7
		3 females	20.0 ±4	24.0 ±3.4	30 ±7

The initial body weight (BW) for control animals were 20.3 kg compared to 18.7 kg and 18.9kg for treatment 1 and treatment 2, respectively. The final BW for control animals were 23.8 kg compared to 22.0kg and 21.4 kg for treatment 1 and treatment 2, respectively. No treatment difference (P>0.5) was observed on BW and average daily gain.

Conclusion

The results obtained in this study provides nutritive value of the browse species and potential for replacing 50% of concentrates in the diet with browse species such as *A.lebbeck* and *L.monopetala.* Because our numbers were small, repetition of the study is warranted.With renewed interest in using

shrubs/trees in ruminant feeding as a means to reduce use of costly concentrate, more research in required for optimal introduction of these feed resources in feeding systems.

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MESOPOTAMIAN BUFFALO IN IRAQI MARSHES: CHALLENGES AND DEVELOPMENTAL PATHS

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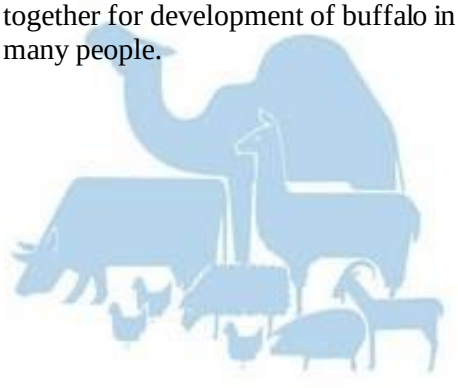
Iraq's Mesopotamian marshes were the cradle of civilization" according to the many historians. Muslims and Christians alike have referred to the area as the Garden of Eden. The marshes were also famous for their biodiversity and cultural richness. These marshlands are both regionally and globally significant for cultural, political and economical.

Archaeological evidence suggests that people brought water buffalo to the marshlands around 2,500 B.C. The most important thing buffalo provided was dung. The marsh Arabs mixed dung with reeds to make fuel for cooking and heating fires. The slightly acrid smoke also repelled flying insects. They also used dung to waterproof roofs and poultice wounds. More than 60% from Iraqi buffaloes were in the southern regions of Iraq and a third were in middle regions, but only limited numbers were present in north. The way of life in marsh areas helps to the explain the distribution of these animals. Buffaloes farming was once an important way for people who living in the marshes. Also, the buffaloes were considered very important for economic wealth, through their production. In the marshlands, adult water buffaloes are not usually slaughtered for their meat, but instead provide milk, butter, yogurt , cheese and other dairy products. The nutrition of water buffalo is considered a main factor in age of puberty and calving interval. Most buffalo owners in the marshes of Iraq nourish their animals on concentrated feed with different amounts , and in most cases on only small amounts of green roughages.

Management, reproduction, veterinary services and availability of dairy factories are the biggest problems related with breeding of Mesopotamian buffaloes. Many efforts by Iraqi ministries and institutes have been made to support development of buffalo production. The Ministry of Agriculture in Iraq has implemented several of buffalo projects and organized national conferences. Also, national and international organizations have worked toward development of buffalo production in Iraq, such as the project of artificial insemination (AI) supported by the Food and Agriculture Organization of the United Nations (FAO), office in Iraq. International Buffalo Federation (IBF) with FAO, Iraq organized the First International Scientific Conference of Mesopotamian Buffalo (FISCMB) from 22 to 23 March 2019. The recommendations of the conference were adopted by the Iraqi government institutions to determine the paths of developing this sector. It is very important to work together for development of buffalo in the Iraqi marshlands, as it contributes to livelihood and lives of many people.



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STATUS OF GENETIC VARIABILITY AMONG INDIGENOUS CATTLE POPULATIONS IN SRI LANKA

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Abstract

The present study focused on the genetic characterization of six indigenous cattle populations (Thamankaduwa-Th [2 distinct populatins], Thawalam-TW, North Central-NC, Southern-So and Northern-No) in Sri Lanka. Data from 189 samples were analysed using a panel of 11 microsatellite markers where 121 microsatellite alleles were detected, indicating a higher allelic diversity. The overall mean number of alleles per locus was 11, and varied from 7.27 (Th) to 8.36 (No). There were high observed and expected heterozygosities per locus with overall mean values of 0.745 and 0.797, respectively. Overall mean estimated inbreeding coefficient (F_{IS}) was 0.043 (0.020 Th to 0.073 TW). All six populations showed a considerable level of inbreeding and the observed highest heterozygosity deficit in TW cattle population clearly explained by their breeding management. Only 3.4% of the total genetic variation was (F_{ST} = 0.034) due to between population differences. Pair-wise Cavalli-Sforza and Edwards chord distances among cattle populations clearly showed that TW cattle, which are pack animals with specific body confirmation, were separated from other populations. However, there was no apparent discrete genetic structure among the six populations.

Introduction

Indigenous cattle in Sri Lanka form an important farm animal genetic resource, which has been identified as a separate category of cattle (Silva *et al.*, 2010). Owing to their low economic significance reflected by production traits, upgrading with improved exotic breeds has been a regular practice for decades. This has invariably caused a loss of important genetic characters of indigenous cattle populations. Except the studies done on phenotypic characterization and farming systems (Wijeweera *et al.*, 2014), only one study reported (Silva *et al.*, 2010) an attempt on genetic characterization. Considering the importance of conservation of indigenous genetic resources as a means of facing challenges under varying climatic scenarios (FAO, 2007), the current study was designed to conduct in-depth genetic analysis of six geographically distinct indigenous cattle populations in Sri Lanka.

Materials and methods

Sample Collection and Microsatellite Genotyping

A total of 49 blood samples were collected from two cattle populations; Thamankaduwa (Th) cattle (22) white cattle in eastern region and Thawalam (TW) cattle (27) or pack animals of Sri Lanka. Sixteen samples collected during the previous study of Silva *et al.* (2010) were also included in the present study

for genotyping to facilitate merging genetic information from 140 samples from the previous study of Silva *et al* (2010), which consists of North Central-NC (38), Southern-So (28), Northern-No (37) and North Eastern/ Thamakaduwa 1-Th1 (37). The Th cattle from two studies were considered as two cattle populations as they were from two separate locations (Th and Th1). DNA extraction was carried out using the Masterpure® DNA extraction kit. Eleven microsatellite markers (ISAG-FAO) were used for genotyping by capillary sequencer (ABI 3730 DNA Analyzer – Applied Biosystems) at the Animal Genetic Resources Research Center, National Institute of Animal Science, South Korea. The electropherogram analysis for allele size determination was carried out using GENEMAPPER software (Applied Biosystems).

Data Analysis

The data sets of Silva *et al*. (2010) and the present study were aligned for allele size. Observed number of alleles, observed and expected heterozygosity and fixation index were calculated using MICROSATELLITE ANALYZER version 4.05 (Dieringer and Schlotterer, 2003). Deviations of heterozygosities from Hardy-Weinberg Equilibrium (HWE) were estimated using GENEPOP software version 4.1.3 (Raymond and Rousset, 1995). A between population dendrogram and radial tree were constructed employing the UPGMA algorithm using PHYLIP version 3.5 (Felsenstein, 1993). Principal Component Analysis (PCA) was carried out using SPSS software version 13.0 with Pair-wise F_{ST} derived from allele frequencies. The population structure and the level of admixture of individuals were determined by Bayesian clustering analysis of STRUCTURE version 2.3.4 (Pritchard *et al*., 2000) and web-based STRUCTURE HARVESTER software (Earl and vonHoldt, 2012). The results were post-processed using CLUMPP software version 1.1.2 (Jakobsson and Rosenberg, 2007) and visualized using Microsoft Office Excel 2010.

Results and Discussion

Genetic Diversity and HWE in Different Cattle Populations

A total of 121 microsatellite alleles were detected, indicating a high allelic diversity across the six populations. Mean alleles per locus was 11, ranging from 6 (ILST005) to 15 (CSSM66), whereas between populations value varied from 7.27 (Th) to 8.36 (No). Those values were higher than the values reported for indigenous cattle in India (Pandey *et al*., 2006) and Europe (Schmid *et al*., 1999). The high allelic diversity in Sri Lankan indigenous cattle population reflects lack of directional selection.
The mean observed and expected heterozygosities per locus across populations were 0.745 and 0.797, respectively indicating the similarity in genetic variability across populations. A summary of diversity measures of all populations is given in Table 1.

Table 1. Summary diversity statistics for cattle populations

Parameter	Th	Th1	TW	NC	So	No
n_o	7.2	7.7	7.4	7.7	8.0	8.4
H_o	0.753	0.765	0.716	0.749	0.738	0.736
H_e	0.768	0.785	0.771	0.783	0.755	0.775
F_{IS}	0.020	0.030	0.073	0.050	0.026	0.062

Overall estimated inbreeding coefficient (F_{IS}) was 0.043, varying between 0.020 (Th) to 0.073 (TW). The exact test for HWE revealed significant deviations ($P < 0.05$) in 13 out of 66 breed-locus combinations owing to heterozygosity deficit. Estimates of F_{IS} indicate important properties of the mating system within populations. Accordingly, heterozygosity deficits observed in all populations reflects level of inbreeding. The observed highest heterozygosity deficit in TW cattle population, is well-explained by the breeding management of the population. In general, Pack animals are castrated.

However, as described by Kumaravithana (2014) superior animals are not castrated in TW population and used for natural breeding. Thus, leading to inbreeding as effective population size of is low.

Genetic Differentiation and Population Structure

Only 3.4% of the total genetic variation (F_{ST} = 0.034) was due to between population differences. Pair-wise F_{ST} ranged from 0.0095 to 0.065 (Table 3), where high genetic variations were observed between TW and NC, So and No cattle populations (Figure 1) justified by the geographical separation of TW from other populations. The clustering of No, NC and So populations well justifies the separation of phenotypic category of cattle called "Lankan Cattle" or "*Batu harak*" which is a non-descript category. Two separate population structures could be observed at K=3, depicting similarities in TW and Th (Figure 2). Similarly, principal component analysis also showed clustering of TW and Th together, separating from other four populations including Th1. Both Structure and principal component analysis reflect the possible sampling time effect too.

Conclusions

Sri Lankan indigenous cattle carry high allelic diversity, low genetic differentiation among geographically separated populations and high within population variation. Pack animals form both a phenotypically and genetically separate group. Findings of the current study could contribute to the knowledge on genetic diversity of Sri Lankan indigenous cattle populations.

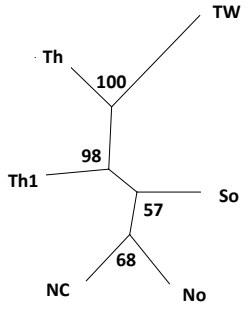

Figure 1. UPGMA Radial tree

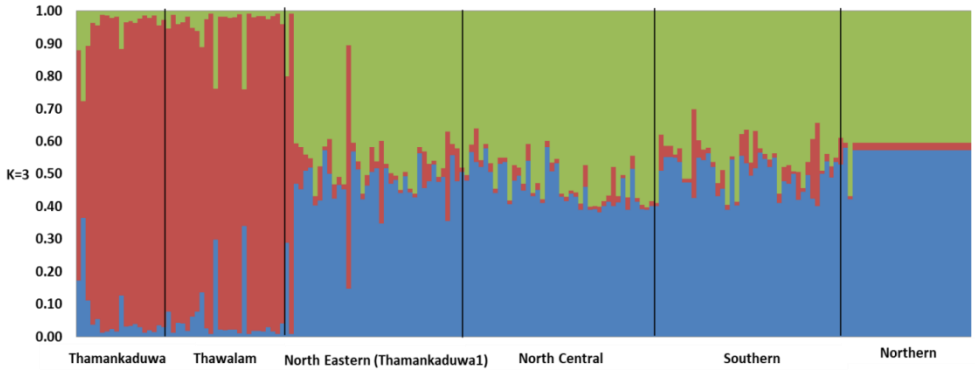

Figure 2. Summary bar plot of Q estimates at best K=3. Each animal is represented by single vertical line broken into *K* colored segments with lengths proportional *K* inferred clusters

Table 3. Pair-wise F_{ST} (upper triangle) and pair-wise Cavalli-Sforza and Edwards chord distance (lower triangle) among Sri Lankan cattle populations

	Th	TW	Th 1	NC	So	No
Th	-	0.0096	0.0478	0.0650	0.0588	0.0610
TW	0.2773	-	0.0438	0.0599	0.0557	0.0608
Th 1	0.3531	0.3391	-	0.0095	0.0191	0.0113
NC	0.3844	0.3800	0.2271	-	0.0245	0.0162
So	0.3588	0.3533	0.2381	0.2349	-	0.0172
No	0.3062	0.3771	0.2411	0.2340	0.2440	-

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NUTRITIVE VALUE OF FODDER RESOURCES IN MAURITIUS TO ENHANCE LOCAL KNOWLEDGE FOR RUMINANT DIET FORMULATION

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Abstract

The nutritional worth of fodder resources in Mauritius for ruminants is not well documented and consequently ration formulation and concentrates supplementation to meet nutritional needs are haphazard. The chemical composition of 60 types of fodder collected on farms was compiled to enhance local knowledge on the forage base in Mauritius. It is aimed that these data would aid in more accurate formulation of diets to optimise nutrient utilization for enhancing animal productivity. The suitability of use of selected fodder species as hay or silage is also mentioned. Trees/shrubs are promising as alternatives to concentrates with potential for integration into livestock-based agroforestry systems for sustainable production.

Keywords: fodder, shrubs, local knowledge, nutritive value, agroforestry

Introduction

The smallholder ruminant system in Mauritius is based on cut and carry with high dependence on fodder collected from marginal and secondary forest lands or sugarcane fields. These are exotic and naturalised species growing mostly in the wild, while some are cultivated. The nutritional worth of these species is not known to the farming community and concentrate supplementation is thus haphazard. The increasing interest in fodder cultivation from farmers, calls for the need to enhance local knowledge on the diversity and nutritional worth of fodder for ruminants in view of optimizing animal productivity.

Materials and Methods

Eight hundred fodder samples were collected from 2008 to 2018 from farms in different agro-climatic regions, dried at 52°C for dry matter (DM) determination and ground to 3mm for chemical analysis of crude protein (CP), crude fibre (CF), acid detergent fibre (ADF), neutral detergent fibre (NDF), lignin (ADL), ether extract (EE), calcium (Ca) and phosphorus (P_2O_5) (AOAC, 1990; Van Soest *et al*, 1991). A database was established in MS Excel for analysis.

Results and Discussion

Sixty species comprising cultivated grasses (6), trees/shrubs (13), leguminous species (3), wild grasses (6), creepers (7) and crop residues (5) were used as forages for ruminants. Trees/shrubs have highest CP (16.9%) and crop residues the lowest (5.3%). Results are summarized in Table 1.

Table 1. Chemical composition (% DM) of fodder categorized by type

	DM	CP	CF	ADF	NDF	ADL	EE	Ash	Ca	P_2O_5
Cultivated grass	28.4	7.2	34.3	43.7	68.1	6.2	1.4	7.9	0.2	0.4

Trees/shrubs	36.3	16.9	27.2	42.9	54.6	18.5	2.3	7.6	1.0	0.7
Leguminous species	25.5	15.0	32.9	44.0	54.9	15.7	2.6	7.4	0.9	0.6
Wild grasses	29.8	7.2	34.7	46.2	71.4	7.3	1.4	9.8	0.3	0.4
Creepers	23.9	13.2	30.3	41.3	60.6	12.9	1.8	10.7	0.9	0.6
Crop residues	33.6	5.3	34.9	43.2	70.1	7.0	1.7	6.4	0.2	0.3
SEM	0.33	0.21	0.20	0.25	0.42	0.24	0.02	0.09	0.02	0.06

SEM: Standard error of the means

Cultivated grasses

These are cultivated for cut and carry with *T. laxum* (5.8% CP), *P. purpureum* (9.8% CP) and *Z. mays* (8.7% CP) and suitable for conservation as silage. *S. sphacelata*, *B. brizantha* and *C. plectostachyus* are appropriate for both grazing and cut and carry and for conservation as hay.

Table 2. Chemical composition (% DM) of cultivated grasses

Common name	Scientific name	DM	CP	CF	ADF	NDF	ADL	EE	Ash	Ca	P_2O_5
Brachiaria	*Brachiaria brizantha*	27.4	6.4	35.1	44.6	69.7	7.4	1.5	7.1	0.3	0.4
Elephant grass	*Pennisetum purpureum*	31.6	9.8	33.8	45.1	69.2	7.1	1.4	10.8	0.3	0.5
Guatemala grass	*Tripsacum laxum*	26.4	5.8	37.5	47.3	71.5	6.3	1.3	7.5	0.1	0.3
Maize	*Zea mays*	30.7	8.7	26.7	34.3	57.5	5.1	1.5	6.8	0.2	0.5
Setaria	*Setaria sphacelata*	25.0	8.8	35.3	45.5	71.6	8.9	1.6	8.4	0.3	0.4
Stargrass	*Cynodon plectostachyus*	36.8	8.5	32.3	42.2	75.1	6.9	1.3	9.3	0.3	0.5
	SEM	0.60	0.21	0.43	2.64	0.76	0.19	0.03	0.19	0.01	0.01

Trees and shrubs

Tree/shrub species namely, *L. leucocephala*, *A. lebbeck*, *M. azedarach* can be grouped as high protein (22.8, 21.5 and 18.5% CP, respectively) and low protein, namely, *L. glutinosa*, *L. monopetala* and *S. terebinthifolius* (12.8, 12.4 and 10.6% CP, respectively) (Table 2). They can also be used as leaf meal.

Table 3 Chemical composition (% DM) of trees/shrub species

Common name	Scientific name	DM	CP	CF	ADF	NDF	ADL	EE	Ash	Ca	P_2O_5
Acacia	*Leucaena leucocephala*	36.4	22.8	24.1	36.1	51.1	14.9	2.1	7.8	1.1	0.4
Bilimbi	*Averrhoa bilimbi*	35.7	18.1	30.9	48.8	72.6	19.8	1.7	8.6	1.3	0.6
Bois d'Oiseaux	*Litsea glutinosa*	41.7	12.8	32.2	42.1	60.9	15.5	2.6	5.9	0.8	0.4
Bois Noir	*Albizia lebbeck*	37.1	21.5	29.5	36.0	53.5	12.1	2.9	7.5	1.3	0.4
Calliandra	*Calliandra calothyrsus*	36.0	18.6	23.7	44.1	43.0	20.2	1.8	6.6	0.9	0.6

Chandan	*Santalum album*	37.5	13.0	21.3	35.0	51.9	13.7	3.2	12.8	1.5	0.4
Conde	*Cordia macrostachya*	27.2	13.4	29.8	49.4	57.3	12.9	1.6	12.7	1.2	0.5
Coqueluche	*Pongamia pinnuto*	32.6	19.7	35.8	46.4	79.1	20.3	2.4	7.0	0.7	0.4
Gliricidia	*Gliricidia sepium*	30.0	20.5	28.3	42.6	49.8	17.2	1.6	8.7	0.8	0.6
Gros Feuilles	*Litsea monopetala*	33.7	12.4	28.6	51.5	60.1	25.8	2.4	6.8	0.7	0.7
Lilas	*Melia azedarach*	38.4	18.5	25.2	39.2	49.5	14.9	1.6	11.7	2.1	0.6
Mulberry	*Morus alba*	30.6	17.3	21.9	35.7	52.1	8.6	2.5	12.3	1.7	28.6
Poivre Marron	*Schinus terebinthifolius*	39.5	10.6	18.8	42.7	56.7	22.2	3.0	7.5	1.4	0.5
	SEM	0.48	0.36	0.36	2.97	0.87	0.48	0.06	0.13	0.04	0.22

Leguminous species

These include naturally growing *Trifolium* spp.(Clover) and *Macroptilium atropurpureum* (Siratro) with CP 24.4% and 15.5%, respectively, while *Desmodium intortum* (Desmodium) is a cultivated species with lower CP (11.8%). They are used for both cut and carry and grazing.

Wild grasses

S. capensis and *I. aristatum* had lowest CP (3.8 and 4.3%) while *D. horizontalis* and *C. lacryma-jobi* had highest CP (9.0 and 8.7%) (Table 4). They are used for both cut and carry and grazing.

Table 4. Chemical composition (% DM) of wild grasses

Common name	Scientific name	DM	CP	CF	ADF	NDF	ADL	EE	Ash	Ca	P_2O_5
Fatak	*Panicum maximum*	29.9	8.3	34.5	46.8	71.5	6.8	1.2	11.6	0.4	0.5
Gros Meinki	*Digitaria horizontalis*	24.2	9.0	33.2	42.0	77.8	7.8	1.4	10.9	0.2	0.4
Herbe Bourik	*Stenotaphrum dimitiatum*	29.8	6.8	33.4	44.2	75.7	8.2	1.4	8.5	0.3	0.5
Herbe Collier	*Coix lacryma-jobi*	27.0	8.7	33.6	46.2	67.2	6.3	1.7	10.9	0.3	0.5
Herbe d'Argent	*Ischaemum aristatum*	32.0	4.3	37.3	47.3	71.1	8.2	1.3	6.9	0.2	0.3
Herbe Sikkin	*Sporobolus capensis*	28.9	3.8	34.1	45.2	71.1	6.7	1.5	9.2	0.3	0.5
	SEM	0.82	0.28	0.30	0.32	0.61	0.19	0.04	0.24	0.01	0.02

Crop residues

Crop residues (Table 5) are important roughage sources during fodder shortages. Sugarcane tops and maize stover are residues from sugarcane and cob maize harvest and are both suitable as green fodder

and silage. Sugarcane trash is the by-product resulting from mechanical harvest of sugarcane which is left to dry in the cane fields. It has lowest CP (2.1%) is currently commercially available as hay.

Table 5. Chemical composition (% DM) of crop residues

Common name	Scientific name	DM	CP	CF	ADF	NDF	ADL	EE	Ash	Ca	P_2O_5
Carrot leaves	*Daucus carota*	15.4	15.3	14.2	24.5	40.3	10.4	2.2	21.4	1.4	0.7
Maize stover	*Zea mays*	30.8	5.4	30.2	39.7	63.1	6.1	1.2	6.7	0.2	0.4
Potato vines	*Solanum tuberosum*	21.3	4.4	36.0	43.6	73.9	9.0	1.2	7.4	0.2	0.4
Sugarcane tops	*Saccharum officinarum*	32.8	5.3	35.4	43.4	70.7	7.0	1.7	6.2	0.2	0.3
Sugarcane trash	*Saccharum officinarum*	88.7	2.1	36.7	52.5	73.0	9.1	1.5	7.1	0.2	0.1
	SEM	0.67	0.14	0.24	2.61	0.52	0.12	0.02	0.12	0.01	0.01

Conclusion

A wide diversity of forages is locally available in Mauritius to meet nutrient requirements of ruminants. They can be conserved as either hay or silage to ensure year-round availability of fodder and mitigate seasonal shortages. Trees are promising as homegrown protein sources and have potential for integration into livestock agroforestry systems. This baseline information would benefit farmers, stakeholders, researchers and extensionists for diet formulation for optimizing use of commercial concentrates and nutrient use.

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NUTRITIONAL QUALITY OF THE TROPICAL LEGUME FORAGES COWPEA (*VIGNA SINENSIS L.)*, LABLAB (*DOLICHOS LABLAB L.)* AND JACK BEAN (*CANAVALIA ENSIFORMIS L.)*

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Commonly, tropical forages have limited nutritional value for dairy cattle, particularly regarding crude protein (CP) and metabolisable energy (ME) (Juárez et al., 1999), leading to the need of feeding concentrates to sustain dairy cattle production. In temperate regions, legumes such as alfalfa (*Medicago sativa*) or clover substantially contribute to the quality of diets (Wilkins, 2001). However, in the tropics, including legumes in the diet of cattle is less common, even though it has been reported that tropical legumes such as jack bean (*Canavalia ensiformis*) and cowpea (*Vigna sinensis*) have positive effects on cattle's nutritional status when supplemented to sorghum (*Sorghum bicolor*) silage (Corea et al., 2010a; Corea et al. 2010b).

More recent studies have shown that jack bean or Vigna have positive effects on milk production, nutrients use efficiency and profitability when fed to the dairy cattle (Corea et al., 2017; Castro-Montoya et al., 2018).

The positive effects of these herbaceous legumes is likely related to their higher nutritional value, due to their higher CP and lower neutral detergent fiber (NDF) compared with most tropical grasses, the latter of which results in a higher feed intake (Amiri and Shariff, 2012).

However, information about the relative nutritional value of different tropical legumes, similar to e.g. the comparison between maize and sorghum forage, as well as the nutritional value of different legume plant parts (i.e. stems, leaves) is scarce and could be used to optimize strategies for optimal cattle feeding. Therefore, the objective of the study was to evaluate, nutrient content and digestibility of the plant parts of three tropical annual legumes with potential use in feeding cattle.

For this, a field trial was conducted at the Experimental Station of the Faculty of Agricultural Sciences of the University of El Salvador. Three plots (2,500 m^2) were established with cowpea (*Vigna sinensis*), lablab (*Lablab purpureus*) and jack bean (*Canavalia ensiformis*) planted at a density of 125,000 plants/ha. Crops were grown in the dry season under irrigation and harvested 70 days after planting, yield was estimated by measuring green matter from three subplots of 9 m^2 in each plot.

The fodder of six whole plants was separated into leaves and stems in three replicates; these fractions were analyzed for dry matter (DM), ash, CP, NDF and acid detergent fiber (ADF). Moreover, dried samples were incubated in triplicate for 48 h *in situ* in a ruminally cannulated lactating cow and *In situ* digestibility of DM and OM was calculated following the procedure described by Mehrez and Orskov (1977). *In vitro* ruminal DM digestibility was estimated using an adaptation of the method of Goering and Van Soest (1970) in which the incubation is conducted under conditions similar to the in *vivo rumen*.

Data were analyzed as complete random design with legume species as the main effect using the statistical software InfoStat. Differences were considered significant at p-value < 0.05 and means were compared using the Tukey test.

Whole plant DM content was similar among the three legumes (~165 g/kg fresh matter) but differences in stems and leaves were found (Table 1). Whole plant and stem of the studied species all had similar CP contents, but cowpea leaves were lower in CP compared to the others. No differences in NDF and ADF contents in leaves were found among species; however, cowpea, stems had the lowest concentration of both fiber fractions. As a whole plant, lablab had the highest NDF concentration, cowpea had the lowest and jack bean was intermediate.

The NDF and ADF concentration of the whole reflected on the *in situ* DMD and OMD, with cowpea having the highest and lablab the lowest values (Table 2). Interestingly, the *in situ* DMD and OMD of the stems appeared to relate more to the ADF concentration than to the NDF, showing a negative relationship. Although the extent of DMD and OMD differed between methods the DMD pattern observed for the legume samples between the *in situ* and *in vitro* techniques was similar.

All studied legumes showed a CP content higher than most tropical grasses, indicating that their feeding would decrease the need for supplementation of protein via concentrates. A high *in situ* degradability is also a promising observation that may result in a greater supply of metabolisable energy. Although differences in leaf composition were not remarkable, cowpea appeared to have the most desirable nutritional characteristics among the studied legume species. Nutritional value of leaves in the studied legumes was greater than stems, as leaves contained almost three times more CP, 40 % less NDF, less than half the amount of ADF, and greater *in situ* and *in vitro* ruminal digestibility. This is important information particularly when utilizing legumes under conditions where leaves losses might be high.

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Table 1. Nutritional composition of whole plant, leaves and stems of Cowpea, Lablab and Jack bean forages

	Cowpea	Lablab	Jack bean	SEM	P-value
DM (g/kg)					
Whole plant	162	169	164	3.5	0.74
Stem	149^{b}	159^{b}	340^{a}	107.2	< 0.01
Leaf	192^{b}	197^{b}	282^{a}	50.1	< 0.01
CP (g/kg DM)					
Whole plant	179	164	186	11.1	0.43
Stem	98.7	91.5	95.5	3.60	0.58
Leaf	237^{b}	296^{a}	276^{a}	30.2	< 0.01
NDF (g/kg DM)					
Whole Plant	500^{b}	542^{a}	518^{ab}	21.3	0.02
Stem	527^{c}	622^{b}	666^{a}	71.1	< 0.01
Leaf	337	386	354	25.1	0.09
ADF (g/kg DM)					
Whole Plant	320^{b}	371^{a}	341^{b}	25.3	0.05
Stem	381^{c}	463^{b}	502^{a}	61.6	< 0.01
Leaf	214	220	186	17.7	0.08
Ash (g/kg DM)					
Whole Plant	103^{b}	111^{a}	80.4^{c}	15.8	< 0.01
Stem	146^{a}	104^{b}	55.5^{c}	45.3	< 0.01
Leaf	142^{a}	118^{b}	124^{b}	12.4	< 0.01

SEM, Standard error of the mean.
$^{a\text{-}c}$Means in rows with different superscripts are different by Tukey's test ($P < 0.05$).

Table 2. Ruminal *in situ* 48-h and *in vitro* digestibilities of whole plant, leaves and stems of Cowpea, Lablab and Jack bean forages

	Cowpea	Lablab	Jack bean	SEM	P value
In situ DMD (g/kg)					
Whole plant	762^{a}	620^{b}	677^{b}	71.5	< 0.01
Stem	681^{a}	583^{b}	481^{c}	99.7	< 0.01
Leaf	844^{a}	836^{a}	803^{b}	21.8	<0.01
In situ OMD (g/kg)					
Whole plant	748^{a}	594^{c}	665^{b}	77.0	< 0.01
Stem	637^{a}	537^{b}	418^{b}	109.6	< 0.01
Leaf	854^{a}	841^{ab}	821^{b}	16.7	0.04
In vitro DMD (g/kg)					
Whole plant	704^{a}	614^{b}	591^{b}	59.7	< 0.01
Stem	681^{a}	495^{b}	413^{b}	137.4	< 0.01
Leaf	682	774	631	72.7	0.07

SEM: Standard error of the mean.
$^{a\text{-}c}$Means in rows with different superscripts are different by Tukey's test ($P < 0.05$).

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PREPARATION AND EVALUATION OF DUAL PROTECTED NUTRIENTS FOR DAIRY CATTLE: AN *IN VITRO* STUDY

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Abstract

An *in vitro* trial was carried out to study the degradability following dual protection of protein and fat. The dual protection of nutrients was done by oil and aldehyde treatment. The crude protein content of Soybean meal (SBM) was determined. SBM was first coated with rice bran oil at a 2:1 ratio and stored. Finely ground soybean meal was treated with formaldehyde at 1% of its crude protein content and kept overnight. One part of oil coated SBM was mixed with 2 parts of formaldehyde-treated SBM and mixed properly. Four replicates for each mixture sample were subjected to two-stage *in vitro* degradability studies against a control for three times. The substrate was analyzed for contents of dry matter (DM), organic matter (OM), crude fat (CF) and crude protein (CP) content. The collected data were subjected to t-test statistical analysis. The *in vitro* CP and CF digestibility in the protected mixture (36.23, 25.68%) was lower than unprotected (52.82, 72.89%). The *in vitro* dry matter, organic matter digestibility were significantly ($P<0.05$) lower in the protected group than the unprotected group. It could be concluded that oil and formaldehyde treatment together will reduce ruminal degradability of both protein and fats in SBM.

Keywords*: In vitro*, dual protection, oil, degradability, formaldehyde.

Background

Rumen microbes degrade dietary protein, regardless of quality, into ammonia and amino acids and then incorporate these products into microbial protein. Ruminants derive their amino acids jointly from dietary protein (escaping rumen degradation) and microbial protein synthesis (from degraded proteins) in the rumen. The amount of protein and amino acids that escapes rumen degradation varies greatly among feeds, depending on their solubility and the rate of passage to the small intestine. In the case of high producing animals, the microbial protein alone is usually not sufficient to meet protein requirement. In such cases, protected protein or bypass protein and bypass fat is needed to meet requirements. Various methods are used for the protection of each of these nutrients but not for both. The study was therefore undertaken to evaluate the i*n vitro digestibility* of dual protected protein and fats.

Material and methods

The proximate composition of soybean meal (SBM) was carried out. The protection method involved two steps. The first step was oil coating of soybean meal, where SBM is coated with rice bran oil (RB oil) in a 2:1 ratio. Similarly, finely ground formaldehyde (1% of CP content) treated SBM was prepared. Then the formaldehyde treated SBM was mixed with the oil coated SBM in a 2:1 ratio. Four replicates for each mixture were taken for *in vitro* studies by Tilley and Terry (1963) against an untreated control. The *in vitro* study was carried out in four replicates for three times. The results were subjected to t- test statistical analysis.

Results

The *in vitro* crude protein and crude fat digestibility in the protected mixture (36.23, 25.68%) were lower than the unprotected (52.82, 72.89%). The *in vitro* dry matter and organic matter digestibility were significantly ($P<0.05$) lower in the protected group than the unprotected control. It can thus be concluded that dual treatment of oil and formaldehyde together helps to reduce the ruminal degradability of both protein and fats, which might be useful during the early lactation period, without the need for separate sources of rumen protected protein and fat.

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COMPARATIVE MOLECULAR DIVERSITY ANALYSIS OF SOUTH INDIAN CATTLE BREEDS

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Introduction

India has 190.9 million total cattle out of which 151 million is indigenous cattle (1). After the white revolution, production driven farming practices has led to the decline of draught purpose animals, which were low producers of milk and earlier used for ploughing, carting and transport. Investigation of molecular genetic diversity is a valuable complement to evaluate phenotypes and production systems. Microsatellites also known as short tandem repeats (STR) are used to differentiate heterozygote from homozygotes and helps to identify rare alleles present in the declined populations. A comprehensive analysis including all the Indian breeds is still lacking. Hence, present study was conducted (i) to evaluate within breed genetic diversity of indigenous cattle populations using microsatellite markers (ii) to estimate genetic admixture and population structure of indigenous cattle populations.

Materials and Methods

A total of 542 unrelated animals representing 10 indigenous cattle populations: Kangayam, Umblachery, Bargur, Alambadi, Pulikulam, Deoni, Ongole, Hallikar, Vechur, two exotic breeds: HF and Jersey and their crossbreds with each breed not less than 30 in number were collected from different regions of the native tract. Amplified PCR products were genotyped for 27 FAO recommended bovine specific microsatellite labelled primers in an automated DNA analyzer using a capillary sequencer (ABI 3730 DNA Analyzer-Applied biosystems).

The allele size data for each sample was extracted and corrected in GENEMAPPER™ software. The microsatellite markers were tested for selective neutrality using LOSITAN software (2) and radial tree was constructed using PHYLIP v.3.5 (3) with pair-wise Nei's genetic distance. Phylogenetic tree was visualized in TREEVIEW v.1.6.6. AMOVA was done to evaluate the distribution of microsatellite variation as a function of breed, geography and phylogeny using ARLEQUIN version 3.1 (4) and Bayesian clustering analysis was employed using STRUCTURE version 2.3.4 (5). The population was tested for mutation-drift equilibrium following sign test, standardized differences test and Wilcoxon sign rank test under different models of microsatellite evolution as implemented in BOTTLENECK program (6). A qualitative test of mode shift was also done to detect whether the population has undergone any recent bottleneck.

Results

A total of 13,932 genotypes were analysed over 14 breeds and 27 microsatellite markers in the study. The mean observed number of alleles ranged between 9.11 (Jersey crossbreds) and 4.7 (Jersey purebreds) respectively in over all breeds whereas it was between 8.07 (Pulikulam) and 5.93 (Punganur) among the native breeds studied. The mean observed heterozygosity ranged from 0.598 (Kangayam) to 0.747 (HF crossbreds). Hallikar showed the highest mean observed heterozygosity (0.687) among the

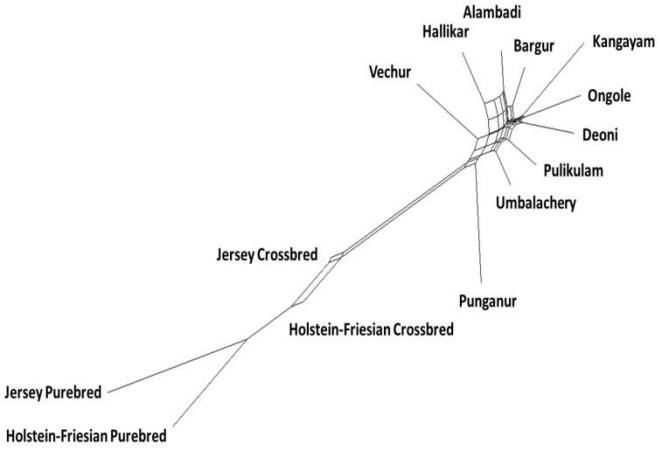
indigenous breeds. The diversity in terms of heterozygosity was moderately higher in all the breeds analysed.

Hardy-Weinberg Equilibrium

Out of 378 loci tested for Hardy Weinberg Equilibrium (HWE) 69 loci over 14 breeds deviated from HWE due to heterozygosity deficit and 12 loci due to heterozygosity excess. The proportion of loci deviating from Hardy-Weinberg equilibrium was lower compared to earlier studies. In Pulikulam the 12 out of 18 loci deviating from HWE in a study (7) have reduced to eight out of 27 loci in the present study.
The F_{IS} value was positive for all the breeds analyzed indicating significant heterozygosity deficiency. The mean F_{IS} value was highest in Pulikulam (0.108) and lowest in Jersey purebreds (0.017). But when the exotic breeds and crossbreds were excluded the breed with lowest F_{IS} was Hallikar (0.027). The mean global F_{IT}, F_{ST} and F_{IS} among zebu, exotic and crossbred cattle were 0.143, 0.101 and 0.047 respectively.

Genetic Relationship between Breeds

The pair-wise F_{ST} and Nei's genetic distance were calculated for all possible pairs of zebu, taurine and crossbred cattle of South India. The closest breeds as per pair-wise genetic differentiation value was HF purebreds and HF crossbreds (F_{ST}=0.007) whereas Kangayam and Jersey (F_{ST}= 0.262) were the distant related breeds. Among the indigenous breeds lowest differentiation was seen between Hallikar and Alambadi (0.023) and it was highest between Punganur and Kangayam (0.107).
The relationship was further elucidated using phylogenetic tree, principal component analysis and multidimensional scaling. The radial tree following UPGMA algorithm and neighbournet tree constructed based on the pair-wise Nei's genetic distance revealed separate grouping of indigenous cattle, purebred exotic breeds and crossbreds of south India into three distinct clusters with 100 per cent bootstrap value (Figs. 1a and 1b). This supports the separate evolutionary history of indicine and taurine population (8). The phylogenetic proximity of Hallikar, Alambadi and Bargur was confirmed from the genetic tree constructed based on the pair-wise Nei's genetic distance. Apparently, Ongole and Deoni were closer compared to other breeds in the present study.



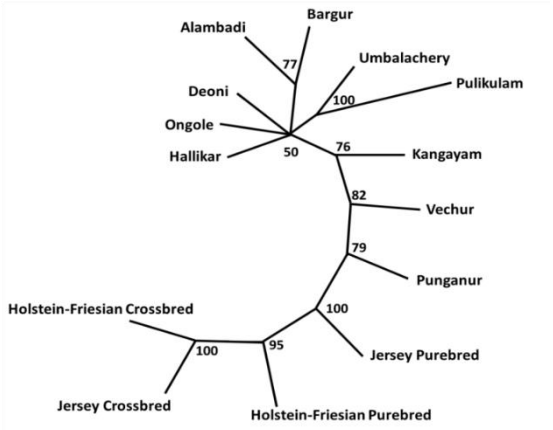


Fig 1. (a) UPGMA(left) and (b) Neighbor Net (right) tree derived from pairwise Nei's genetic distance of zebu cattle breeds (numbers at nodes indicate percent bootstrap values of 10000 resampled data sets).

Further, three dimensional scatter gram using SPSS 13.0 revealed clustering of exotic and crossbreds together and was separated from the indigenous breed cluster. Among the indigenous breeds some animals of Kangayam showed separation from the zebu cattle cluster. The clustering pattern was in harmony with the geographic location and evolutionary history. Multidimensional scaling of taurine, crossbreds and indigenous breeds is confounding with the PCA observations. A substructing was observed in Kangayam, Punganur and Vechur separated from the cluster of remaining seven breeds.
The multidimensional scaling plot derived from pair-wise F_{ST} values developed including zebu, crossbreds and exotic breeds revealed separate clustering of the zebu cattle and exotic cattle. The multidimensional scaling plot of the zebu cattle alone revealed clustering of the Tamil Nadu cattle breeds with Deoni breed of Karnataka except Kangayam. Analysis of molecular variance (AMOVA) revealed

a high between group variation when the grouping was done based on phylogeny with Hallikar and Alambadi in one group, Pulikulam, Umblachery and Bargur in one group, Deoni and Ongole in another group and Vechur, Punganur and Kangayam individually in separate groups. Similar grouping based on phylogeny was found to be best fit in case of Asian goats (9).

Population Structure and Admixture

The population structure and level of admixture was estimated using Bayesian clustering without a priori knowledge of the ancestors. The delta K versus K graph showed a peak at K=2, which represented the optimum K value for the investigated population based on the second order rate of change of likelihood function with respect to K(ΔK). HF and Jersey were assigned to the first cluster and all the indigenous breeds to the second cluster. Crossbreds showed admixture from both clusters. It was also observed that the level of exotic blood was beyond the permissible range (62.5 per cent) in some of the HF crossbreds and Jersey crossbreds. Further, breeds like Pulikulam, Umblachery and Alambadi showed high level of admixture with other south Indian breeds, despite sampling was done from animals with characterized morphological features. Breeds like Kangayam, Hallikar, Deoni and Ongole were comparatively pure to its type. The availability of purebred semen for artificial insemination can hence be a reason for maintaining the purity of breeds.

Summary

All indigenous breeds studied nurture medium to high genetic diversity but there is inbreeding within population that might have happened due to consanguineous breeding practices and small effective population size. Absence of discrete population structure observed among the breeds can be attributed to overlapping breeding or grazing tract. The genetic relatedness revealed between Hallikar and Alambadi is indeed the reason why microsatellite remains a potential tool for genetic characterization of breeds. Higher than permitted level of exotic inheritance observed in the crossbreds might predispose the animals for lower disease resistance, heat tolerance and reproductive performances. There was no bottleneck present in any of the breeds despite of the declining population size.

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EFFECT OF REPLACING UREA WITH NITRATE AS A NPN SOURCE, WITH OR WITHOUT TANNIN, ON THE HAEMATOLOGY AND SERUM BIOCHEMICAL PARAMETERS OF MERINO LAMBS

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Recent interest in the use of nitrate salts has been due to its potential in mitigating enteric methane, apart from also being an NPN source. Most of the studies on tannin and nitrogen interaction in ruminants have focused on protein digestibility and growth performance, while their impacts on the physiology and health status of animals have not been widely reported. In this study, the effect of replacing urea with calcium nitrate as NPN sources in a total mixed ration (TMR) with or without Acacia tannin extract (ATE) inclusion was evaluated based on the haematology and serum biochemical parameters of merino lambs after consuming the diets for 81 days pre-slaughter.

Twenty-four ram-lambs and sixteen ewe-lambs (34.7±4.0 kg) were blocked for sex, stratified and allotted as two animals per pen in a randomised complete block design with 5 blocks and 4 dietary treatments: 1) urea-based TMR, 2) urea-based TMR+42 g/kg DM ATE, 3) calcium nitrate-based TMR, and 4) calcium nitrate-based TMR+42 g/kg DM ATE. Diets were formulated with 60:40 concentrate: forage ratio consisting of Lucerne and Eragrostis hay. At the end of the growth period, 5 mL of blood was collected from each animal via the jugular vein to determine the haematological profile and serum biochemical variables. Using the GLM procedure of SAS, data was analysed and treatment means compared using the Tukey test.

Data on the growth performance and feed utilization of the lambs had been published as reported by Adejoro et al. (2020). No clinical or sub-clinical signs of morbidity or tannin intoxication symptoms were observed across the treatments. Animals on the nitrate-based TMR had similar haematocrit, RBC count and haemoglobin concentration with lambs on the urea-based TMR (Table 1). However, tannin reduced plasma LDH and cholesterol concentration, but had no effect on creatinine concentration in the lambs. Alanine aminotransferase level was higher in those lambs fed on the nitrate-based TMR diets compared to those lambs fed on the urea-based TMR diets ($p < 0.05$). Compared to reference standards, creatinine, total protein, ALT, AST and LDH levels were within normal ranges in all treatments. The haematological result is similar to the observation of Cherdthong et al. (2014) on the effect of nitrate in beef cattle. The results of this study suggests that neither using calcium nitrate as an NPN source, nor tannin supplementation, at the dosages used here, did not elicit any serious negative consequences to the health of the lambs.

Table 1. Haematological and serum biochemical parameters of merino lambs fed a total mixed ration diet containing urea or nitrate as NPN source, with or without acacia tannin extract

	Urea diet		Nitrate diet		[2]SEM	[3]p-values		
[1]Parameter	No tannin	With tannin	No tannin	With tannin				
N	10	10	10	10		N	T	N*T
Haemoglobin (HB; g/L)	116.9	119.9	122.5	125.0	1.79	0.094	0.629	0.895

RBC count ($\times 10^{12}$ cells/L)	9.97	10.6	10.6	10.8	0.17	0.089	0.348	0.518
Haematocrit (HCT; L/L)	0.33	0.34	0.35	0.34	0.01	0.083	0.877	0.372
MCV (fL)	33.1	32.1	33.2	32.1	0.27	0.869	0.077	0.80
WBC count ($\times 10^{9}$ cells/L)	7.56	7.46	7.11	7.43	0.23	0.721	0.668	0.222
Creatinine, µmol/L	57.9	58.0	67.3	65.1	1.23	0.054	0.692	0.99
Total serum protein, g/L	62.9	60.5	63.1	62.1	0.48	0.759	0.272	0.852
Cholesterol, mmol/L	1.34	1.10	1.41	1.31	0.04	0.818	0.029	0.511
Aspartate amino-transferase (AST), U/L	106.8	105.1	97.7	107.6	2.81	0.388	0.677	0.660
Alanine amino-transferase (ALT), U/L	14.0	15.6	17.9	18.8	0.58	0.001	0.351	0.656
Lactate dehydrogenase (LDH), U/L	917.8	805.9	946.3	872.1	18.6	0.204	0.012	0.781

[1]RBC, Red blood cell; MCV, Mean corpuscular volume; WBC, White blood cell.
[2]SEM, standard error of mean.
[3]N, effect of NPN source; T, effect of tannin; N*T, interaction effect (NPN source+ tannin inclusion).

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PRODUCTIVE PERFORMANCE, INTESTINAL MORPHOLOGY AND IMMUNITY OF BROILER CHICKENS FED GINGER AND NETTLE AS ANTIBIOTIC GROWTH PROMOTER SUBSTITUTION

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Abstract

Use of antibiotics as feed additives in poultry has caused emergence of new pathogenic strains. Herbs and phytogenic compounds have attracted a lot of attention for their potential role as alternatives to antibiotic growth promoters (AGP) in poultry. This experiment was conducted to investigate the effect of ginger *(Zingiber officinale)*, nettle *(Urtica dioica)* and mixture of both as an AGP substitution on growth performance, carcass characteristics, intestinal morphology and immunity of broiler chicks. A total of 375 one-day-old chicks (Ross 308) were allocated to 5 dietary treatments with 5 replicates of 15 chicks in a completely randomized design. Dietary treatments consisted of basal diet as control; AGP (Flavophospholipol); 2 g/kg nettle powder; 4 g/kg ginger powder and 2 g/kg ginger+2 g/kg nettle powder. Results showed that AGP, ginger, nettle and ginger + nettle all elevated body weight in the overall growth period compared with the control ($P < 0.05$). Feed consumption of broilers fed nettle or ginger were increased in the entire growth period ($P < 0.05$). The best feed conversion ratio was observed in the AGP group ($P < 0.05$). Antibody titers against Newcastle and Influenza viruses and sheep red blood cell were not affected by dietary treatments ($P > 0.05$). Carcass yields were not affected, but abdominal fat decreased in the ginger group. The lowest jejunum villous height and villous height to crypt depth ratios were observed in the ginger group ($P < 0.05$). In conclusion, dietary supplementation with either ginger or nettle improved productive performance and can be applied as alternatives to AGP in broiler diets.

Key words: Broiler chick, Nettle, Ginger, Antibiotic, Performance, Immunity.

Introduction

Antibiotic growth promoters (AGP) have been used in poultry diets for more than 50 years to promote growth and prevent diseases. The growing concerns about antibiotic residues in final poultry products and the risk of bacteria acquiring resistance to these specific antibiotics has become a controversial issue around the world. Thus, new alternatives, including herbal products, have been suggested in the poultry industryas possible AGP (Toghyani et al., 2010).

Ginger *(Zingiber officinale*), is a monocotyledonous herbaceous plant and one of the most common food-flavoring spices used worldwide. The main important compounds in ginger are gingerol, gingerdiol and gingerdione, which have the ability to stimulate digestive enzymes and affect the microbial activity and have antioxidative activity (Dieumou *et al.*, 2009). Ginger has been reported to enhance the growth performance and digestibility in broilers and be effective in treating and controlling coccidial infection (Ali et al., 2019; Qorbanpour et al., 2018). Nettle (*Urtica dioica* L.*)* is widely grown in different parts of the world and has been used to improve human health. The plant has been reported to have various pharmacological activities, such as antioxidant, anti-inflammatory, anti-colitis, antiulcer, anticancer, antiviral, antibacterial, antimicrobial, antifungal, antiandrogenic, insecticide, immunomodulatory,

hypoglycemic, cardiovascular effects, analgesic, natriuretic, hypotensive and hepatoprotective (Joshi et al., 2014). Recent studies showed that using nettle in broiler diets had positive effects on performance, carcass traits, and blood biochemical and immunity parameters (Ahmadipour and Khajali, 2019; Keshavarz, 2014; Dalvand et al., 2018). However, little information has been published on mixtures of nettle and ginger in broiler diets. Therefore, the aim of this study was to evaluate the effect of ginger, nettle and mixture of both as anAGP substitute on growth performance, intestinal morphology and immunity of broiler chicks.

Materials and method

Herbal plants were purchased from the local market in fresh condition. After identification by the botanical section of the Islamic Azad University Isfahan (Khorasgan) Branch, the plants were dried, powdered and used in the present study.
A total of three hundred and seventy-five one-day-old unsexed broiler chicks (Ross 308) were allocated to 5 dietary treatments with 5 replicates of 15 chicks in a completely randomized design. Birds were raised in floor pens from 1 to 42 d. The lighting program was 23 h of light followed by 1 h in darkness. All chicks had free access to feed and water throughout the trial. Dietary treatments consisted of basal diet as control; AGP (5 mg/kg Flavophospholipol); 2 g/kg nettle powder; 4 g/kg ginger powder and 2 g/kg ginger+2 g/kg nettle powder. Chicks were fed starter diets from d 1 to 14, grower diets from d 15 to 28, and finisher diets from d 29 to 42, based on the Ross 308 catalogue recommendation (Aviagen, 2014).
Chicks were weighed at 1, 14, 28, and 42 d of age. Daily feed intake (DFI) was measured during starter, grower, finisher periods and over the entire experimental period. Mortality was recorded as it occurred to correct DFI. Feed conversion ratio (feed:gain) was calculated.
Two birds from each pen were slaughtered on d 21. Segments of the small intestine were sampled from jejunum. The jejunum samples were evaluated for the villus height, crypt depth and the ratio of villus height to crypt depth as intestinal morphology.
At 9 d of age, Newcastle and influenza antigens were injected to chicks with a dual vaccine of Newcastle-influenza (H9N2 subtype). Two chicks per pen were selected randomly for intraperitoneal injection with a sheep red blood cell (SRBC) suspension at 25 d of age. Five days later, the same wing-banded birds were bled to determine antibody titer against SRBC, Influenza disease virus (IDV) and Newcastle disease virus (NDV). Subsequently, antibody titer against IDV and NDV separately were measured by hemagglutination inhibition (HI) and SRBC by hemagglutination assay (HA) method.
The data were statistically analyzed by the GLM procedure of SAS (2012) in a completely randomized design. Means were separated using Duncan's multiple range test at significance level of $P < 0.05$.

Results and discussion

The performance, intestinal morphology, carcass and immunity indices of control and supplemented chicks are summarized in Table 1. The results showed AGP, ginger, nettle and mixture of ginger and nettle elevated body weight in the overall growth period compared with control ($P < 0.05$). Feed consumption of broilers fed nettle or ginger was increased over the entire growth period ($P < 0.05$). The best feed conversion ratio was observed in the AGP group ($P < 0.05$). Improvement in growth performance by ginger and nettle are associated with the antioxidative effects of naturally-occurring terpenoid phenols in the plant. Carvacrol and carvone are the main terpenoids found in nettle, which account for 46.8% of the oil. These compounds exhibit a broad range of biological properties, such as growth-promoting, antioxidant, antibacterial and antiviral actions (Upton, 2013). The main important compounds in ginger are gingerol, gingerdiol and gingerdione, which stimulate digestive enzymes, affect the microbial activity and have antioxidative activity (Dieumou *et al*., 2009). Antibody titers against Newcastle and Influenza viruses and sheep red blood cell were not affected by dietary treatments ($P > 0.05$). Sharma et al. (2018) and Nasiri et al. (2011) reported dietary nettle did not have any significant effects on immunity parameters of broilers, which is similar to the present study on nettle.

Carcass yields were not affected, but abdominal fat decreased in the ginger group. In agreement, Zhang et al. (2009) showed that the addition of ginger slightly reduced abdominal fat content of broilers. Moorthy et al. (2009) did not find any significant differences for dressing percentage and eviscerated weight in broilers by feeding ginger. The lowest jejunum villous height and villous height to crypt depth ratio were observed in the ginger group ($P < 0.05$). Contrary to our results, Shewita and Taha (2018) reported higher villous lengths and greater crypt depths in broilers by feeding ginger. The differences in the current results with other researchers in relation to growth performance, carcass traits, immunity and morphology of intestine may be due to the different varieties of ginger and nettle used, dosage, drying process and the duration of the experiments.

Conclusions

Based on the results of this study, application of ginger, nettle powder or mixture of both as a growth promoter improved various performance indices but had no effect on immune responses of broiler chicks. Thus, ginger and nettle could be considered as alternatives to in-feed antibiotics in broiler diets.

Table 1. Effect of ginger, nettle and mixture of both on growth performance, carcass characteristics, intestinal morphology and immunity of broiler chicks

Treatment	Growth performance (1-42 d)			Jejunum morphometric (21 d)			Antibody titer (30 d)			Carcass trait (42 d)	
	FI (g/d)	BW (g)	FCR (g/g)	VH (µm)	CD (µm)	VH: CD	IDV (Log2)	NDV (Log2)	SRBC (Log2)	AF (%LW)	CY (%LW)
Control	86.3 b	2327.2 b	1.59 ab	1362 a	191	7.32 a	5.37	4.62	7.12	1.25 a b	65.04
AGP	86.1 b	2536.4 a	1.49 b	1204 bc	189	6.70 ab	5.40	4.70	7.30	1.57 a	64.04
Ginger	92.6 a	2393.4 ab	1.72 a	1115 c	191	6.00 b	5.00	4.50	7.20	1.14 c	65.33
Nettle	95.0 a	2393.8 ab	1.70 a	1263 ab	206	6.12 a	5.25	4.50	7.58	1.41 ab	65.88
Ginger + Nettle	89.7 ab	2411.8 ab	1.59 ab	1256 ab	205	6.36 b	5.00	4.37	7.00	1.37 abc	65.80
SEM	0.983	26.375	0.024	22.57	42.69	0.1436	0.138	0.116	0.089	0.069	0.825

a-d Mean values in column with no common superscripts differ significantly ($P \leq 0.05$).
AGP: Antibiotic growth promoter (Flavophospholipol); FI: Feed intake; BW: Body weight; FCR: Feed conversion ratio; VH: Villous height, CD: Crypt depth, IDV: Influenza disease virus, NDV: Newcastle disease virus, SRBC: Sheep red blood cell, AF: Abdominal fat, CY: Carcass yield, LW: Live weight

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ALUM [ALUMINUM SULPHATE] AS A LITTER AMENDMENT TO CONTROL ODOR IN BROILER HOUSES

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Abstract

One hundred and forty-four finishing broilers were used in a four weeks study to investigate the performance and ammonia reducing potential of Alum when deployed as a litter amendment. The birds were randomly assigned to four treatments with 36 birds per treatment. Each treatment was replicated four times with 9 birds per replicate in a completely randomized design (CRD). The litters were treated with 0, 100, 200 and 300g of alum/square meter in treatments 1, 2, 3 and 4 respectively. Treatment 1 served as the control. Daily feed intake records were measured and body weight measurements of the birds were kept on weekly basis. Litter pH, Ammonia, Nitrate, Volatile Fatty Acids [VFA] and microbial respiration were analyzed using appropriate procedures. Data collected were subjected to analysis of variance [ANOVA] in a completely randomized design using the SPSS 17.0 computer package. Performance indices of the birds increased as the level of Alum in the litter increased. Treatment 4 birds (i.e. 300g of Alum in their litter) had significantly higher values [$P< 0.05$] than the others. Litter pH showed a decline from 8.98 in the control to 2.98 in Treatment 4. Likewise the rate of ammonia volatilization was significantly reduced from 15.78 mg/L in the control birds to 8.0mg/L in Treatment 4. The same trend was observed for data on Nitrates, VFA and microbial respiration. We conclude that litter amendment using Alum improves birds' performance and results in a lower rate of ammonia volatilization. The reduced ammonia volatilization presumably reduced odor emanating from the broiler house.

Keywords: Alum, litter, odor, broiler houses

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FORAGE BASED TOTAL MIXED RATIONS ON MILK PRODUCTION

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Introduction

The total milk production in the year 2018 in Sri Lanka was 471.6 million litres. However, this was only 45% of the national requirement. Thus 99,028 metric tons of milk powder were imported to meet the gap (Central Bank of Sri Lanka, 2018). The above report further stated that the Government has taken measures in 2018 to introduce newly developed pasture species with the aim of feeding the upgraded cattle to increase the domestic milk production. The new species introduced included Napier (*Pennisetum puerperium*) hybrids obtained from India. These hybrid pastures are high in dry matter content and palatability (Premaratne & Premalal, 2006; Somasiri et al., 2010). Thus, they are popular with the dairy farmers. And the grasses are mostly cut and fed or fed as silage.
Feeding forage based total mixed rations (TMR) is a trend in many medium and large-scale dairy farms in Sri Lanka. The main objectives of feeding TMR are to increase the daily milk production and to enhance the cow body condition score (BCS). Sri Lanka has two distinctive drought seasons; January to March and June to September (Punniyawardena, 2008). During these periods body condition score (BCS) of the dairy cows drops drastically due to lack of quality and quantity of feeds. The objective of the present research was to introduce a grass based TMR to enhance the existing milk production and quality.

Materials and Methods

A medium-scale farm situated at the intermediate zone (7.4322° N, 80.4438° E, altitude 66 m) was selected for the research. Two TMRs (TMR1 and TMR2) were formulated according to NRC (2001). The composition of the rations is given in Table 1.

Table 1: Composition (percentage) of the Total Mixed Rations (TMR1 and TMR2)

Raw ingredient	TMR1	TMR2
Chopped maize fodder (*Zea maize*)	55.5	10
Chopped hybrid Napier CO3 (*Pennisetum perpureum X Pennisetum americarnum)*	13.8	25
Chopped guinea grass *(Panicum maximum)*	-	20
Commercial Cattle feed	11.1	-
Beer pulp	11.28	4
Dhal meal *(Cajanus cajan)*	5.55	-
Coconut poonac *(Cocos nucifera)*	-	20
Rice bran *(Oryza sativa)*	-	10
Maize meal	-	10
Mineral mixture	2.77	1

TMR 1 had a crude protein (CP) of 8.2% and metabolizable energy (ME) content of 2603.9 kcal/kg while TMR 2 had CP of 7.5% and ME content of 2616.0 kcal/kg.
Eighteen Friesian crossbred lactating cows of age 3.5 years, having an average body weight of 418±13 kg and Body Condition Score of 2.7±0.05, were selected for the research. The initial average milk yield was 9.5±4.12 litres (mean ± SE). The cows were randomly assigned into two treatments (TMR1 and TMR2). Each treatment had three replicates. The treatments were arranged according to a Randomized Complete Block Design. A preliminary period of 7 days was given for the cows to get used to the TMRs. Machine milking was practised twice a day, early morning (3.30 a.m.) and late afternoon (3.30 p.m). Water was provided *ad-lib*. Data such as daily milk yield and feed intake were collected for 5 weeks.

Results and Discussion

Effect of Treatment on Milk Yield and Composition

Table 2: Effect of TMR1 and TMR2 on milk yield and composition

Parameter	Treatment		
	TMR1	TMR2	SE
Feed intake (kg as fed basis)	33.7[b]	34.8[a]	0.06
Milk yield (L)	10.15[b]	12.01[a]	0.24
Feed conversion efficiency (FCE)	0.643[a]	0.683[a]	0.0008
Milk composition (%)			
Fat	4.54[b]	4.86[a]	0.0016
SNF	8.84[b]	9.05[a]	0.00046
Protein	3.41[b]	3.65[a]	0.0081

[a b] means within the same row with different superscripts are significantly different ($p < 0.05$).

The cows fed TMR2 had higher ($p<0.05$) feed intake and milk yield than the cows fed TMR1 (Table 2). Supporting the above findings higher milk yields have been obtained by Scharen et al., (2016), Hernandex-Ortega et al., (2014) and Mohammad et al., (2017) by feeding TMR based rations than feeding concentrates and roughages separately. Milk composition data were also higher in TMR2 cows than TMR 1. Similarly, Mohammad et al., (2017) reported that cows fed on TMR had higher ($p<0.05$) total milk fat percentage than feeding concentrates and roughages separately. Bargo et al., (2002) reported that TMR feeding produced 38% more fat than cows on pasture feeding.

However, the feed conversion efficiency (FCE) and BCS (TMR1 2.74±0.0014 and TMR2 2.87±0.0014) were not different between the treatments. The two TMRs had similar efficiencies but TMR2 provided comparatively better results than TMR 1. Khan et al., (2010), Verma et al., (1999), Kolver and Muller, (1998) and Devries and Gill, (2012) suggested that the high performance due to TMR may be due to high palatability and enhanced digestion as a result of low particle sizes. However Felton and Devries, (2010) observed a lower feed intake in TMR compared to conventional feeding. They suggest that it may be a result of high moisture content in TMR reducing the voluntary intake. Feeding TMR is very effective in the Sri Lankan context because it avoids the fluctuations of feed quality and quantity in drought season and high rainfall season where it is difficult to allow the animals to graze outside.

In the extensive management systems in the intermediate zone of Sri Lanka, dairy cows are allowed to undertake tethered grazing under coconut plantations or roadsides or in government lands. The milk production is marginal in this system. However, the majority of the farmers practise semi intensive management system where the cows are allowed to graze during the day and stall-fed at night with concentrates and silage. Thus, the milk production is comparatively high. The cut-and-carry system with

stall-feeding at night with concentrates is practiced in the intensive system (Samarajeewa et al., 2003; Premaratne et al., 2013). However, the labour costs of cut and carry are high if family labour is scarce. Total mixed ration feeding avoids sending the animals for grazing thus it reducers the energy waste of walking and consequent compromises on milk production.

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POTENTIAL FOR NUTRITION OF RUMINANTS OF PELAGIC SARGASSUM ARRIVALS ON THE BEACHES OF DOMINICAN REPUBLIC

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Introduction

Sargassum arrivals are a natural phenomenon, but from 2011 to the East coast of Dominican Republic massive amounts of Sargassum have arrived. The events from years 2015 and 2017 have exceded the arrivals of other recent years (Mendez y Rosado, 2019).
In the Southerna and Eastern coasts of Hispaniola, predominantely two species of Sargassum have been found: *Sargassum natans* and *Sargassum fluitans*. These are brown pelagic macroalgae. In smaller amount *Sargassum polyceratum* Var. *Ovatum* has also been identified (Rosado, 2019).
Algae from diverse species are an important source of vitamins, minerals, antioxidants and natural dyes. Even when used in small amounts in animal nutrition, it has been reported that algae improve the immune system, weight gain, reproductive performance, among other benefits that can improve the quality of meat and eggs (Kovac *et al.*, 2013).

Methodology

A descriptive study was made to characterize sargassum that arrives at the coasts of La Altagracia province in the Dominican Republic, and a feeding trial was later performed. At Cabeza de Toro beach the following samples were taken: three (3) samples of fresh Sargassum from over the sand and later sun-dried (SPL); three (3) samples of fresh Sargassum floating on the sea next to the beach and later sun-dried (SML); three samples of fresh Sargassum of fresh Sargassum floating on the sea next to the beach and later oven-dried (SMH); and one (1) sample of fresh Sargassum floating on the sea next to the beach and sun-dried withough washing (SMSL).
The samples were transported to the experimental farm CASTA of UNEV. After weighing and measuring the amount of foreign material, the samples were washed and dried under protected environment (200-micron plastic) with open ventilation. SMH samples were washed and oven-dried at 60°C. All samples were vacuum-packed and sent to the Cumberland Valley Analytical Services, Inc. (EEUU) laboratory.
A feeding trial was carried out with dried and ground Sargassum flour (SF). A completely random design was used with four treatments and 10 repetitions which were male weaned lambs between 4 and 6 months old. All treatments received a basic diet that included forage from tropical grasses and shrubs and 454 gr (1 lb) of supplement feed with or without SF per day. All supplement feeds were balanced to 14% crude protein (CP) and 11 MJ EM/kg MS. The treatments were: T0 with supplement without SF; T1 with supplement including 10% SF; T2 with supplement including 20% SF; and T3 with supplement including 30% SF. After a period of 21 days of adaptation, weights were taken on the first day and at 60, and 152 days. Blood samples were collected from all animals in the experiment, and red blood cells and blood platelets were measured.

Results

The weight of wet Sargassum at collection at the beach has a mean density of 167.26 kg/m^3. The amount of impurities in the Sargassum samples was 6.08% in general. Sun drying lasted 7 days during the month of October of 2015. Average after-drying weight was 15.24% of wet samples. There was no statistical difference among different sources of Sargassum.

Sargassum samples had different humidity levels depending on drying procedure and source of sampling. Oven-dried Sargassum had the lowest percentage of humidity, 6.27±0.07, and the highest was for samples taken from over the sea and sun-dried with 25.1±0.85. The average dry matter (DM) in Sargassum samples was 11.72±1.27%.

Crude protein (CP) content on Sargassum DM was 6.80±0.15%. The highest level was obtained in oven-dried Sargassum with a value of 7.37±0.13%. While the percentage of adjusted protein over DM was 4.88±0.14, with no statistical difference at $p \leq 0.05$.

The Sargassum samples that were collected over the sand and sun-dried had a higher percentage of Acid Detergent Fiber (ADF) with values of 25.8 ± 0.29, which was statistically similar to the samples of oven-dried Sargassum which had an average of 22.57 ± 0.09. Lignin was present on average as 22.31% of DM, and there was no statistical difference related to drying method or source of collection. But lignin as percentage of NDF had statistical difference ($p \leq 0.05$) with values of 81.32 ± 0.63 for oven-dried Sargassum, 71.26 ± 2.48 for sun-dried fresh Sargassum, and 63.06 ± 1.14 for Sargassum collected over the sand.

The average percentage of raw fat was 0.27 ± 0.06, and the content of non-fibrous carbohydrate (%NFC) was 29.02%. Neither had statistical difference for the drying method or collection source at $p \leq 0.05$.

The percentage of total digestible nutrients (TDN) was 26.92±1.36. This is equivalent to 3.05 MJ ME/kg DM according to the formula provided by Moran (2005). Sargassum collected over the sand and sun-dried had the highest percentage of TDN, 32.07±2.1, statistically similar to sea-collected and sun-dried Sargassum, 25.67±1.4, but different than sea-collected and oven-dried Sargassum, 24.83 ± 0.56 ($p \leq 0.05$).

The Net Energy for Lactation (NEL) present in Sargassum is 0.27±0.02 Mcal/lb, and Net Energy Gain was -0.16±0.02 Mcal/lb. In vitro digestibility (DIV) at 30 hours of DM was 22.00±2.35%. There was statistical difference ($p \leq 0.05$), as shown in table 1.

Table 1. Summary of Analysis as Feedstuff of Sargassum collected in the East coast of the Dominican Republic.

Analysis	**Sargassum samples ***		
	SMH	**SML**	**SPL**
Humidity (%)	6.27 ± 0.07 a	25.1 ± 0.85 c	18.13 ± 0.58 b
Dry Matter (%)		9.47±0.38	15.27 ±1.86
Dry Matter in samples (%, w/w)	93.73 ± 0.07 c	74.9 ± 0.85 a	81.87 ± 0.58 b
Crude Protein (% DM)	7.37 ± 0.13 b	6.43 ± 0.19 a	6.73 ± 0.19 ab
Adjusted Protein (% DM)	5.3 ± 0.1 a	4.77 ± 0.32 a	4.57 ± 0.22 a
Adjusted Protein (% CP)	71.87 ± 0.44 a	74.4 ± 2.55 a	67.67 ± 1.4 a
Soluble Protein (% CP)	22.2 ± 3.94 a	17.33 ± 2.56 a	11.9 ± 1.39 a
Protein NDF (%DM)	4.02 ± 0.2 b	3.07 ± 0.06 a	4.02 ± 0.08 b
Acid Detergent Fiber, ADF (%DM)	22.57 ± 0.09 ab	19.67 ± 2.18 a	25.8 ± 0.29 b
Acid Detergent Fiber, ADF (%NDF)	86.03 ± 2.25 b	76.47 ± 3.09 ab	73.43 ± 0.43 a
Lignin (%DM)	21.38 ± 0.66 a	18.33 ± 1.94 a	22.16 ± 0.28 a
Lignin (%NDF)	81.32 ± 0.63 c	71.26 ± 2.48 b	63.06 ± 1.14 a
Crude Fat (%DM)	0.36 ± 0.08 a	0.18 ± 0.04 a	0.33 ± 0.18 a
Non Fiber Carbohydrates (%DM)	26.97 ± 0.82 a	28.47 ± 1.3 a	33.33 ± 2.79 a
Total Digestible Nutrients, TDN (%DM)	24.83 ± 0.56 a	25.67 ± 1.4 ab	32.07 ± 2.1 b
Net Energy Lactation (Mcal/lb) (MJ/Kg)	0.25 ± 0.01 a	0.25 ± 0.02 a	0.32 ± 0.02 b

Net Energy Gain (Mcal/lb)	-0.2 ± 0.01 a	-0.18 ± 0.02 a	-0.07 ± 0.04 b
In vitro Digestibility 30 h (%DM)	12.13 ± 0.70 a	23.10 ± 0.91 b	29.87 ± 0.27 c
N	3	3	3

* *Averages with same leters are not statistically different ($p \leq 0.05$).* Paulino and Bethancourt, 2020.

The percentage of ashes present in Sargassum was 38.79 ± 2.39. The results of Ca, Cr, Cu, Na, S, and Zn showed no statistical difference concerning drying method and source of samples. The Calcium content (DM) in Sargassum is 60,628.89±4,195.11 ppm, Chromium 1.78±0.19 ppm, Copper 4.00±0.12 ppm, Sodium 18,524.27± 1,085.07 ppm, Sulfur 13,569.84± 227.37 ppm, Aluminum 271.81±25.47 ppm; Zinc 7.13±0.28 ppm, and Magnesium 12,594.51±475.87 ppm.
Minerals such as Arsenic, Barium, Boron, Magnesium, Manganese, Phosphorus, and Potassium did show statistical difference ($p \leq 0.05$) depending on Sargassum source or drying method as reported in table 2. Other metals including Lead, Cadmium, and Mercury were not detected by the laboratory.

Table 2. Mineral content in Sargassum samples collected in the East coast of the Dominican Republic.

Analysis	**Sargassum samples ***		
	SMH	**SML**	**SPL**
Ashes (%DM)	43.08 ± 0.25 b	42.39 ± 1.57 b	28.45 ± 2.18 a
Aluminum (ppm)	348.67 ± 57.06 a	235.93 ± 13.43 a	231.73 ± 43.48 a
Arsenic (ppm)	143.03 ± 1.72 c	106.2 ± 6.27 b	53.53 ± 0.49 a
Barium (ppm)	25 ± 0.72 a	26.3 ± 0.51 ab	30.77 ± 1.74 b
Boron (ppm)	161.13 ± 4.02 a	252 ± 15.11 b	428.07 ± 25.87 c
Calcium (ppm)	57,124.13 ± 2,069.1 a	69,587 ± 9,075.81 a	54,701.5 ± 10,637.84 a
Cromium (ppm)	1.73 ± 0.33 a	1.4 ± 0.1 a	1.87 ± 0.42 a
Copper (ppm)	5.33 ± 0.88 a	3.67 ± 0.33 a	6.67 ± 1.45 a
Hierro (ppm)	33.47 ± 2.12 a	107.97 ± 8.94 b	160.23 ± 14.37 c
Magnesium (ppm)	11,000.93 ± 93.21 a	12,806.87 ± 854.82 a	13,535.6 ± 794.78 a
Manganese (ppm)	24.8 ± 0.42 a	52.3 ± 1.14 c	38.97 ± 1.39 b
Phosphorus (ppm)	1,151.43 ± 8.01 b	1,031.07 ± 79.52 b	568.53 ± 66.95 a
Potassium (%DM)	9.54 ± 0.16 c	7.6 ± 0.26 b	1.76 ± 0.15 a
Potassium (ppm)	91,672.23 ± 2771.06 c	72,025.47 ± 2012.29 b	16,683.03 ± 1116.72 a
Sodium (ppm)	16,946.03 ± 369.02 a	20,001.6 ± 1545.57 a	16,076 ± 233.94 a
Sodium (%DM)	1.74 ± 0.01 a	2.03 ± 0.15 a	1.63 ± 0.06 a
Sulfur (ppm)	13,770.87 ± 428.3 a	13,955.17 ± 248.35 a	12,932.57 ± 478.48 a
Zinc (ppm)	6.57 ± 0.09 a	6.57 ± 0.58 a	8 ± 0.21 a
N	3	3	3

* *Averages with same leters are not statistically different ($p \leq 0.05$).* Paulino and Bethancourt, 2020.

The feeding trial was designed with diets at a basic nutritional level, even so, weight gain was seen with an increase of 4.05 kg at 60 days and 10.51 kg at 152 days for an average of monthly increase of 2.07 kg. Treatments T0, T1, T2 y T3 had increased weight of 3.02, 4.04, 4.38, and 4.97 kg at 60 days, and 10.43, 10.54, 10.26, and 10.85 kg at 152 days, respectively. T3 (with 30% SF in the supplement) had the highest weight gain.
Daily weight gain (DWG) at 60 days was between 50.35 and 82.75 gr, and at 152 days, 67.49 and 71.40 throughout all treatments. At 60 days DWG increased according to the inclusión of SF in the diets. This did not occur at 152 days as T2 had lower DWG that the other treatments. Even so, T3 consistently had the highest DWG.
Blood samples were collected from all lambs in the feed trials at 61 days after initial feeding with SF. Results show that red blood cells for T0, T1, T2 y T3 were 24.6, 27.2, 28.2, and 32.4, respectively. While in the same order blood platelets were 198.8, 277.0, 192.2, and 337.8, respectively. T3 had the highest leve of red blood cells and blood platelets.

Discussion

Crude protein in marine brown macroalgae is in the range of 2.4 to 16.8%, which is lower than reported for green and red macroalgae (Øverland *et al.*, 2019). Our results for CP were within that range, but lower than the 15.4% reported from Sargassum in African coasts (Oyesiku and Egunyomi, 2014) and the 12.8% reported by Millege andy Harvey (2016) citing Tiwari and Troy (2015). Although it was similar to the 7.7% of PC in *Sargassum* spp. found in México (Casas-Valdez, 2006).
Studies performed with tropical grasses of various genera typically used as dairy cattle, which were cut at an advanced state, had a mean of 6.5% CP (Mlay *et al.*, 2006). Thus pelagic Sargassum thus not qualify as a protein feedstuff.
Tropical forages usually have a high level of NDF. Results of various genera of grasses and legumes yielded an average of 69.8% of NDF for grasses and 37.8% for legumes (Mlay *et al.*, 2006). ADF in *Brachiaria* spp., *Panicum* spp. y *Pennisetum* spp. Grasses reported by Ortega-Gómez *et al.* (2011) was in the range of 41.6 and 48.7%.
Sargassum had a lower NDF and ADF tan tropical grasses. It can be compared to other feedstuffs such as rice bran and wheat bran, which have 33.5 and 49.0% of NDF, respectively. The average ADF of Sargassum (25.8%) was higher than the 15.0% reported for wheat bran (Mlal *et al.*, 2006; Schulze *et al.*, 1994). Since ADF represents the least digestible compoments in a plant, including lignin, this may be a limiting factor for voluntary intake of Sargassum.
The 3.05 MJ ME/kg DM in Sargassum is low enegy level for feed that has to be dried and transported. A minimum of 48% TDN is expected in tropical forages, which is equivalent to 7.0 MJ EM/kg MS. The minimum expected in tropical legumes is between 50 and 73% of TDN (Moran, 2005).
Dry matter digestibility of tropical forages is between 50% and 65% (Aminah and Chen, 1985; Moran, 2005). Low digestibility of Sargassum is associated with its low energy level, which indicates that its use in animal diets should be complemented by other high energy ingredients to avoid nutritional deficiencies.
The potential of Sargassum, as with other algae, is based on its mineral content, where the levels of calcium, magnesium, and potassium in Sarsgassum are 6.06%, 1.26%, and 5.83%, respectively. The inclusion of Sargassum in animal feed may allow for a reduction in the use of minerals from inorganic sources. The amount of arsenic, cadmium, and lead is un the maximum permitted in the present regulation of the European Union.
In the feed trials with lambs, T3, with 30% inclusion of SF in the supplement, equivalent to 151 gr per day of dried Sargassum, had the highest levels of weight gain, daily weight gain (DWG), red blood cells, and blood platelets. This indicates a superior performance. The number of red blood cells increased in a directly proportionate manner to the inclusion of SF in the diet, so an antiparasitic effect is appreciated which is assumed is due to the compounds present in Sargassum. The fact that a basic nutritional level was used in the feeding trial suggests a favorable effect in performance and animal health can be attributed to Sargassum flour in the feed.

Conclusion

Sargassum collected in the East coast of the Dominican Republic consists primarily of *Sargassum fluitans* and *Sargassum natans*, and has the potential to be used in animal nutrition although it has low levels of PC (6.8%) and energy (NDT: 26.92%; 3.05 MJ EM/kg MS). Particular interest is in its potential as a source as minerals such as Calcium, Potassium and Magnessium. Its salt content may represent a limit to its inclusion in diets. Favorable levels of animal health and performance with a basic diet that included Sargassum flour was obtained. Therefore the use of Sargassum as ruminant feed is recommended.

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MATERNAL ABILITY AND DAILY BEHAVIOUR OF KOSTA AND BOERKA GOATS

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Indonesia has various types of goat livestock genotypes, one of them is the Kosta goat that endemic in Banten Province. The uniqueness as a characteristic of Kosta goats is the line design on the fur on the face parallel to the left and right. The population of Kosta goats in 2000-2014 is estimated to be around 1,000-1,500, but it is drastically decreased in the 2014-2015 to only around 100-150 head. Boerka goat is a result of crossing of boer goats with Indonesian local goats, Kacang goat. These crosses were produced by the Goat Research Station, Sei Putih. There is little information about the behavior of kosta and boerka goats, especially how the ability of kosta and boerka mothers in caring for their children. Birth of kids is one thing that is expected in livestock farming activities. However the birth events are often followed by deaths of kids both before as well as after weaning. The maternal ability is also one of the determining factors in a kids's ability to survive. And efforts to conserve kosta goats will be harmonious if equipped with a lot of information about this. The purpose of this study was to identify the characteristics of the daily behavior of mothers and kids of Kosta and Boerka goats so that information was obtained about their ability to care for their offspring.
The research was conducted at The Breeding Research Facilities of Goat Research Institute, Sei Putih, North Sumatera using 9 Kosta and 9 Boerka does with 13 Kosta and 15 Boerka kids of three weeks old, resepectively. The study was carried in two stages, the first stage was a macro observations, to investigate the daily behaviour patterns of the does and kids, while the second stage was the micro observations that were observed to determine the process during suckling .
Among seven types of does behaviour observed in the study, only rejecting kid, feeding, ruminating and walking showed significant differences ($P<0.05$) among breeds (Table 1). This rejecting behaviour of does to the kids was in line with higher feeding and ruminating behaviour of the does. This indicated that does felt uncomfortable to nursing the kids when they were feeding or ruminating, that occured in the morning around 07:00 am and 16:00 PM in the afternoon. The rejecting behaviour was investigated when kids reached 2-5 weeks old and accelarated according to the increasing of kids age until the end of lactation period.
Among 12 behavioural types of kids during observation, it showed that only sleeping, grouping, looking for does and playing, had significant responses ($P<0.05$) (Table 2). Clustering the behaviour activities of sleeping, grouping and playing of Boerka kids, it showed that at 20:00-04:00 PM, most of the period was used for sleeping and from 04:00-20:00 PM was used for playing and grouping with others. On the other hand sleeping, playing and grouping of Kosta was much lower and there was no specific time to do activities and tended to express their activities across the periods of time. Its showed the kids rejecting and walking activities of the does of Kosta compared to Boerka.
The successfull of suckling process was shown finally with kids kneeling and nagging the tail. The avarage occasion of kids mooing was 0.18 times and was not significantly affected by breed types as well as interval of suckling which was morning or afternoon (Table 3). Kosta had higher frequencies to look for the does compared to Boerka kids ($P<0.05$). This indicated that Kosta does were much more protecting the kids compared to Boerka does. After kids can find their does, the next step was teat

seeking with an average time spent about 0.6 times and breeds significantly (P<0.05) affect the effort to teat seeking. There was no significant influence of breed as well as period of the day to teat nuzzling, with an average of 3.8 times. In the morning, both breeds significantly (P<0.05) influenced during the process to reach the teat they prefer to suckle, however in the afternoon this attempt was not seen again. Attempts to suckle, was initiated by teat licking with an average of 0.17 times and significantly affected (P<0,05) by breeds and period of activities. When the attempt of suckle was success, kids kneeled with an average of 0.58 times and significantly affected (P<0.05) by breeds of goat. In the afternoon session, Boerka kneeled longer (0.67 times) compared to Kosta kids (0.48 times). The average of tail nagging was 0.83 times with significant influence (P<0.05) of breed, Boerka kids had longer frequency for nagging (0.86 times) compared to Kosta kids (0.75 times).

Among seven behaviour types during nursing , licking kids, stand still, moving apart , caring and agresivenes significantly influenced (P<0,05) by breeds and / or interval of observation (Tabel 4). Both breeds licked the kids in most of the nursing time, and in average, Kosta showed higher licking frequencies compare to Boerka does in most of the occurence. Nursing activities, occured when does were eating, however does nursing during eating was not significantly affected between Boerka andKosta does. Mooing, was an of initial step from the series of nursing that was conducted by the does and/or suckling that was also done by the kids. An interesting behaviour of Boerka and Kosta occured as seen in Figure1, where the pattern of kids suckling during lactating periods were higher in Boerka (total of 50.3 times/lactating period) compared to Kosta (total of 34.5 times/lactating period), respectively. On the other hands, the duration of suckling was also higher for Boerka (total of 159.3 second/lactating period) compared to Kosta kids (total of 98.6 second/lactating period).

Table 1. Daily behaviour of Kosta and Boerka does

Interval	Breed	Nursing	Rejecting Kid	Feeding	Ruminating	Excreting	Agresiveness	Walking
16.00-20.00	Kosta	3,5	**4,0[a]**	**4,4[a]**	3,4[a]	0,3	3	**7,2[a]**
	Boerka	4	**1,1[b]**	**7,0[b]**	0,4[b]	0,3	1,2	**3,0[b]**
20.00-00.00	Kosta	1,9	1,7	1,2	6,6[a]	0,7	2	**4,2[a]**
	Boerka	3,9	1	1,7	4,7[b]	0,2	0,7	**1,1[b]**
00.00-04.00	Kosta	0,9	1,9	0,8	7,8	0,1	1,1	2,2
	Boerka	2,1	0,5	2,2	0	0,2	0,2	0,4
04.00-08.00	Kosta	2,9	**5,1[a]**	**6,8[a]**	**5,6[a]**	0,7	2	4,7
	Boerka	3,4	**0,7[b]**	**1,7[b]**	**9,9[b]**	0,2	0,8	3
08.00-12.00	Kosta	3,7	3	16,1	0,6	0,9	2,8	**6,4[a]**
	Boerka	3,7	2,4	12,7	0,2	0,6	1,6	**2,9[b]**
12.00-14.00	Kosta	1,1	2,4	9,7	0,4	0,4	1,1	**3,8[a]**
	Boerka	2	0,9	8,1	0,2	0,4	0,7	**0,4[b]**

Notes : a,b : significantly different (P<0.05)

Table 2. Daily behaviour of Kosta and Boerka kids

Interval	Breed	Eating	Sheltering	Sleeping	Investigating	Grouping	Agresivenss	Excreting	Suckling	Walking	Looking for does	Playing	Does' licking
16.00-	Kosta	0.0	0.3	5.4	2,9	1,2	0,2	0,3	3,5	7	4,2[a]	2,5[a]	0,7
20.00	Boerka	1.7	0.3	7.5	1,1	1,5	0	0,1	2,7	5,5	1,1[b]	5,9[b]	0,7

20.00-	Kosta	0.0	0.0	4.9[a]	0,5	1,6	0,1	0,1	1,8	3,6	2,2	1,4	0,7
00.00	Boerka	0.4	0.2	7.4[b]	1,6	0,6	0,1	0	2,2	3,9	0,9	2,8	1,1
00.00-	Kosta	0.5	0.1	3.5[a]	0,5	1,7[a]	0	0,4	1,1	1,8	1,5	2,1	0,7
04.00	Boerka	0.5	0.0	8.1[b]	0,6	0,4[b]	0	0	0,9	1,5	0,5	1,2	1
04.00-	Kosta	0.2	0.5	4.6	1,2	2,2	0,5	0,2	2,8	4,4	2,6	4,8	1,5
08.00	Boerka	0.1	0.0	6.5	1,5	1,3	0	0	2,1	4,5	1,3	4,4	0,7
08.00-	Kosta	1.2	0.8	8.7	0,9	0,5[a]	0,1	0,6	2,5	3,4	1,6	2,6[a]	0,5
12.00	Boerka	0.9	0.0	7.5	2,1	1,9[b]	0,8	0,2	3,2	4,8	1,9	4,3[b]	0,3
12.00-	Kosta	1.1	0.8	5.8	1,5	0,5[a]	0	0,3	0,8	2,9	1,5	1,6[a]	0,7
16.00	Boerka	0.4	0.0	8.1	1,4	1,7[b]	0,2	0,1	1,2	3,4	1,3	4,0[b]	0,6

Notes: [a,b] : significantly different (P<0.05)

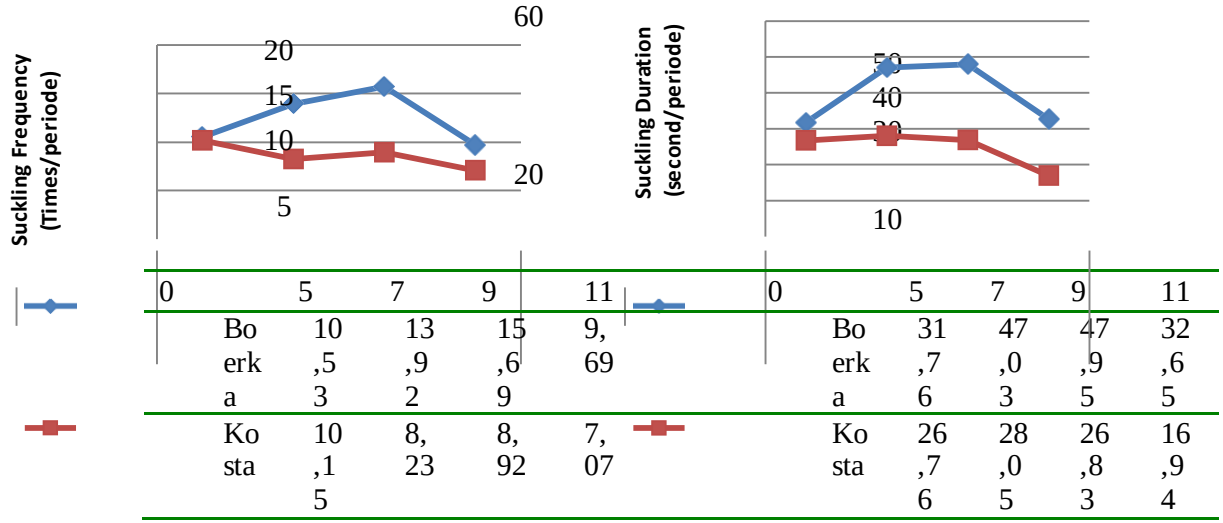

Figure 1. Frequency and duration of suckling based on kid's age

Table 3. Kids behaviour during suckling activities

Interval	Breed	Mooing	Does seeking	Teat seeking	Teat nuzzling	Snatching	Teat licking	Kneeling	Tail nagging
08.00-00	Boerka	0,11	0,62[a]	0,46[a]	3,47	0,16[a]	0,25[a]	0,60	0,83
	Kosta	0,17	0,74[b]	0,64[b]	3,67	0,28[b]	0,13[b]	0,54	0,87
16.00-00	Boerka	0,24	0,55	0,64[a]	3,70	0,27	0,19[a]	0,67[a]	0,86[a]
	Kosta	0,2	0,49	0,51[b]	4,22	0,33	0,1[b]	0,48[b]	0,75[b]

Notes : a, b: significantly different (P<0.05)

Table 4. Behaviour of Boerka and Kosta does during nursing.

Interval	Breed	Licking kids	Eating	Stand still	Moving apart	Mooing	Caring	Agresiveness
08.00-12.00	Boerka	0,32[a]	0,23	0,79[a]	0,87[a]	0,05	0,11	0,05[a]
	Kosta	0,54[b]	0,22	0,55[b]	0,99[b]	0,08	0,05	0,19[b]
16.00-20.00	Boerka	0,40[a]	0,05	0,79	0,86[a]	0,05	0,14[a]	0,03[a]
	Kosta	0,66[b]	0,03	0,85	0,96[b]	0,06	0,04[b]	0,13[b]

Notes : a, b: significantly different (P<0.05)

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IMPACT OF URBANIZATION AND LAND USE CHANGE ON PASTORAL LIVESTOCK FARMING IN NEPAL

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Abstract

Pastoralist populations are facing more pressures to their way of life than ever before. Population growth; loss of pastureland to private farms, ranches, game parks, and urban areas; increased commoditization and rising inequality within the livestock economy; out-migration of poor pastoralists; and periodic dislocations brought about by drought, inaccessibility and search for employment are collectively threatening a way of life that has proved in the past to be a highly adaptive food production system in arid lands.

Nepal has huge rangeland resources (22.6 % of the country area). Rangelands are located in the high mountains and Himalayan regions at 2500-5000 masl. In Nepal, existing pasturelands are practically natural and thus, the terms rangeland and pastureland have been used as synonymous. Approximately 78 percent of the rangelands are located at high altitude regions in the northern belt of Nepal bordering Tibet. About 50 percent of total range/grassland is found in high mountains, 29 percent in high hills, and almost 50 percent of range land is found in mid-western region and about one-fourth in the western region.

Livestock production supported through the pasture and rangeland area provides the major support for livelihoods in high-hills and mountain regions. The livestock sector contributes almost half of the total agriculture income (i.e. 47.3%) of the mountain livelihood, whereas contribution of the livestock sector is comparatively less in other eco-zones. Range livestock species are hardy, and capable to resist changes, although they are less productive. Therefore, breeding and feeding management are the areas where interventions have to be focused.

Nepal is facing the problem of land-use change. The young generation tends to migrate out of the hilly arena for study and employment. Urbanization and city center activities attract people who are suffering from unavailability of basic resources in pastoral life.

For sustainable development, pastoral livestock management in arid lands is productive, rational, and an essential way of utilizing scarce and patchy resources. Pastoral strategies of herd diversity,mobility, and residential flexibility offer a means to convert irregular, seasonal, and scarce vegetation into calories and protein for human consumption in arid and marginal lands. Policies to support such strategies now need to be more specific and flexible than ever. Pastoral risk management ought to be supported through strategies and interventions to increase preparedness of herders and local authorities for drought and other climatic risks. Furthermore, pastoralists need better access to credit and savings institutions to improve animal husbandry in pastoral areas for livelihood improvement of rural people. Promotion of indigenous knowledge, agro-based tourism, commercialization of range-livestock products, market improvement and government support are essential in forthcoming years.

Keywords: Rangeland, Pastoral, commercialization

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SURVEY, CHARACTERIZATION AND REGISTRATION OF A NEW BUFFALO BREED– A CASE STUDY UNDER NATIONAL ACTION PLAN ON ANIMAL GENETIC RESOURCES IN INDIA

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Introduction

Buffalo populations can be classified as – The African Buffalo (Syncerus), wild and Asian Buffalo (Bubalus), mostly domesticated. Most of the Indian buffalo population are riverine with few populations of swamp buffalo distributed in the eastern part of the country. Buffalo contributes about 49.2% of India's total milk production of 176.3 MT (Annual Report, AHD&F, 2017-18). Apart from their contribution to total milk, buffaloes also contribute about 19.80% of the country's meat production. Among all livestock108.7 million are buffaloes (21.23%) and the female buffalo population has increased by 7.99% over the previous census. Out of seventeen recognised buffalo breeds of India, two unique breeds viz., Toda and Bargur belong to the state of Tamil Nadu. Toda buffalo, reared by a local tribe called "Todas" of the Nilgiri district, whereas the Bargur is maintained by the Lingayat, an indigenous Kanada speaking community in the Bargur hills of Erode district in Tamil Nadu. The Lingayats are pure vegetarians.

Materials and methods

This survey of Bargur population was conducted in the villages of Anthiyoor and Sathyamangalam taluk in Tamil Nadu and in Kollegal taluk of Chamragnagar district in Karnataka. Body biometry for 17 traits, phenotypic characteristics, reproduction performance, morphometric traits, utility and management practices on 209 adult Bargur animals and 28 calves of both sexes were recorded from 107 households in the Bargur hills and on 27 adult buffaloes from the Bargur foothills. Blood samples from unrelated animals were collected for isolation of genomic DNA. Eight blood samples were utilized for karyological analysis and 48 genomic DNA samples were genotyped by PCR for 24 buffalo specific microsatellite markers. Genotypic data generated through automated DNA sequencing were used to determine genetic variability by use of POP32 gene and GenAlEx6.5 software. Mitochondrial D-loop analysis was also conducted on 10 DNA samples and data were analysed for phylogeny.

Results and Discussion

Breed Distribution: The majority of Bargur buffaloes are distributed in hamlets of Bargur village of Bargur hills (part of Western Ghats) in Anthiyoor taluk and in a few hamlets of Sathyamangalam taluk in the Erode district of Western Tamil Nadu. The buffalo population in the foothills of both the sides of Bargur hills (i.e. southern and northern sides) were found to be phenotypically distinct from the buffaloes distributed in the hills. Bargur buffalo is exclusively reared by Lingayat an indigenous Kanada speaking community settled in Tamil Nadu few centuries ago.

Phenotypic characterization: Bargur buffaloes are small to medium sized domesticated buffaloes. Local names are "Malai Erumai" and "Malai Emmai", which mean "hill buffalo" The coat colour of the sampled animals varied from brown (32%) to brownish black (46%), and completely black (22%). Skin

colour also ranged between light brown to blackish brown (71%) and black (29%) and the hair coat ranged from medium to long. In most of the animals, predominantly in females (>75%), greyish white colouring (stockings) was observed from the carpal/tarsal joint to the fetlock. Male animals generally had brownish black, medium to long coats and brownish black skin. Horns are medium to long in size and sickle shaped with curved backward and inward orientation. As the age progress, the horns either touch each other or overlap. Bargur buffaloes have small sized udders that are mostly round in shape (76%), with the remaining animals having bowl shaped udders. The teats are cylindrical in majority of the animals (81%) with rounded (65%) and pointed tips (35%), whereas the milk vein is less prominent in the females. The buffaloes averaged 102.83±0.68 cm in height and 093.33±0.93 cm in body length. Their small and compact bodies enable them to easily graze in the hilly terrain. The buffaloes from the foot hills were phenotypically different from those of the hilly region. Most of the traits showed significant differences between the two buffalo populations (body height, neck length, neck circumference, body length, chest girth, paunch girth, face length, ear length, horn circumference, distance between horns, hip bone length, and pin bone length). The foothill buffaloes are bred mostly through natural service, and to some extent through artificial insemination. They are maintained under a semi-intensive system of rearing. Bargur buffalo is mainly utilized for manure, milk and cara-beef (male calves). The dung is utilized as fertilizer. The milk yield of the animals ranged from 1.5 to 2.0 litres per day. Twice a day milking is practiced through hand milking only and the milk is mainly used for household purposes like curd making and preparation of buttermilk. The excess milk is typically sold in the local area (@ Rs 30/kg). The milk contained 8.59±0.62% fat on average, which indicates that it is good for preparation of dairy products like butter and ghee. The fat percentage is comparable to that of Toda and Bhadawari breeds and higher than Murrah and Surti. Protein percentage was observed to be 3.31±0.07% which is less than that of Toda buffalo milk. The mean SNF content was 9.10±0.19%. The adult male animal can be sold at a price of Rs 10,000 to 15,000, whereas the females fetch prices of Rs 20,000 to 30,000 through the local market at Anthiyoor or to middle man. The cows are bred mostly through natural service (>95%) with the available breeding bulls. Breeding males are referred to as "Konan". The age at first calving ranges from 3 to 4 years with an average lactation length of 240-250 days and calving interval from 16 to 18 months. Age at first breeding in males is more than 3 years. Murrah semen is being used in the hamlets near to the Veterinary Dispensary at Bargur, resulting in dilution of this unique germplasm. This crossbreeding needs to be addressed and appropriate conservation measures intiated to avoid the germplasm dilution.

Genetic Characterization

Karyological analysis: Karyological analysis was to done to ascertain the chromosome numbers in Bargur buffaloes. The karyological analysis of Bargur buffalo revealed a common fundamental number (2n=50) and confirm it as a riverine buffalo. The first five pairs of autosomes are submetacentric and the remaining 19 pairs are acrocentric. The third pair is more metacentric than submetacentric. In males the X chromosome is the longest among the acrocentric, like those of females. The Y chromosome is among the smaller acrocentric.

Microsatellite marker based diversity analysis: All the loci studied were polymorphic. Overall average number of alleles was 8.00±0.55 and average effective number of alleles was 3.85±0.25. The average observed (H_O) and unbiased expected (uH_e) heterozygosity were 0.66±0.04 and 0.71±0.032, respectivley. The observed heterozygosity in the studied population was found to be lower than the expected heterozygosity. The F_{IS} was 0.056±0.037. Polymorphism information content varied between 0.115 (ILSTS 019) to 0.808 (HEL013) with a mean value of 0.660±0.130.

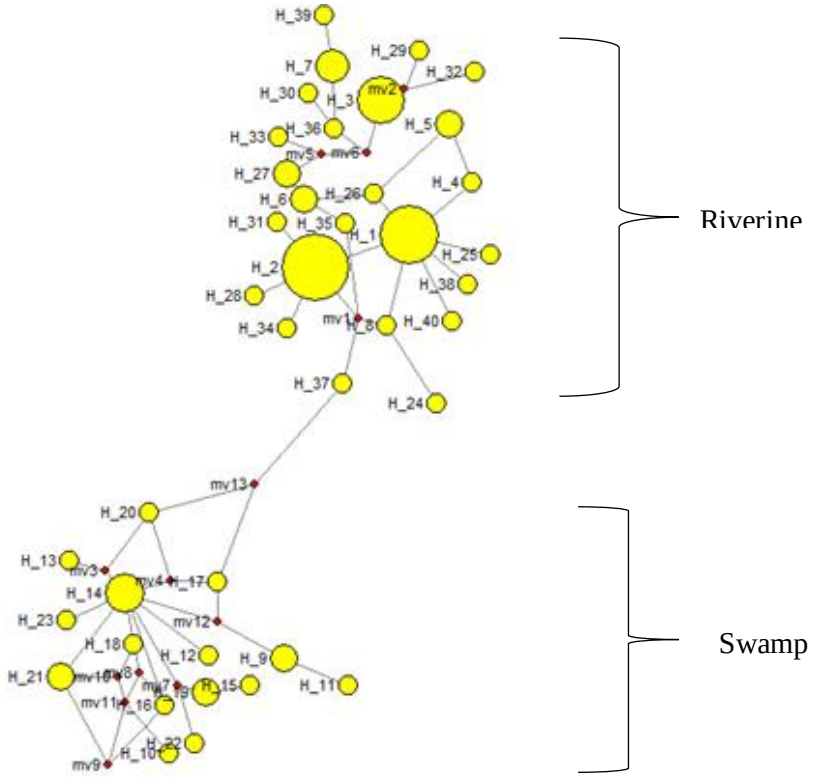
*Heterozygosity deficit and Hardy-Weinberg equilibrium:*The average heterozygosity deficit across 25 microsatellite loci was 0.056±0.037 and was significantly positive ($P<0.05$). Fifteen out of the 25 loci investigated were found to have positive F_{IS} values. Although the average heterozygosity deficit was found to be significantly positive, it was found to be comparatively much less than that of other buffalo breeds (Mishra *et al.*, 2009; Kathiravan *et al.*, 2009). It is interesting to note the relatively lower F_{IS}

values in Bargur buffaloes, despite the population being very limited and restricted to a narrow geographical area. The test for Hardy-Weinberg equilibrium (HWE) revealed significant deviations in all except seven loci, viz. ILSTS019, ILSTS089, ILSTS028, ILSTS058, CSSM019, ILSTS033 and ILSTS030. The possible reasons for deviations from HWE could be the presence of null alleles, sample size and relatedness of the sampled animals (in the absence of parentage records). The quantitative test for mode shift revealed the normal L shaped distribution of allele frequencies, suggesting that Bargur buffaloes have not lost much of their rare alleles.
Mitochondrial DNA variation: Mitochondrial D-loop analysis revealed 40 haplotypes with an overall haplotype diversity of 0.9524 and nucleotide diversity 0.03894. Three unique haplotypes were observed (Hap_38: 1, Hap_39: 1 and Hap_40: 1) with a haplotype diversity of 0.9111 and nucleotide diversity of 0.01826. Median joining network analysis revealed clustering of the Bargur with the riverine group (Figure 1) which substatiates the result of the karyological analysis.

Breed Registration: Bargur buffalo was reistered as the fifteenth buffalo breed of India and has been notified in The Gazette of India with the accession number INDIA_BUFFALO_1800_BARGUR_01015. This registry recognizes the need for an authentic national documentation system of valuable sovereign genetic resource with known characteristics. This would provide protection for the valuable animal genetic diversity and facilitate its access for genetic improvement. This mechanism is the sole recognized process for registration of animal genetic resources material at national level.

Conclusions

From this study we can conclude that the distribution of Bargur buffalo breed is limited to the Bargur hills only. It is an important breed with distinct morphological characteristics, playing significant role in the social and cultural life of Lingayat community in Bargur hills. The animals are maintained under a low input system and provide milk for family consumption as a nutritional support to Lingayat. Even though the Lingayats are pure vegeterians, sale of aged animals and male calves for slaughter fetches substantial income and makes raising buffalo sustainable. The genetic diversity analysis revealed the existence of variablility and absence of recent bottlenecks, suggesting that the Bargur buffalo have not lost many of its original alleles. Mitochondrial diversity revealed three unique haplotypes in this breed, suggesting that it might have evolved sperately as compared to other buffalo breeds. We recommend to the Animal Husbandry Department of the Government of Tamil Nadu, India to avoid using Murrah semen in the Bargur breeding tract, as Murrah crosses cannot be sustained due to non-availability of fodder crops. State Animal Husbandry official should take necessary action for conducting regular deworming, vaccination and awareness camps. Both *In-situ & Ex-situ* conservation programme need to be initiated to provide access to superior male germplasm. Opportunities for value addition such as organic milk production and facilities for milk sales may be created to make reearing of Bargur buffaloes more remunerative and sustainable. We further recommend to establish a Bargur buffalo breeders association in the native tract for creating awareness on scientific management and rearing of this buffalo breed.



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MANAGING FEEDING RESOURCES AND OPPORTUNITIES FOR IMPROVING FEED USE EFFICIENCY IN PERI-URBAN DAIRY PRODUCTION SYSTEMS IN PAKISTAN

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Abstract

Dairy farming is an important activity for many urban dwellers in Asia who try to serve the increasing urban demand for milk due to the growing urban population. During 12 months, data on demographic events, types and amounts of feeds offered, milk and body weight changes were collected in 15 mixed buffalo and cattle dairy herds in Faisalabad, third largest city of Pakistan. Herds were semi-commercial small scale mixed (SSM), semi-commercial small scale dairy (SSD) and commercial small scale dairy (CSD); their animals were mainly stall fed. Regularly collected samples of feeds offered were analyzed for their nutrient composition.

Among the green feeds, maize and berseem (Egyptian clover) were of major importance. While maize was used in all seasons, berseem was commonly fed during winter and spring only. Green sorghum and sugar cane tops were sometimes fed alone, but mostly mixed with either berseem or maize. All green feeds were chopped to 2 - 3 cm length and mixed with the other diet constituents. Almost all farmers were using wheat middling as the main concentrate feed, followed by dried bread pieces, cottonseed cake, by-products from pulses, wheat bran, wheat flour, maize oil cake and rapeseed cake. On all farms, finely ground wheat straw also contributed between 7% and 18% (on DM basis) to the diet, but with its high NDF and low CP, CDOM (cellulose digestible organic matter) and ME concentration mainly served as a 'filling' material.

The offer of feed dry matter and crude protein were significantly different ($P<0.05$) between the three production systems across the four seasons of a year. The overall average body weight of female adult buffaloes and cattle was 579 kg (480-807) and 434 kg (339-630), respectively. Daily milk production (corrected to 4% fat) per animal was 13.5 liters in buffaloes and 8.1 liters in cattle; while milk extracted for sale from buffaloes varied ($P<0.05$) between seasons for the SSD and CSD production system, this was not the case in cattle. On a yearly basis, buffaloes received approximately 0.6 kg and 0.7 kg DM less roughage feed per day on SSM and CSD farms, respectively, than on SSD farms. A lactating buffalo was exposed to a daily ME deficit of -7.0 MJ and -8.5 MJ on SSM and CSD farms, and to a more or less balanced energy supply (1.7 MJ) on SSD farms. For cattle a daily ME oversupply of 14.0 MJ, 18.7 MJ and 17.1 MJ was calculated for SSM, SSD and CSD farms, respectively. Gross margins of selling milk and occasionally young stock and culled females was higher on SSM and CSD farms than on the resource poor SSD farms, whereby the only variable costs accounted for were those of feed. It was concluded that more efficient feed utilization in these dairy peri-urban dairy production systems is possible through separate feeding of groups of buffaloes and cattle, according to physiological and productive needs of different species (buffalo and cattle), and, for SSM and CSD farms, the adoption of silage and hay making. Yet, for the both improvements advice and the technical support for farmers should envisaged by the responsible governmental bodies.

Keywords: Buffalo; cattle; energy balance; gross margin, milk production.

Introduction

The global dairy sector has seen a major intensification during the past five decades, with the increase in scales and efficiency of production driven by the demand from a growing human population and increasing incomes of parts of the population (FAO, 2011a). The increase in milk output was achieved through advances in animal nutrition and breeding, feed use efficiency, health management, housing, and automation strategies, along with supporting policies (FAO, 2011b). In Pakistan's peri-urban commercial dairy farms, feed accounts for more than two thirds of the operational costs (Habib et al., 2007), because animals are stall-fed year-round on wheat straw, purchased green fodder, and concentrate feeds which farmers obtain from the markets. Seasonal variations in quantity and quality of the roughage feeds are a major concern to farmers, especially during the scarcity periods (Gillah et al., 2012), and high feed costs negatively affect the profitability of peri-urban dairy enterprises. In consequence inadequate nutrition of lactating animals is considered one of the major limitations to dairy production in peri-urban areas (Olafadehan, 2007), and may even lead to morbidity and mortality of high yielding animals (Jalil et al., 2009). Low gross margins of milk sales due to the high feed costs (Garcia et al., 2003) often push farmers to unethical practices such as adulteration of milk with water, ice, or other additives in order to improve their economic situation (Jalil et al., 2009).
In spite of the substantial contribution of livestock to the national economy, per head productivity of dairy animals under present farm conditions is relatively low in Pakistan. Among dairy buffaloes, 98% are producing less than 10 liters of milk per day (Khan et al., 2012), and the country's major increase in milk yield during the last few decades has resulted from an increased number of cattle and buffaloes, while the increase in milk yield per head has contributed relatively little to overall growth of milk production (Habib et al., 2007). High productive and reproductive efficiency of livestock can only be achieved if animals receive the required quantity of feedstuffs providing all nutrients in the needed proportion (NRC, 2001), and are well-managed in terms of health and environmental conditions (Oltenacu and Broom, 2010). A sound intervention strategy to increase income from dairy animals should focus on two fronts: firstly, lowering feed costs, and secondly, increasing individual animal productivity (Habib et al., 2007). Both require adequate nutritional management and a high efficiency of feed utilization. Given the paucity of information regarding the nutritional status of lactating animals in Pakistan's peri-urban dairy units, specific measures that help dairy farmers to adequately address the previous issues are difficult to devise. This study therefore evaluated the feeding practices for lactating buffaloes and cattle in peri-urban dairy units of Faisalabad, in order to determine the supply and conversion of feed and energy into milk, body weight (maintenance) and offspring, and by this identify shortcomings and potential improvements.

Conclusions

The above situation analysis identifies two major but connected nutritional problems: on the one hand limited feed and energy supply is hampering productivity of high yielding buffaloes, and on the other hand over-supply of feed and feed energy to buffaloes and cattle in late lactation adds to feed costs without substantial increase in milk yield; both under- and over-feeding adversely affect the animals' fertility and health. A more efficient feed utilization in Faisalabad's peri-urban dairy production systems should therefore aim at synchronizing qualitative and quantitative feed supply with the requirements of different species (buffalo and cattle) and physiological stages; for this group feeding of animals in similar production stage is a first and easy to adopt step. However, extension services should provide farmers with the respective knowledge wherever necessary. Coping with feed shortage periods through silage making is an issue of increasing interest in Pakistan and India; however this option may not be easily adopted by smallholders.

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GROWTH PERFORMANCE AND CARCASS QUALITY OF LOHI LAMBS REARED UNDER DIFFERENT FEEDING SYSTEMS IN PAKISTAN

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Abstract

A study was conducted to evaluate the effects of different feeding systems on growth performance and carcass composition of male Lohi lambs. Sixty-four Lohi breed lambs were divided in two groups (A and B) of thirty two animals in each group and kept in subgroups of eight in four pens. Half of the lambs were castrated. The group A was offered a concentrate diet (85g of DM/kg of metabolic body weight/day) containing 15 % of crude protein and 3010 kcal/kg of ME along with hay (100 g/lamb/day). Group B was offered fresh green forage *ad libitum* and supplemented with a concentrate (400 g/lamb/day) containing 20 % of CP and 2940 kcal/kg ME. Feed offered and refused were sampled and their DM contents determined. Feed intake was measured/day and animals weighted/week. At the end the feeding period, all animals were slaughtered; a half carcass was sampled from each pen for carcass composition determination. The concentrate diet significantly affected daily DM intake (P=0:001), FCR (P<0:05), daily weight gain DWG (P<0:05), final live weight (P<0:001), carcass weight (P<0:001), dressing percentage (P<0:001), conformation scores (P<0:05), internal viscera weight (P=0:001), feet (P<0:05), total bones (P<0:05), buttock (P<0:05) and fat score (P<0:001) as compared to the fodder fed lambs. However fodder fed lambs had longer finishing period (P<0:001), heavy total bones (P<0:05), weight of *m. longissimus dorsi* (P<0:05), lean:bone ratio (P<0:05) and more leaner carcasses (P=0:06) than concentrate fed lambs. Entire lambs had significant daily DM intake (P=0:001), FCR (P<0:05), DWG (P<0:05), liver weight (P<0:05), lungs weight (P<0:05) and lean:bone ratio (P=0:05) than castrated lambs however the castrated lambs had longer finishing periods (P<0:05) and more total fat contents (inter-muscular and subcutaneous fat) but these values were non-significant. There was no interaction found between sexual status and feeding systems. In general, the concentrate feeding system showed good results for growth performance and carcass composition but economic decisions and intensive management must be regarded. The results of this trial show that the sex had an effect on growth and carcass composition and castration may only increase the fatness of carcass but lean remains lower.

Keywords: Carcass, Growth, Lamb, Meat Quality, production System, Sex

Introduction

Growth and carcass traits are well-known factors that influence financial returns in sheep enterprise. But they are also influenced by other factors such as the breed (Kremer *et al*., 2004), the sex and age of the animal (Barone *et al*., 2007) and the feeding regime (Jacques *et al*., 2011). All these factors are key components of any production system. Diet composition significantly influence retained fat and protein and its partition (Afonso and Thompson, 1996). While overfeeding may lead to excessive production of fat in the carcass, underfeeding provides poor quality products and can prevent the animals from

developing their genetic potential in terms of stature and weight (Croston and Pollatt, 1994). Mutton production system in Pakistan is traditional and has to be transformed into a highly commercialized system in order to meet the domestic needs and to produce mutton for export (Khan *et al*., 2003). While castration improves the quality of meat, non-castration allows males to utilize feed more efficiently and finish quicker with leaner carcass. Maghoub *et al*. (1998) noted that intact and cryptorchid sheep had higher average daily gains than castrates. According to *Pena et al*. (2005), the sex of the animal is the primary factor that affects the quality of all type of fat deposits. This experiment is designed to identify the most suitable production system for the lambs of Lohi breed with the specific objectives of evaluating: i) the effect of feed regimes on growth performance and carcass composition of lambs and ii) the effect of sexual status (entire or castrated) on growth performance and carcass composition of lambs.

Conclusions

In general, the concentrate feeding system showed good results for growth performance and carcass composition but economic decisions and intensive management must be regarded. The results of this trial show that the sex had an effect on growth and carcass composition and castration may only increase the fatness of carcass but lean remains lower. We can conclude that although the concentrate feeding system showed better results for lamb growth performances and carcass composition, economic decisions must guide the choice of the appropriate feeding system.

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BLOOD METABOLITE STATUS AND REPRODUCTIVE PERFORMANCE IN GRAZING SANGA AND FRIESIAN-SANGA COWS SUPPLEMENTED WITH CONCENTRATE DURING THE POSTPARTUM PERIOD

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Introduction

Sanga and Friesian-Sanga crossbred cows are important for meat and milk production in the extensive grazing system of production in Ghana. These breeds however suffer nutritional deficiencies resulting from inadequate nutrition and supply of quality feed especially during the dry season culminating in poor growth, low milk yield, less than optimum reproductive performance and decreased profitability of the production enterprise (Obese et al., 2010). There is therefore the need to improve nutrition of cows by providing supplementary feeds during periods of pasture or nutrient deficit to increase dry matter intake and improve productivity. Feed supplementation of cattle has been demonstrated in a number of studies elsewhere to improve growth, milk yield and reproductive performance in cattle grazing tropical and subtropical forages (Ortega et al., 2020; Renaweera et al., 2020). Such information is limited in the extensive smallholder cattle production system in Ghana.

Objectives

This study evaluated the effects of feed supplementation on milk yield, blood metabolite concentrations, resumption of ovarian activity and conception in grazing Sanga and Friesian-Sanga cows during the postpartum period.

Materials and Methods

Forty grazing Sanga and Friesian-Sanga cows were used in the study. Twenty out of a total of 40 cows (10 Sanga and 10 Friesian-Sanga cows) were supplemented with 2.5 kg of concentrate per day for 16 weeks after calving. The two herds were housed separately in open kraals and were grazed separately, but on plots within the same field of natural pasture with similar nutritive value. The supplementary diet was provided to each cow in the supplementary groups before grazing. Partial milk yield was determined by collecting milk from two quarters of the udder. They were monitored for oestrus by visual observation two times per day while at pasture. Resumption of postpartum ovarian activity and conception were determined by measuring the progesterone concentrations in plasma samples from cows from week 1 to week 16 postpartum using a commercial ELISA Kit. Cows were classified as having resumed ovarian activity when plasma progesterone concentration of ≥ 1 ng/ml was recorded in a plasma sample. Also, the concentrations of blood biochemical indices such as glucose, total protein, albumin, triglyceride and urea were determined in the plasma using the Mindray BA-88A Semi-Auto Chemistry Analyser.

Statistical Analysis

The effects feed supplementation had on milk yield, and plasma concentration of metabolites measured in the Sanga and Friesian-Sanga cows were determined using repeated measures analysis of variance

procedure of GenStat Release 12th Edition (VSN International, 2009). The chi-square test was used to determine the association between resumption of ovarian activity and breed or dietary regime.

Results and Discussion

Partial milk yield was higher (P<0.001) in supplemented than non-supplemented cows (2.07 versus 1.60 kg/day; Figure 1) indicating the beneficial effect of feed supplementation in improving milk yield of cows as have been observed in some studies (Idris et al., 2015; Obese et al., 2018). Friesian-Sanga cows had higher (P<0.001) partial milk yield than Sanga cows (2.05 versus 1.61 kg/day) probably due to channeling more of their dietary energy for milk production. Supplemented cows had higher total protein (86.7 versus 81.3 g/l; P<0.01) and globulin (53.0 versus 47.7; P<0.05) concentrations than non-supplemented cows. This is attributed to improved amino acid absorption arising from increased microbial protein synthesis from additional nitrogen provided by the feed supplement. Sanga cows had higher plasma glucose (4.43 versus 4.09 mmol/l; P<0.05), total protein (84.0 versus 73.1 g/l; P<0.001) and globulin (47.5 versus 40.01 g/l; P< 0.001) than Friesian-Sanga cows. The higher glucose concentration in the Sanga is probably due to their lower energy requirement. The higher total protein and globulin concentrations of the Sanga cows suggest better protein status, ability to resist diseases and higher adaptability to the environment than the Friesian-Sanga crossbred cows.
A lower proportion of supplemented cows did not resume ovarian function compared to non-supplemented cows (20 versus 55%; P<0.05). Also, the improved nutritional status in the supplemented than non-supplemented cows contributed to the shortening of their interval from calving to conception (95.8 versus 106, days; P < 0.05). This may have been mediated by higher plasma concentrations of protein and globulin in the supplemented than the non-supplemented cows as improved nutritional status enhances metabolic status and conception in cattle (Almeida, 2017). Friesian-Sanga cows had higher plasma progesterone concentrations at 1st progesterone rise (3.34 versus 1.32 ng/ml; P<0.05) and shorter interval from calving to conception (96.7 versus 106 days; P<0.05) than Sanga cows suggesting the ability of the Friesian-Sanga to conceive more easily once it has resumed ovarian activity.

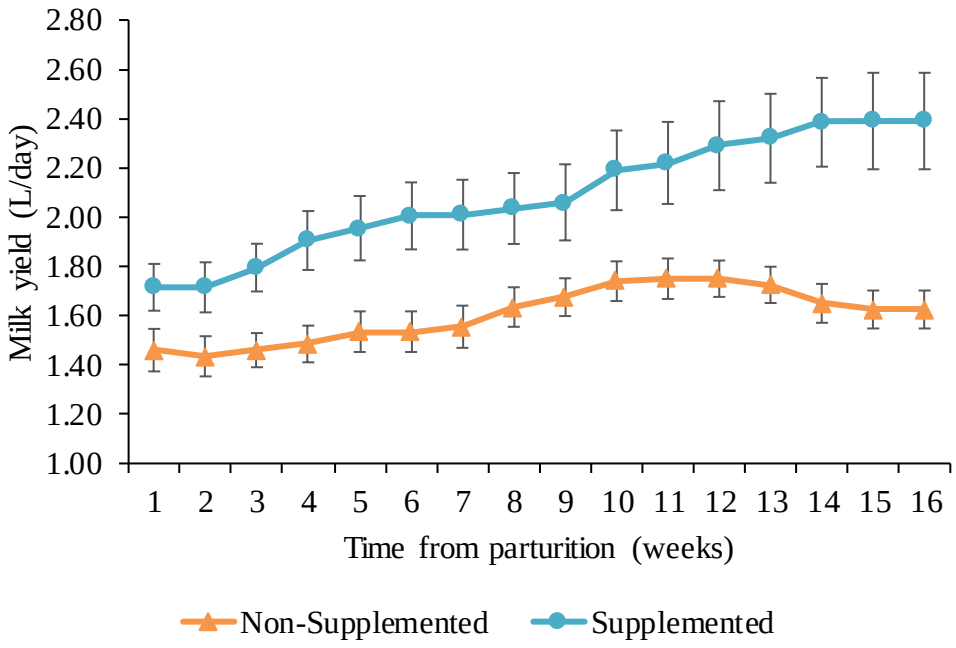

Figure 1. Changes in partial milk yield in supplemented and non-supplemented Sanga and Friesian-Sanga cows during the postpartum period

Conclusion

Feed supplementation improved milk yield, total protein and globulin status, and reduced cyclicity problems and days open in supplemented than non-supplemented cows during the postpartum period suggesting the beneficial effects of feed supplementation. Also, feed supplementation had a more beneficial effect in the
Friesian-Sanga cows evidenced by higher milk yield and shorter days open.

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EXPLOITATIONS METHODS, PHENOTYPE CHARACTERISTICS AND THE POTENTIAL OF BLACK BELLY SHEEP IN CENTRAL AFRICA FOREST ZONE

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Abstract

This study was carried out from the 1st April to the 31th November 2019 on 299 breeders in three countries: Cameroun, Congo, Gabon. to assess the exploitations methods. A total of 288 sheeps (204 females and 84 males) were scored for 22 body measurements and 12 body indices were calculated for phenotypic diversity. This study aims to assess the exploitation methods, phenotype characteristics and the potential of Black belly sheep in Central Africa forest Zone. Results shows that, Black belly farmers are predominantly men (91.6%) of between 40 and 60 years old (48.2%), and married (79.3%). Black belly breeders mainly use family labor (97.0%) and are not register in a socio-professional organization (98.7%). Many farmers raise Black belly for a double objective one part for consumption and the other part Marketed (79,9%). Black belly participates up to 150,000 FCFA per year in the household's revenue of most of the breeders. In general, the herd size is 1 to 5 animals or 6 to 10 animals but 35% farmers in Congo have flocks of more than 30 sheep. average structure of herds consists of 1,903 ± 2,852 adult males, 7,736 ± 14,068 adult females and 2,699 ± 3,519 lambs**.** Peasants farming system is largely used (95.0%), raising animals in common with other animals and graze on communal land in crops season in sedentary breeding (94.0%). Many farmers have at least a simple accommodation for their animals (58.9%). These housing are pens (26.1%) and warehouses (21.4%), made of wood (46.2%), often associated with concrete block (15.0%), and iron (25.0%) in Congo. Breeders have no equipment in their farms for Black belly. But in Congo almost all breeders have a minimum of equipment, in general feeders and drinker made either in half barrel or half can. Animals are generally fed on pasture (58**.9%).** access to water is unlimited (51.8%), but food resources are limited to some periods of the year. Mating are uncontrolled and females are very earlier, 86.0% in puberty between 5 and 8 months with first lambing between 10 and 13 months (62.2%). Two lambing per year (40.5%) of 1 to 2 lambs per litter, with 31.8% lambing of more than 2 lambs are found. Only 28.8% of breeders practice the selection of replacement animals, mainly replacement rams (15.4%) but in Congo 66.7% of farmers select both rams and replacement ewes. The most common diseases are bacterial, viral and fungal diseases. Ticks (14.7%) are the main type of ectoparasites while helminths (43.1%) and protozoa are the most encountered endoparasites. A total of 76.6% never vaccinate their animals. In case of illness only 27.4% of Black Belly breeders use a treatment. Almost all breeder loss animal's (94.6%), due to the diseases and theft (29.4%). Significant phenotypic dimorphism has been establishing between Black belly of different countries, with the highest and heaviest animals coming from Congo (HW= 62.217 ± 5.288 et BW = 27.447 ± 6.081kg), longest from Gabon (TBL = 84.691 ± 8.704) and the larger from Cameroon. Majority of Indices (SGI, ELI, CI, MI, IT, IL, FI, DTI, EI) were higher in Cameroon sheep. Excepted IT and FI the coefficient of variation of all the other indices were higher in Cameroon. The PCA reveals six main components from 21 measurements of our study which explain 73.1% of the variations observed within the Black belly populations in Central Africa. The first two components had eigenvalues greater than 3 and explained

25.44% (CP1) and 17.41% (CP2) of the variations observed on body measurements, and can be considered for improvement and selection program. Hence the back height and the thoracic circumference appear to be the most interesting measure to be consider for selection and conservation. three classes of Black belly have been identified in Centrale Africa. Class 1 includes small animals with an elongated neck. Class 2 includes the longest animals (TBL =86.108 cm) with a longer and larger head, the body and the trunk longer than the others classes, the ears and tail are long and the nipples are more developed. Class 3 includes large sheep with a voluminous chest (TC =78,424cm and CW = 15.121cm) and heavy weight (BW=33.053kg). Black belly farmers of Centrale Africa have a large among of good practices that are adapted to their environmental conditions and have being transfer through generations. However, these practices need to be improved by appropriate training in various aspects of farming. It is also important to organized these famers in professional groups to improve sharing of good practices, conservation of the genetic material and facilitate market accessibility. Selection practices base on the biometric measurements of this study most be important aspect to develop in Black belly farms and improve animal's productivity and farmer's profitability.

Keywords: Exploitation, Measurements, Black belly, Biodiversity, Phenotype, Central Africa

Introduction

Sheep is an integral part of the activities of the African's peoples. Sheep farming is practiced by several peoples for various reasons, but the most important remains the economic profitability, which is generally accompanied by the self-consumption Tchouamo *and al* (2003). Black belly sheep have been one of the widely distributed in the world because of its higher adaptability capacities in various climatic condition (Meka *and al.*, 2019). The farming practices of Black belly in the forest zone of Central Africa have enabled this resource to be conserved for generations, but current environmental changes are necessarily causing changes in the farming practices of the populations. But it is true that no animal resource can be efficiently exploited without a qualitative and quantitative genetic zoo-genetic inventory. To this end, several indicators of genetic diversity are commonly used, namely: breed inventories, inbreeding measures and genetic markers (Louis Ollivier and Jean-Louis Foulley, 2013). The presence of the Black belly around the world demonstrates the importance and the interest given to this zoo-genetic resource by the developed country (Meka *and al.*, 2019). But in its original native area, Central Africa, there is very few information about this breed and its exploitations methods. Hence the aim of this study, which is to improve the knowledge of Central Africa small ruminant resource by Assessing its exploitation methods and biometrics Characteristics.

Materials and methods

Description of the study area

The location of our study mainly involves three Central African countries, namely: Cameroon, Gabon and the Congo. A total of 8 regions are included in this study, the Center, South, East, Littoral regions in Cameroon, the Estuary and Wouleu-Ntem in Gabon and Kouilou and Niari in Congo. These areas are mainly occupied by the Bantu populations. These localities include similar climatic characteristic of humid tropical forest and equatorial, extend to the South of Cameroon, and the southwest of the Central African Republic to Congo, DRC, Gabon and Equatorial Guinea, with an average rainfall between 1 400 to 1500 mm of precipitation per year (European Commission. 2007). The climate is hot and humid with temperatures ranging between 22 ° C and 30 ° C (Tsalefac *and al.*, 2015).

Data collected

Data was collected between April to November 2019, a total of 299 Black belly farmers (267 in Cameroon, 20 in Congo and 12 in Gabon) were selected in Hazard by snowball method and Survey

framework was used as well as observations method for data collection. The principles information collected include: the socioeconomic characteristics of breeders, the exploitation methods of the breeders. For biometric characteristics, a total of 288 animals (204 females and 84 males) were measured using the random sampling method, 252 animals in Cameroon, 20 in Congo and 12 in Gabon. After screening to avoid unhealthy and pregnant animals, the Ages were obtained by examination of dentition. Twenty-two (22) biometric traits were measured using a graduated measuring stick (in cm) and a barymetric tape according to the FAO (2013) and AU-IBAR (2015) guidelines. The traits were classified into 3 categories (cephalic, trunk and limb).

Data analysis

The descriptive statistics made it possible to determine the frequencies, means, and percentages of the socio-economic characteristics and exploitation methods of the Black belly farmers in Central Africa using SPSS 21. To assess the causes of genetic variability in the sheep population studied, the principal component analysis (PCA) was used on the 22 biometric measurements to determine the linear relationships between these different characteristics (FAO, 2013).The Discriminant Factor Analysis (DFA) was used to identify the genetic types that found in the studied population . The Ascending Hierarchical Classification protocol (HAC) was used to establish the existing genetic relationships between the genetic types using SPSS 21 and R 3.6.1.

Figure 1. Central Africa Black belly ewe (a) and ram (b)

Results

Socials and economic characteristics of breeders of Black belly in Central Africa

Breeders of Black Belly in the forest zones of Central Africa are predominantly men (91.6%), between 40 and 60 years old (48.2%), and married (79.3%). Generally, they have 6 to 10 years (33.4%) and 11 to 15 years (25.5%) of experience. All of the practices use by breeders came from the knowledge transferred over generations or from self-learning because almost all of these breeders **(97.7%)** have never received a training in the field of breeding. Man is responsible for all decisions on the scale of production (56.9%), sale and purchase (65.9%) , intensity of production (44.8%), Target market (46.5%) and reproductive objectives (46.2%). Black belly breeders mainly use family labor **(97.0%)** and almost all breeders do not belong to a socio-professional organization **(98.7%)**. The production intended for both market and self-consumption and the target is local market (73.9%). Black belly sheep is mainly used for meat, the major by-product use is the manure; this is due to the fact that the majority of the stockbreeders practice agriculture as principal or second activity. Black belly participates up to 150,000 FCFA per year in the households of most of the breeders. In Congo, it represents an even greater value, contributing 150,000 to 450,000 FCFA per year for 30% of breeders and 450,000 to 750,000 FCFA per year for 25% of farmers.

Exploitations methods of Black belly sheep in Central Africa

Black Belly Breeders generally had a flocks' size of 1 to 5 or 6 to 10 animals, average structure of herds of Black belly consists of 1,903 ± 2,852 adult males, 7,736 ± 14,068 adult females and 2,699 ± 3,519 lambs. The farms are mostly peasants farming (95.0%), the animals being raised in common with other animals and graze on communal lands in crops season in sedentary breeding (94.0%). Most of raisers (58.9%) has at least a simple accommodation for their animals, most of them are under not fully controlled atmosphere (30.4%), insuring a basic protection against heat and against cold (20.7%). Main housing are pens (26.1%) and warehouses (21.4%) in the three countries, mainly made of wood (46.2%), which in Congo is very often associated with concrete block (15.0%), and iron (25.0%). Most of breeder has no equipment in their farms and when present it mostly feeders and drinkers made of half barrel or half can. Black belly is mostly fed on pasture (58.9%). Animals are generally fed at voluntary (93.6%) due to the often-poor quality of pasture and the scarcity in the dry season, access to water in the majority is unlimited (51.8%). the majority of breeders only uses natural pasture as food for animals while in Gabon farmers use natural pasture and crop residues (66.7%), in Congo, the association mainly used is natural pasture, crop residues (straw, stubble, etc.) and industrial by-products (35.0 %), but also with food supplements (20.0%). Black belly ewes of Central Africa are very earlier (86.0% in puberty between 5 and 8 months with first lambing between 10 and 13 months (62.2%), two lambing per year (40.5%), from 1 to 2 lambs per litter, with 31.8% lambing of more than 2 lambs. The majority of breeders do not practice pedigree identification (69%) and only 28,8% of farmers practices selection of replacement animals. Bacterial diseases are most commonly encountered in Black Belly farms in Central Africa but the majority of breeders (76.6%) never vaccinate their animals. Almost all breeders of Black Belly record cases of animal's loss (94.6%) due to the diseases and theft (29.4%).

Biometric Characteristics of Black belly sheep in Central Africa

For cephalic measurements average HWh, NL and EL were higher in Congo, while HLh and CN were higher in Gabon. only Ear Length and neck circumference were significantly affected ($p < 0.05$) by countries. For body measure the total body length (84.691± 8.704cm) and the trunk length (62.573 ± 5.885 cm) were higher in Gabon animals, while the sheep of Congo have the highest average HW, HB, TC and CD. But the average CW, RL and RW were higher in Cameroon. The ANOVA of these parameters shows that, except the height at the withers, the thoracic circumference, the width of the rump, all the other measurements of the trunk (TBL, TrL, HB, HR, CD, CW, RL) was significantly ($p < 0.05$) influenced by the country. For the measurements of the limbs, extremities and live weights, the average LFL, LHL, CB and LN were higher in Cameroon sheep but the animals of Congo had the highest live weight. The averages of most of Indices (SGI, ELI, CI, MI, IT, IL, FI, DTI, EI) were higher in Cameroon sheep. Excepted the massiveness index, the Dactylo-thoracic Index and the Caudal Index, all the other index (SGI, ELI, IF, CI, IT, IL, FI, BI, EI) were significantly different ($p < 0.05$) between countries. Excepted IT and FI the coefficient of variation of all the other indices were higher in Cameroon. correlation between measures range from -0.297 (between Neck length and the height at the back) to 1.00 (between the edge of the barrel and the live weight). There is a very strong correlation (0.94) between the height at the withers and the height at the back. Body weight is significantly ($p < 0.01$) correlated with height at the withers (0.72), back (0.70) and rumps (0.66).

Genetic variability of Black belly sheep in Central Africa

Six principal components with eigenvalue greater than 1 were extracted. components 1 and 2 have their eigenvalue 5.598 and 3.835 respectively and cumulatively represent 42.87% of the phenotypic variability observed on the body measurements of Black belly in Central Africa. However, 4 other components have an eigenvalue greater than 1 (component 3, 4, 5 and 6) and represent, with the first two components, 73.109 % of the phenotypic variability observed in this population. Two main groups

of variables express variations between Black belly of different countries that are correlated with each other (NL, LFL, LHL on the F2 axis and HW, HB, HR, BW on the F1 axis) It appears that, the height at the withers, the height at the back, the height at the rump, the thoracic circumference and the live weight are positively and strongly correlated between them, but also very correlated to the axis F1. While, the length of the rump, length of the front leg and length of the hind leg are strongly correlated with each other and with the axis F2.
Tree Classes of Black belly were determined. The most discriminating factors that characterize class 1 sheep are NL, LFL and LHL. For sheep of class 2, is HWh, HL, DC, EL, TBL, TrL, HB, CD, RL, RW, TL, CB and LN. For Class 3 it is HLh, HW, HB, TC, CW and BW. class 2 generally has the highest measurements. But the average live weight and chest circumference are higher in class 3. this population of Black belly sheep is made up of two subgroups, the first consisting of the genetic types T3 and the second consisting of the genetic types T2 and T1. Types 1 and 2 are therefore closely related while type 3 is more distant.

Conclusion

Black belly of Centrale Africa plays an important role in the socials and economics life of population; however, this activity has to be well organized to insure resilience in theses production practices. Training farmers can be and important aspect to harmonized practices and improve production. However, organization of breeders into production groups can lead to rapid and sustainable improvement of production and profitability form the Black belly production activities. There is great phenotypic variation between sheep of various countries. The longest animals were form Gabon while the highest and largest and heaviest were from Congo. The Black belly sheep of Central Africa are small in size and are light meat type. Thoracic circumference can efficiently be used for weight selection as we obtained a perfect correlation between them. In the light of this result it is noted that, apart from the biometrics characterization, genomic and biotechnologies have also to be used in order to improve and conserve the performances of the central Africa Black belly.

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INVENTORY AND NUTRITIONAL VALUE OF LOCAL FODDER RESOURCES OF SMALL RUMINANTS IN THE DRY SEASON IN WEST CAMEROON

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Abstract

Between January and June 2020, a study was conducted in the Menoua division, more specifically in five (5) subdivisions, which aim at developing a database on the inventory and nutritional value of local fodder resources in the dry season. A survey followed by direct observation on a grazing area or in the field, with the agreement of the farmers, was carried out in order to collect information on the types of fodder resources grazed by small ruminants, the segments generally grazed, their preferences and the phenological stage of the fodder at the time of grazing. A 100g sample was taken and sent to the Animal Production and Nutrition Research Unit where the chemical composition and the nutritional values were determined including Milk Forage Unit (MFU) and Meat Forage Unit and the protein inputs (the Digestible Protein in the Intestine allowed by Nitrogen - DPIN)) and the Digestible Protein in the Intestine allowed by Energy (DPIE). The results of this study showed that 21 fodder species are used in the diet of small ruminants during the dry season, of which the best known are *Pennisetum purpureum, Desmodium uncinatum, Musa paradisiaca and Manihot esculenta.* The species belonging to the grass family have the highest cellulose contents ranging from 25.87 to 36.25% DM; on the other hand, those belonging to the legume family have the highest crude protein contents ranging from 25.02 to 36.72% DM. Legumes and unconventional resources record the highest contents of MFU (varying respectively from 58.47 to 77.51 UF/100kg of DM and 72.12 to 107.83 UF/100kg of DM), Meat Forage Unit (varying respectively from 48, 95 to 71.97 UF/100kg of DM and 62.4 to 101.92 UF/100kg of DM), DPIN (ranging 6.81 to 23.07 g/kg DM and 8.47 to 18.48) and DPIE (ranging 37.78 to 80.66 g/kg of DM and 47.65 to 87.81 g/kg of DM, respectively). A total of 21 forage species were inventoried and their nutritional value determined. These resources constitute a main support for the synthesis and dissemination of knowledge on the nutritional value of fodder for feeding small ruminants during the dry season.

Keywords: Inventory, chemical composition, nutritional value, forage, small ruminants, Cameroon

Introduction

In developing countries, there has always been a balance between the agricultural production system and the small ruminants' husbandry, the latter making use of agricultural residues such as straw, tops, cereal stubble and other peelings (Lemoufouet et *al.*, 2012). This valorization is particularly done during the dry season when the animals are left to roam and during the cropping season when majority of their feed originates from marginal lands unsuitable for cultivation (Pamo et *al.*, 2007). However, human population growth in developing countries is leading to a decrease in the availability of land for fodder production (Archimede et *al.*, 2011). Also, the use of these resources by pastoralists is irrational because their nutritional values according to seasons and agro-ecological zones are poorly known and the lack

of data on the inventory of available fodder plant species in a locality is an impediment to the planning and formulation of fodder range plans (Pamo et *al*., 2007). However, a good knowledge of the nutritive value of the local fodder resources available in an agro-pastoral area could allow the formulation of appropriate feed formulations based on the nutritive requirements of the animals. Hence the objective of this work, which is to improve the knowledge of small ruminant feeding through the development of a database on local fodder resources available in the dry season, together with their nutritional values in the Menoua division, West Cameroon.

Materials and methods

Study area

The study took place in the Menoua division (Figure 1) which geographical coordinates are: 5°27'0"N/ 10°4'0'E. The study was conducted from January to mid-March for data collection in the field and from June for chemical composition analyses at the Animal Nutrition and Production Unit.

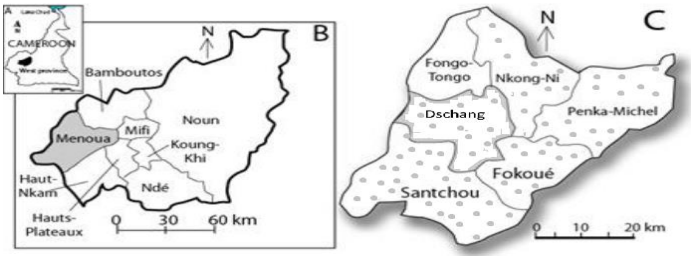

Figure 5 : Study area

Methodology

The study was carried out in five (5) subdivisions in Menoua Division, namely Dschang, Nkong-Ni, Penka-Michel, Fokoué and Santchou. An identification of at least three (3) villages in which small ruminant keepers were the most represented was carried out. More than fifty (50) owners were interviewed in order to collect information on the types of fodder resources grazed by small ruminants, the parts generally taken and their preferences. With the farmers' permission, direct observation of the animals on the grazing area was carried out to identify the grazed resources and their phenological stage at the time of grazing. The species and segments grazed were snapped, and a 100g sample was taken for chemical composition analysis in the laboratory. These samples were identified using Geerling's Woody Guide (1982), Van der Zon's Grasses of Cameroon (1992), Merlier and Montegut's Tropical Weeds (1989). Newspapers was used to wrap species that could not be identified on site to be sent to the National Herbarium of Cameroon for taxonomic identification.

Parameters studied

The parameters studied are as follows:

- Taxonomic identification, vernacular name, preference and part of the plant grazed by small ruminants;
- The citation frequency (FC) which reflects the regularity in the distribution of a species within a locality. It is expressed as the percentage of citations of a species in relation to the total number of people surveyed. The citation frequency for each of the taxa surveyed is calculated by the formula of Gbekley et *al* (2015) and Orsot (2016) :

FC=n/N, n: number of persons citing the species, N: total number of respondents

- Chemical composition was determined according to the AOAC method, 2000. The nutritive value of the herbaceous forage species inventoried was estimated from the chemical composition, using the equations of Throught of Peyraud, (1988).

Statistical Analysis

The data were analyzed and processed in an Excel spreadsheet version 2013

Results

Taxonomic identification and vernacular naming of the species identified

The findings gave a total of 21 species belonging to 10 families. The largest number of species recorded belonged to the family Poaceae (8), followed by Fabaceae (5) as illustrated in Table 1 below.

Table 1: Name of species inventoried, their family and vernacular name.

Family	Species names	Vernacular names	French common names
Poaceae	*Bracharia ruziziensis*	/	/
Poaceae	*Cynodon dactylon*	/	/
Poaceae	*Panicum maximun*	/	/
Poaceae	*Digiatria ciliaris*	/	/
Poaceae	*Pennisetum purpereum*	rouseau,mesouson, chouchoun	Sussongo
Poaceae	*Trypsacum laxum*	/	/
Poaceae	*Imperata cylindrica*	Keneum, fixtree	/
Fabaceae	*Desmodium uncinatum*	/	Mariage forcé
Fabaceae	*Arachis glabrata*	/	/
Fabaceae	*Calliandra calothyrsus*	/	/
Fabaceae	*Leucena leucocephola*	/	/
Fabaceae	*Gliricidia sepium*	/	/
Lauraceae	*Persea america*	Feh piah	Feuille d'avocat
Convolvulacea	*Ipomea batatas*	Feh metong	Feuille de patate
Myrtaceae	*Psidium guajava*	Feh goya	Feuille de goyave
Meliaceae	*Entandrophragma cylindricum,*	/	Sapelli
Euphorbiaceae	*Manihot esculenta*	Feh kessala, eyeu cassava	Feuille de manioc
Asparagaceae	*Draceana fragrans*	Keun	Arbre de paix
Asteraceae	*Vernonia amygdalina*	Mekan	Feuille de Ndolé
Musaceae	*Musa paradisiaca*	Feh quiguien, eyeu abeneu	Feuille de bananier

Table of chemical composition and nutritive values of forage resources in the Menoua division

The Table 2 presents the chemical composition and nutritional value of the different resources.

Table 2: Table of chemical composition and nutritional value of the different resources

	Studied parameters											
Species	DM (%)	As (%DM)	OM (%DM)	Gl	Fat (%DM)	CP (%DM)	CC (%DM)	Water content (%)	UFV (UF/100 kg per DM)	UFL (UF/100 kg per DM)	PDIN (g/kg DM)	PDIE (g/kg DM)
Bracharia ruziziensis	90.04	8.76	81.28	75.16	1.40	4.72	36.25	40.44	46.39	58.47	2.96	33.62
Cynodon dactylon	89.80	11.80	78.00	51.15	2.70	24.15	25.87	63.83	71.97	81.03	15.17	41.32
Digiatria ciliaris	89.26	17.49	71.77	47.75	1.80	22.22	27.07	57.78	68.12	77.51	13.95	36.95
Panicum maximun	90.40	10.2	80.20	64.69	3.01	12.60	31.04	67.41	60.39	70.79	7.91	41.13
Pennisetum purpereum	85.91	8.73	77.18	60.01	0.20	16.97	34.75	69.93	48.95	61.01	10.66	17.10
Imperata cylindrica	84.67	7.94	76.73	56.16	1.20	19.36	33.41	60.70	53.27	64.97	12.16	18.38
Trypsacum laxum	90.85	8.65	82.20	66.05	5.30	10.84	32.78	66.56	59.15	70.34	6.81	37.78
Gliricidia sepium	89.68	11.11	78.57	39.15	2.70	36.72	13.69	66.58	99.56	104.46	23.06	70.07
Arachis glabrata	90.40	9.60	80.80	52.78	3.00	25.02	23.31	57.78	78.03	86,14	15.71	51.94
Calliandra calothyrsus	90.79	6.01	84.78	57.66	0.70	26.42	16.80	59.35	89.62	95.17	16.59	80.66
Leuceana leucocephala	91.30	9.08	82.22	54.28	0.90	26.15	21.20	60.91	79.91	87.25	16.40	59.07
Desmodium uncinatum	83.87	7.85	76.02	48.38	2.40	25.24	21.96	66.59	80.27	87.85	15.85	54.24
Centrosema pubescens	88.27	9.34	78.93	42.61	8.50	27.82	18.36	75.34	95.79	102.34	17.47	66.55
Entandrophragma cylindricum	89.11	7.97	81.14	66.47	1.20	13.47	29.10	50.99	62.40	72.12	8.46	48.30
Ipomea batatas	89.33	8.34	80.99	56.11	4.84	20.04	26.02	78.86	74.01	83.00	12.58	49.49
psidium guajava	90.78	5.725	85.05	67.46	1.10	16.49	19.49	46.88	83.58	89.71	10.36	87.81

Manihot esculenta	89.26	12.06	77.2	37.89	9.88	29.44	16.4	70.54	101.92	107.83	18.48	70.53
draceana fragrans	88.45	11.48	76.97	45.71	5.86	25.40	26.44	70.70	74.64	84.11	15.95	36.39
Musa paradisiaca	90.35	10.97	79.38	54.58	1.40	23.39	24.83	67.34	72.63	81.19	14.69	47.65
vernonia amygdalina	89.26	12.06	77.20	42.23	5.54	29.43	16.38	52.75	96.63	102.36	18.48	70.53
Persea america	91.24	7.36	83.87	67.06	1.50	15.31	22.99	50.15	76,32	83.76	9.61	73.38

DM: dry matter (%), OM: organic matter (%DM), CP: crude protein (%DM), MG: fat (%DM), As: ash (%DM), Gl: carbohydrates (%DM), CC: crude cellulose (%DM), UFV: fodder unit meat (UF/100Kg of DM) UFL: milk fodder unit (UF/100Kg DM), PDIN : Digestible Protein in the Intestine allowed by Nitrogen (g/kg DM), PDIE : Digestible Protein in the Intestine allowed by Energy (g/kg DM).

Conclusion

At the end of this study:

- Twenty-one (21) fodder species are used in the feeding of small ruminants during the dry season, of which the best known by small ruminants' keepers *are Pennisetum purpureum, Desmodium unicatum, Musa paradisiaca* and *Manihot esculenta;*
- Grasses have the highest cellulose content, followed by unconventional resources and legumes. Species belonging to the legume family have the highest crude protein content, followed by unconventional resources and grasses. Species belonging to the non-conventional resources have the highest levels of UFV and UFL, followed by pulses and grasses. *Manihot esculenta* is the most energetic resource; with regard to nitrogen inputs, species belonging to the legume family record the highest levels of PDIN, followed by non-conventional resources and grasses. *Gliricidia sepium* has the highest PDIN content; Species belonging to the non-conventional resources have the highest PDIE content, followed by legumes and then grasses. *Psidium guajava*, records the highest PDIE content.

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EFFECTS OF DIFFERENT RATIOS OF ROUGHAGE AND CONCENTRATE IN DIETS ON *IN VITRO* METHANE GAS PRODUCTION AND DIGESTIBILITY IN GOATS

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Abstract

This study was conducted to investigate an *in vitro* methane gas production of the diets containing different ratios of roughage and concentrate, and to determine nutrient digestibility of goat fed on the diets which had low methane production. Rice straw (RS), sorghum stover (SST) and groundnut straw (GNS) were used as basal roughages and mixture of 50% groundnut meal (GM) and 50% pigeon pea pod residue (PPR) were used as concentrate (C) in this experiment. Roughage and concentrate ratio of feed mixtures were 30:70, 50:50 and 70:30. The methane gas production (ml/200 mg DM) of feed mixture $SSTC_{30}$ was significantly higher ($P<0.05$) than those of others except $SSTC_{50}$, $SSTC_{70}$ and $GNSC_{50}$. However, the methane concentration (%) of feed mixture RSC_{30} was significantly higher ($P<0.05$) than those of others except $SSTC_{30}$. According to the findings of *in vitro* methane gas production, three diets ($RSC_{50,}$ $SSTC_{50}$ and $GNSC_{30}$) from each roughage based-diets which had the lower methane production were selected to conduct the digestion trial. The diets $SSTC_{50}$ and $GNSC_{30}$ showed significantly ($P<0.05$) higher values in DMD and OMD compared to RSC_{50}. No significant effects on CPD, NDFD and ADFD of goats were found among the experimental groups.

Key words: *in vitro* methane gas, digestibility, ratio of roughage and concentrate, goats

Introduction

As Myanmar is an agricultural-based country, cereal crops such as rice, maize, groundnut, sorghum and pigeon pea are cultivated widely in all parts of the country. Generally, ruminant animal use crop residues, agricultural byproduct as main roughage sources which are low quality forages because they have high crude fibre content and low crude protein. Apart from being a greenhouse gas, methane also represents significant energy loss to the animal. Mitigation of methane emissions from ruminants can lower greenhouse gases and increase the efficiency of livestock production (Kumar *et al*. 2009). Methane production primarily depends on the quantity and quality of the feed that affects the rate of digestion and the rate of passage in the fermentation process (Dougherty, 1984). Dietary manipulation such as concentrate and roughage ratio, roughage quality, type of carbohydrates fed and feed processing on methane production have been widely done (Moe and Tyrrell, 1979). Therefore, this study was aimed to investigate an *in vitro* methane gas production and nutrient digestibility of goats fed on diet containing the different ratios of roughage and concentrate.

Materials and methods

The *in vitro* gas analysis was done by the methods described by Menke and Steingass (1988). Fistulated bull with 340 kg body weight was used as a donor of rumen inoculum. The experimental feed mixtures with three replicates were used for *in vitro* methane gas production. The feedstuffs used were rice straw

(RS), groundnut straw (GNS), sorghum stover (SST), groundnut meal (GM) and pigeon pea pod residue (PPR). The concentrate mixture consisted of ground nut meal and pigeon pea pod residue. Levels of concentrate used in feed mixture were 30, 50 and 75%, respectively for each roughage source. The experimental feed mixtures were RSC_{30}, RSC_{50}, RSC_{70}, $SSTC_{30}$, $SSTC_{50}$, $SSTC_{70}$, $GNSC_{30}$, $GNSC_{50}$ and $GNSC_{70}$. The *in vitro* methane production was determined by the method of Fievez *et al*. (2005). In the digestion trial, nine male goats were randomly divided and allocated into three treatment groups with three replications in a completely randomized design (CRD). Three diets (one from respective roughage) that had the lower methane production and closer crude protein contents were selected from the results *in vitro* methane gas analysis. They were RSC_{50} (Group 1), $SSTC_{50}$ (Group 2) and $GNSC_{30}$ (Group 3). The experimental animals were fed on diet at a restricted level (2% of animal live weight). The experimental period was lasted for 45 days. Digestion trial was done by total collection method. During the digestion trial period, the faece voided by each animal were collected for 5 consecutive days. The weight of feed offered and residues and faeces voided were recorded for the measurement of digestibility. All of the chemical analyses of feedstuffs and faeces sample were done by the method described by AOAC (1990) and Goering and van Soest (1970). The data were subjected to the analysis of variance (ANOVA) using SAS (version 9.0) software (SAS, 2002) and the significance of differences between treatments means were compared by Duncan's Multiple Ranged Test (DMRT) at $p<0.05$.

Results and Discussion

The methane concentration (% of total gas production) of RS supplemented with concentrate at the level of 50% and 70%, respectively was significantly reduced (Table 2). However, in SSTC group ($SSTC_{30}$, $SSTC_{50}$ and $SSTC_{70}$), mthane production was tended to reduce by increasing level of concentrate. IN GNSC group ($GNSC_{30}$, $GNSC_{50}$ and $GNSC_{70}$), methane concentration was not significantly influenced by roughage to concentrate ratio (Table 2). Eun *et al*. (2004) found that methane production was highest with high (70%) forage diet compared to medium (50 %) or low (30 %) forage diets. The methane concentration (%) of SST-based feed mixtures was higher than those of other feed mixtures except RSC_{30} which had the highest methane concentration (%). In this experiment, the variation in methane concentration among the feed mixture might be due to different contents (%) of NDF and ADF (Table 1). Santoso and Hariadi (2009) reported that methane production is influenced by NDF content of the feedstuff.

Table 1. Chemical compositions (%) of experimental feed mixtures

Description	DM	OM	CP	NDF	ADF	EE
RSC_{30}	94.05	87.40	10.70	60.03	40.11	1.78
RSC_{50}	93.63	88.68	16.03	54.89	37.25	2.67
RSC_{70}	93.56	91.15	19.63	47.99	35.25	3.57
$SSTC_{30}$	93.27	95.05	9.34	61.82	40.81	2.62
$SSTC_{50}$	94.32	94.25	15.30	54.94	36.96	3.27
$SSTC_{70}$	93.95	94.90	17.31	49.76	34.04	3.83
$GNSC_{30}$	93.16	93.92	15.71	53.73	44.28	1.52
$GNSC_{50}$	94.29	94.16	19.49	49.85	39.61	2.48
$GNSC_{70}$	93.70	94.35	23.41	48.27	35.26	3.47

Table 2. Total gas production, methane production (ml/200mg DM) and methane concentration% of experimental feed mixtures at 24h incubation

Description	Total gas	Methane gas	Methane concentration%
RSC_{30}	27	15^{bc}	54^{a}
RSC_{50}	35	11^{d}	30^{d}

RSC_{70}	39	12^{cd}	30^{d}
$SSTC_{30}$	40	19^{a}	46^{ab}
$SSTC_{50}$	42	16^{ab}	39^{bc}
$SSTC_{70}$	43	16^{ab}	39^{bc}
$GNSC_{30}$	49	13^{bcd}	27^{d}
$GNSC_{50}$	47	15^{ab}	31^{cd}
$GNSC_{70}$	47	13^{bcd}	29^{d}
SEM	-	0.82	3.05
p value	-	0.0011	0.0326

[a, b)]Significantly differences between treatment means within the same columns are indicated by dissimilar superscript (P<0.05).

In this study, the nutrient digestibility (%) (DMD, OMD and CPD) of the diets $SSTC_{50}$ and $GNSC_{30}$ were significantly higher (P<0.05) than those of diet RSC_{50}. Although no significances (p>0.05) in NDF and ADF digestibility were observed among dietary treatment groups (Table 3), neutral detergent fibre digestibility (NDFD %) of $SSTC_{50}$ was numerically higher than that of RSC_{50} and $GNSC_{30}$ (Table 3) and the methane concentration % of $SSTC_{50}$ was significantly higher (P<0.05) than RSC_{50} and $GNSC_{30}$ (Table 2). This finding was supported by Santoso *et al*. (2006) who reportedthat methane production is greatly influenced by fibre fraction (mainly NDF) digestibility and there was strongly relationship between methane and NDF digested. Somkid and Promma (1987) stated that limitation of rice straw was low palatability, low digestibility and low digestible protein.

Table 3. Digestibility of nutrients (%) of experimental diets

Description	Treatments			SEM	p value
	RSC_{50}	$SSTC_{50}$	$GNSC_{30}$		
DMD	61.63^{b}	69.74^{a}	70.72^{a}	1.66	0.0048
OMD	66.13^{b}	71.04^{a}	73.06^{a}	1.19	0.0116
CPD	68.77^{b}	71.69^{ab}	75.07^{a}	1.05	0.0517
NDFD	47.37	51.08	46.92	0.76	0.2854
ADFD	40.49	42.54	47.49	1.20	0.2386

[a,b)]Significantly differences between treatment means within the same rows are indicated by dissimilar superscript (P<0.05). DMD=dry matter digestibility,OMD=organic matter digestibility,CPD= crude protein digestibility, NDFD=neutral detergent fibre digestibility,ADFD=acid detergent fibre digestibility

In conclusion, the NDF was the most important factor in methane production because of positive relationship between with methane concentration and NDF contents experimental of feed mixtures as well as NDF digestibility of experimental diets.

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A SURVEY STUDY ON FEEDING PACKAGES ADOPTION BY SMALL-SCALE BEEF CATTLE FARMERS UNDER MIXED FARMING SYSTEM IN EGYPT

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Abstract

This study aimed to characterize feeding package (silage, hay, untraditional green forages(UGF), urea and molasses) adoption by the small-scale beef cattle farmers under mixed farming production system(crop-livestock). The cross-sectional survey of 200 cattle farmers was carried out in 4 governorates (50 each) through semi-structured interviews with questionnaires. In conclusion, adopters of feeding package recorded significantly higher productivity compared to non-adopters.

Introduction

In Egypt intensive and semi-intensive system comprise almost 7 % and 60 % of the total bovine population of the country, respectively, the smallholder system is characterized by less than 11 head per farmer **(FAO, 2018).** Livestock production plays a vital role in poverty reduction of poor farmers in developing countries **(Stür *et al.*, 2013).** Egypt has a relatively large population of ruminants about 18.247 million heads **(MALR, 2015)**, but with limited sources of agricultural land and water resources. There is a necessity to cover animal nutrition requirements by maximum utilization of crop residues. Practical and simple feed packages that are beneficial and easy to adopt by farmers are important to improve cattle productive efficiency, and moreover, will help in reducing cost of feeds.
Using of feed packages resulted in a 45% increase in the area planted with Berseem and 19.8% increase in the cash crop area, and feeding costs were reduced by 29% and 44% for growing and fattening animals, respectively (**El-Wardani *et al.*, 2005**). The annual per capita consumption of red meat by Egyptians reached approximately 11 kg **(MALR, 2015).** In developing countries, population growth, changing consumer preferences, economic progress and urbanization increase demand for animal products **(Delgado *et al*, 2020**). However, to increase the country's meat output, a judicious strategy of focusing on feed packages should be adopted for improved beef cattle development. The present study was therefore undertaken to obtain base-line information of current feeding packages adoption by beef cattle farmers.

Methodology

The study was carried out in four governorates in Egypt, two in the Nile Delta region (Sharkia and Menoufia) and two in the middle Egypt region (El-Fayoum and Menia). A total of 200 small-holdings were included in the present survey (50 small-holders from each governorate). The questionnaire was initially tested in the field and modified before extensive application.

Results and discussions

Adoption strategy of feeding packages

The main cause of reduced productivity of livestock in Egypt is the seasonal insufficiency of feed and fluctuating quantity and quality. Farmers depended mostly on the forage and crop residues produced on their lands. Fodder storage is crucial in guaranteeing that animals have enough feed all over the year. The vast majority of farmers had fully adopted the feeding packages. Corn silage adoption recorded the highest percentage (39.5%) followed by hay being 20.5 % as compared to the other feeding packages. Corn silage adoption recorded the highest percentage in Menoufia and Menia being 54% and 38 %, respectively. The highest percentage of hay adopters (34%) was found in Sharkia governorate. On the other hand, El-Fayoum had the highest percentage of untraditional green forage (UGF) adopters as compared to others governorates, being 32%. It could further be seen that urea and molasses were adopted by the farmers to a limited extent being 4% and 2%, respectively, as indicated in Table1. Urea treatment is not used on a wide scale because of inadequate extension efforts to popularize the technology, and unavailability of cash to purchase urea. Similar findings reported by **Nguyen (2004)**. The lack of the adoption of urea and molasses technologies is an indication that these packages are not playing sufficient part in extension programs. Farmers may lack knowledge about the utilization and advantages of these feeding packages, and sometimes they are not even aware of these packages. Moreover, uses of molasses is very limited due to problems related to transport and storage.

Table 1. Adoption status of feeding packages under the studied regions and governorates

Feed Packages	Nile Delta				Middle Egypt				Total	
	Sharkia		Menoufia		El-Fayoum		Menia			
	N	(%)	N	(%)	N	(%)	N	(%)	N	(%)
Corn silage	17	34	27	54	16	32	19	38	79	39.5
Hay	17	34	11	22	5	10	8	16	41	20.5
UGF*	7	14	2	4	16	32	9	18	34	17
Urea	2	4	1	2	4	8	1	2	8	4
Molasses	2	4	1	2	1	2	1	2	5	2.5
Non adopters	5	10	8	16	8	16	12	24	33	16.5

Differences between region and governorates for adoption are significant ($\chi 2$ =200.00, P<0.05)
* Untraditional green forages

Frequency of feeding packages

It seemed that most farmers adopted silage either for one (100 and 100%), two (71.43and 66.67%), three (66.67 and 40%), or four times and more (50.0 and 42.86%) in Menoufia and Menia, respectively. Silage was the first nutritional package being adopted by 80% of farmers for only one time, followed by hay, which was the second most common nutritional package being adopted either for two (50%), three (37.5%%), or four times and more (46.15%) in Sharkia governorate. On the other hand, silage and untraditional green forages (UGF) recorded the highest adoption percentage for one (33.33 and 33.33%), two (27.27 and 54.55%), three (50 and 28.57%), and four times and more (36.36 and 36.36%) respectively, in El-Fayoum governorate.

Beef production

The longest fattening period (360 day) was detected for indigenous and crossbred cows reared under non-adopters. The heaviest finished weight of indigenous cows, was detected for farmers who adopted urea and UGF being 400kg, whereas the lightest finished weight (337.5kg) was for those belonging to non-adopters. The same trend was observed for crossbred cows. Indigenous cows, crossbred cows, and buffaloes for non-adopters of feeding packages had the lowest live weight gain being 0.48, 0.52, and 0.59 kg/day as compared to those belonging to feeding package adopters as indicated in Table 2. These results agree with those reported by **Allam *et al.* (2009)** who found that feeding treated wheat straw with urea, molasses and enzymes to growing male goats significantly increased live weight gain. **Ma *et***

***al.* (1990)** reported that responses in live weight gain of cattle to ammonization of wheat straw were significant. Cattle fed with corn silage gave the highest body weight gain Nazli, 2018.

Table 2. Least square mean ± standard error of fattening traits of beef animals under adopters of feeding packages and non-adopters

Item	Fattening period	Weight at beginning	End weight	Weight gain kg/day
	M±SE	M±SE	M±SE	M±SE
Indigenous cows				
Silage	210.00±12.69^{b}	208.50±9.38^{ab}	396.25±9.64^{a}	0.89±0.02^{a}
Hay	232.50±21.02^{b}	200.00±18.89^{ab}	393.75±27.44^{a}	0.83±0.00^{ab}
UGF	190.00±10.00^{b}	242.50±13.76^{a}	400.00±12.90^{a}	0.83±0.03^{ab}
Urea	320.00±40.00^{a}	166.66±16.66^{b}	400.00±28.86^{a}	0.74±0.04^{b}
Molasses	-	-	-	-
Non adopters	360.00±35.00^{a}	162.500±12.50^{b}	337.50±12.50^{b}	0.48±0.04^{c}
Crossbred cows				
Silage	236.12±13.03^{b}	205.00±8.87^{ab}	428.12±11.43^{a}	1.02±0.04^{a}
Hay	282.85±22.17^{ab}	197.14±22.32^{ab}	435.71±22.50^{a}	0.87±0.04^{bc}
UGF	300.00±29.27^{ab}	197.14±25.51^{ab}	428.57±18.44^{a}	0.78±0.03^{d}
Urea	280.00±40.00^{ab}	233.33±44.09^{a}	483.33±44.09^{a}	0.92±0.11^{b}
Molasses	360.00±25.22^{a}	200.00±19.12^{a}	500.00±50.22^{a}	0.83±0.02^{c}
Non adopters	360.00±13.11^{a}	143.33±6.66^{b}	333.33±16.66^{b}	0.52±0.05^{c}
Buffalo				
Silage	204.68±21.43^{c}	167.68±17.09^{c}	389.28±34.16^{b}	0.98±0.05^{a}

Hay	230.16±36.70[b]	176.83±30.04[b]	392.00±54.07[b]	0.92±0.03[ab]
UGF	200.66±50.20[d]	151.00±85.73[d]	317.66±57.99[d]	0.83±0.04[b]
Urea	180.00±0.00[dc]	350.00±0.00[a]	500.00±0.00[a]	0.83±0.00[b]
Molasses	360.00±50.13[a]	150.00±40.39[d]	450.00±23.12[b]	0.83±0.04[b]
Non adopters	140.66±71.46[c]	167.66±82.33[c]	251.33±24.50[c]	0.59±0.03[c]

[a-b-c-d] Means, within the column, with different superscripts difference significantly (P<0.05)

Conclusion and recommendation

Corn silage adoption recorded the highest percentage followed by hay**,** however the majority of farmers adopted corn silage for only one time and hay for more than one time. Urea and molasses were adopted by the farmers to a limited extent. Adopters of feeding packages recorded significantly higher weight gain and finished weight for their cattle. Raising awareness about feed packages, providing technical support, tailored training is the way to spread the suitable technology and increase adoption rate, based on a bottom-up approach taking into consideration needs, objectives and abilities of the poor small farmers.

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RUMINANT LIVESTOCK FEED RESOURCES IN DIFFERENT AGRO-ECOLOGICAL ZONES OF CAMEROON

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Abstract

Documenting the indigenous sources of nutrition for ruminant livestock is a key to overall development of the sector in Cameroon. This paper highlights the forage and fodder potentials of Cameroon's native pastures in the major agro ecological zones **(**AEZs).These resources present regional dominance depending on climate and rainfall patterns. Dominances range from Pennisetum "Elephant Grass" in the North West to Hyparrhenia/Panicum in the savannas of East and Adamawa via *Andropogon gayanus* predominant in northern and far northern regions. *Andropogon gayanus* is also found in the eastern region. Agricultural residues complete these natural resources in use by the ruminants of the pastoral system. These are various tops (peanuts, millet and cowpeas), leaves and stalks of millet, rice straws, corn cobs, rice husks etc. The rational exploitation of land resources has great potential for fodder resources and agro-industrial by-products for animal feed in Cameroon.

Keywords: Agricultural residues, fodders plants, graminea, leguminous, ruminant.

Introduction

In Cameroon, the production of livestock and their productivity is, far below the population's requirement for animal protein (MINEPIA, 2016). In all the agro-ecological zones of Cameroon, pasture from unimproved rangelands serves as a major source of feed for ruminant livestock. Under smallholder management systems, feed resources available for small ruminants range from those obtained by scavenging around households to grazing and browsing on natural vegetation in natural pasture, fallow lands or along roadsides. Under confinement systems these same materials have to be collected by the farmer and fed to the animals.
Forage and fodder crops include pasture and range vegetation, as well as crop residues derived from farm crops (Cook *et al.*, 2005).The use of crop residues and agro by-products also play an important role in the nutrition of ruminant livestock and ensure year-round availability of feed. It is also the cheapest way of reducing the rising cost of feeding ruminants in the tropics (Archimède et *al.*, 2011; Suheel *et al.*, 2015).Documenting the indigenous sources of nutrition for ruminant livestock is a key to overall development of the sector in the country. This paper highlights the forage and fodder potentials of Cameroon's native pastures in the major agro ecological zones **(**AEZs).

Agro ecological zones

Cameroon consists of five major agro-ecological zones that include:

-Sudano-Sahelian (I) in the north and extreme north region. This zone is characterized by unimodal rainfall with annual levels between 650 and 1000 mm. There is a distinct wet season of 3–4 months and a dry season of about 8–9 months (September to May). Throughout most of this zone, the relative humidity is consistently below 60%.
-Sudano-guinea (II) in the Adamaoua Plateau is characterized by unimodal rainfall with annual levels around 1500 mm.
-Western High Plateau (III) in West and North-west region. The Western High Plateau lies at altitudes ranging between 1,100 m and 2,000 m above sea level (ASL). The rainfall distribution is bimodal and range from 1,000 to 2,000 mm per annum, with two cropping seasons in the valleys and one on the mountains areas. This is the coolest part of the country, with temperatures ranging from 18 to 25 °C. The long wet season of 8–9 months from March to November and the high humidity generally over 80% ensures continuous presence of moisture in the air.
-Humid Forest with unimodal rainfalls (IV) in the Littoral and Southwest region, and
-Humid Forest with bimodal rainfalls (V) in Central and Eastern part of the country. The Rainforest AEZ (IV and V) in the southern part of the country lies at altitudes ranging between 0 and 800 m ASL. This AEZ is characterized by forest/savanna mosaic vegetation with rainfall between 1,200 and 2,000 mm per year from March to November. The temperature ranges from 22 to 32°C and the relative humidity is normally around 80%.

Forage resources

The country has a great diversity of fodder species spread over the 5 agro-ecological zones. These resources present regional dominance depending on climate and rainfall. Dominances range from Pennisetum "Elephant Grass" in the North West to Hyparrhenia / Panicum in the savannas of East and Adamawa via *Andropogon gayanus* predominant in northern and far northern regions. *Andropogon gayanus* is also found in the eastern region. Fodder shrubs complete these natural resources in use by the ruminants of the pastoral system (MINEPIA, 2016).
Tables 1 to 4 below summarize the forage species present in the different regions of Cameroon as well as the time of their availability during the year.

Table1: Forages and fodder in Sudano-Sahelian agro-ecological zone

Forage species	Time of their availability during the year
Grasses	
Hyparrhenia rufa	In the dry season mainly
Andropogon gayanus	
Andropogon pseudocarpus	
Digitaria	
Hyparrhenia dissoluta	
Panicum sp	
Sesbania pubescencs	
Fodder shrubs	

Hymenocardia acida *Danellia oliveri* *Stereospermum kunthianum* *Strychnos spinosa* *Ficus dekdekena* *Securidaca longipedonculata* *Ziziphus mauritiana* *Balanites aegyptiaca* *Ptericarpus erinacens* *Ficus sp.* *Zama sp* *Gardenia rubescens* *Gardenia termifelia*	Leaves eaten by cattle and especially goats during the long dry season (6 to 9 months)

Table 2: Forages and fodder in Humid Forest agro-ecological zones

Forage species	Time of their availability during the year
Pennisetum purpureum	Available all year round
Imperata cylindrica	On fallow after 2nd crop cycle
Panicum maximum	Available all year round
Setaria megaphylla	
Hyparrhenia diplandra	
Hyparrhenia rufa	
Panicum phragmitoides	

Table 3: Forages and fodder in Western High Plateau agro-ecological zone

Grasses	
Hyparrhenia diplandra	In the dry season
Melinis minutiflora	
Sporobolus pyramidalis	
Pennisetum clandestinum	Available all year round
Sporalobus pyramidalis	
Fodder shrubs	
Hymenocardia acida	Available all year round
Lophira sp.	
Piliostigma thonningii	

Table 4: Forages and fodder in Sudano-guinea agro-ecological zone

Forage species	Time of their availability during the year
Loudetia kagerensis	Available all year round
Hyparrhenia bracteata	
Andropogon schirensis	
Urelytrum fasciculatum	
Hyparrhenia rufa	
Hyparrhenia diplandra	
Brachiaria brizantha	
Setaria sphacelata	
Panicum phragmitoides	
Beckeropsis uniseta	

Schizachyrium platyphyllum	Rainy season
Paspalum orbiculare	
Setaria anceps	
Fodder shrubs	
Deniellia oliveri	Available all year round
Ficus thonningii	
Gardenia ternifolia	
Phyllanthus muellerianus	
Piliostigna thonningii	
Bridelia ferruginea benth	
Bridelia ndelensis beille	
Bridelia of speciosa muli arg	
Canthium venosum hiern	
Carissa edulis vahl	
Erythrina sigmoides hua	
Ficus glumosa var. Glaberrina	
Ficus glumosa	
Hymenocardia acidatul.	
Lannea schimperi engl.	In the dry season mainly
Lophira lanceolata van tieg	
Mussaenda arcuata lam ex poir	
Nuttosporum viridiflorum sims	
Spondianthus preussi var. Glaber (toxic)	
Tricalysia okelensis var. Oblan.	
Vitex doniana sweet	
Vitex madiensis oliv.	

To the fodder resources summarized above, there are agricultural residues which are highly prized in the northern part (north and far north). These are various tops (peanuts, millet and cowpeas), leaves and stalks of millet, rice straws, corn cobs, rice husks etc.

Photo 1: Peanut tops

Photo 2: Corn bran

Conclusion

For livestock and agriculture, Cameroon has immense land resources estimated at 47 million ha of which 9.2 million ha are used for agricultural purposes and 19 million ha can accommodate pastoral activities. The rational exploitation of these land resources has great potential for fodder resources and agro-industrial by-products for animal feed.

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EVALUATION OF TRAIT PREFERENCE AS A PREREQUISITE FOR CAMEROON NATIVE GOAT IMPROVEMENT

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Abstract

Goat is one of the most important livestock for African countries where the development of sustainable improvement strategies are expected to address more efficiently, nutritional and economic challenges. Among these strategies, selection of high potential adapted native breed such as West African dwarf Goat (WADG) is one of the key actions to improve flock productivity under traditional breeding systems. However, the success of the improvement program is highly dependent of the real involvement of main stakeholders especially producers in the process of designing the breeding goals. The study aims at assessing the trait preferences of goat keepers in order to design the breeding goal achievements. The result of the focus group discussions shows that farmers are interested by varieties of qualities for females or males. In females, the main qualities are respectively: high litter size (fecundity); conformation; high weight; coat colour and disease resistance. In males, the desired traits are: conformation; virility (stamina); weight; origin; disease resistance. The genetic determinisms of some of those traits have been already subjected to investigation using novel molecular genetic approaches like genome wide association studies in the perspective of improvement initiatives. These preliminary results are available but to be blended and being implemented, under significant and relevant monitoring and appropriate resources.

Introduction

Based on current FAO data, there are nearly 1.2 billion goats around the world with almost 95 % percent located in Asia and Africa. This numerical productivity is paradoxically relative to the production systems, mostly extensive with less investment and care. This suggest that, the native breeds are specifically well adapted to their harsh environment. Potential productivity of goats is constrained by poor understanding of their diverse values and of strategies for improved natural resources management in target environments (Mamabolo and Webb, 2005).

In Africa, such specificities could have been used by decision markers as a basis to build up their strategies to address issues of animal protein deficiency and poverty alleviation. Improving the genetic ability of local breed is among the main step. In this line, Kosgey *et al.*, (2006) have suggested within-breed selection of the adapted indigenous genotypes as a viable option.

So far, the constraints of breeding improvement of goat in smallholder production systems as reported by various authors (Peters, 1989**,** Wollny *et al.*.2002) are persisting. Peters (1989) reported single sire flocks as an issue of confounding of sire and flock effects in on-farm study in traditional small ruminant breeding system while Wollny *et al.*, (2002) listed small animal populations, single sire flocks, lack of systematic animal identification, inadequate animal performance and pedigree recording, low levels of literacy and organizational shortcomings. Improvement attempts require good definition of the breeding goal. However, the success of the improvement program is highly depending on the real implication of main stakeholders especially producer in the process of designing the breeding goal. The study aims at assessing the trait preferences of goat keepers in order to design genuine breeding goals.

Methodology

Study area

Data were collected in the Western highlands zone of Cameroon. The first zone is located between 5° to 8° latitude North and 9°45 to 11°15 longitude East and the second, 2° to 4° latitude North and 11°15 to 16 ° longitude East (ASEB, 2010)

Data collection

For females: the trait preferences were assessed after undertaking a consultative process. Three discussion groups of 10-15 farmers each from the goat's innovation platform where constituted randomly. They were asked to report some traits used to select the reproductive animals. Then they were invited to rank those traits according to their relative importance.
For males: The trait preferences have been discussed in a plenary session given the fact that the majority of household do not keep buck. The rank proposed by breeders has been simply reported.

Data analysis

The investigated traits were analysed by descriptive statistics. In each group, each trait was affected by a coefficient corresponding to its weight, from the highest (3), the fairly (2) to the lowest (1) and 0 when the trait is not selected. The consensus trait importance among the three groups was determined by ranking the mean obtained from the average coefficient from the highest to the lowest.

Results

Table 1 give the classification of traits/characteristics criteria used by goat's keeper in selecting for empirical selection.

Table1: Trait preferences for female and males in Cameroon native goat

Sex of animal	Criteria/Traits	Mean for the three groups of discussion	Rank
Female	High Litter size /prolificacy	2	1
	Body size/conformation	1.66	2
	High Weight	1	3
	Coat colour	1	3
	Disease resistance	0.33	4
	Growth rate	0	-
	Precocity	0	-
Male	Body size/ conformation	-	1
	Virility	-	2
	Weight	-	3
	Origin	-	4
	Disease resistance	-	5
	Coat colour	-	-
	Growth rate	-	-
	Father performance	-	-
	Mother performance	-	-

The result of the focus group discussion shows that farmers are interested by varieties of qualities either for females or for males. For female, the main qualities they would like are in term of importance: high litter size; conformation; high weight; coat colour and disease resistance. For male these qualities are classified in order of preference as: conformation; virility; weight; origin; disease resistance. These

traits usefulness in the process of designing the breeding goal in a breeding program is then demonstrated.

Surprisingly, the growth rate has not been reported in the top criteria. The explanation might be that according to the production system (free range and low investment) producers do not paid so much attention. They cannot even evaluate it objectively because of the lack of follow up and record keeping. Another explanation could that the relative importance of numerical productivity over individual productivity.

Male coat colour is of less importance for goat keepers but not in females. An attempt of explication is that the low input system on production does not yet constraint goat breeders to pay attention to indicators such as the internal rate of return. Regarding the growth, farmers may be still more interested on the numeric productivity rather than the individual productivity. An objective breeding goat design should not ignore the growth rate. Those criteria represent a useful baseline in the process of designing a consensual breeding goal in any structured improvement program.

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ESTIMATING ENTERIC METHANE EMISSION USING EMPIRICAL MODELS IN GRAZING INTEGRATED PRODUCTION SYSTEMS IN THE AMAZON BIOME OF CENTRAL BRAZIL

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Introduction

The low efficiency of animal production systems in degraded pastures or in degradation process allows the achievement of low productive indexes. This makes the livestock one of the activities with the highest emission of greenhouse gases (GHG) among agricultural activities, causing negative impacts on the environment, as it presents large amounts of GHG emissions per kilogram of animal product produced (SEEG, 2017).
Considering the importance of livestock for Brazil's economy, it is necessary to establish sustainable strategies that increase productivity and concomitantly reduce GHG emissions, especially enteric methane (CH_4), as this is directly correlated with the efficiency of energy use by animals and their performance (Berchielli et al., 2012; Berndt et al., 2020).
The mitigation of CH_4 emissions by ruminants in integrated systems is an effect provided by the potential for carbon sequestration that forage, trees, and the increase in organic matter in the soil. This interaction increases the ability to compensate the CH_4 emission resulting from the enteric fermentation of herbivores, generating a positive carbon balance in the integrated systems (Pontes et al., 2018).
The use of prediction equations has been widely used to estimate for CH_4 emissions, as a way to reduce the need to use expensive and complex equipment's (Kebreab et al., 2008). In this work, empirical models were used to compare predictions of enteric CH_4 emissions from two integrated production systems. The objective was to identify strategies that mitigate CH_4 emissions from forage-based livestock production.

Material and methods

Data on animal performance and composition of diets were collected over 4 months, in the rainy season (December 2017 to March 2018) on 2 integrated systems: livestock (L) and livestock-forestry (LF). The experimental design was a randomized complete block, with two systems and four replicates, totaling 8 experimental units.
In the L system, Marandu palisadegrass was used as a monoculture. The LF system was established in the summer of 2011/2012 when the trees [*Eucalyptus urograndis* (hybrid of *E. grandis* W. Hill ex Maiden and *E. urophylla* S. T. Blake) clone H13] were planted in triple rows (intra-row spacing 3 m, inter-row spacing 3.5 m) in the east-west direction and the distance between groves was 30 m, resulting in a density of 270 trees/ha. Subsequently, Marandu palisadegrass was established among the tree groves. In September 2016, 50% of the trees were thinned to improve the light environment, resulting in a density of 135 trees/ha, with 21% PAR reduction (Gomes et al., 2020). In both systems, each paddock has an area of 2 ha, and in the LF system, 0.5 ha was occupied with trees and 1.5 ha with forage.
Datasets contained 36 records for L system and 33 records for LF system.

Mean daily CH_4 emissions (g d^{-1}) and Y_m value, both estimated monthly, were compared within models. Four models were used to predict CH_4 (g d^{-1}) and methane conversion factor (% GEI).

1) IPCC1 (IPCC, 2006 – Tier 2): (0.065 x GEI)/0.005565, where GEI = gross energy intake (MJ/d).
2) IPCC2 (IPCC, 2019 – Tier 2): DMI x MY, where DMI = dry matter intake (kg/d); MY = methane yield (kg CH_4/kg DMI).
3) VL (van Lingen et al., 2019): -16.4+ 12.1 x DMI + 2.1 x NDF, where NDF = dietary neutral detergent fiber (% of DM)
4) RIB (Ribeiro et al., 2020): (0.938 + 0.0368 x GEI + 0.0098 x BW) x 55.65, where BW = body weight (kg).

For equations that used DMI, this variable was calculated using the IPCC equation:

$$DMI = BW^{0.75} \times \left[\frac{(0.2444 \times NE_{ma} - 0.0111 \times NE_{ma}^{2} - 0.472)}{NE_{ma}}\right]$$

Table 1. Summary of database by dietary variables and DMI between livestock and livestock-forestry systems.

Variable	n	Mean	SD	Min	Max
Livestock					
BW (kg)	36	463	28	408	518
DMI (kg d^{-1})	36	12	0.5	10	13
GEI (MJ d^{-1})	36	160	26.8	98	221
CP (% of DM)	36	12	1.3	11	18
NDF (% of DM)	36	57	2.2	55	62
Livestock-forestry					
BW (kg)	33	460	25	411	517
DMI (kg d^{-1})	33	11	0.5	11	13
GEI (MJ d^{-1})	33	156	27.7	98	204
CP (% of DM)	33	13	1.2	11	15
NDF (% of DM)	33	58	1.9	56	60

BW: body weight; DMI: dry matter intake; GEI: gross energy intake; CP: crude protein; NDF: neutral detergent fiber.

The comparisons were conducted using the Kruskal-Wallis test and nonparametric multiple comparisons among means were made by pairwise two-sided multiples comparison analysis ($P \leq 0.05$). Statistical software used was SAS version 9.4.

Results

In the livestock (L) system the Y_m was 18% and 27% higher for IPCC2 and VL compared to RIB and IPCC1, respectively (Table 2). RIB was 8% higher than IPCC1. The CH_4 emission was 25% and 17% higher for IPCC2 and VL than IPCC1 (193.3) and RIB (207.9), respectively.

In both systems, IPCC2 and VL predicted higher Y_m values compared to the other models. Regardless of the system, no model has estimated a Y_m similar to that of IPCC1. Although the IPCC2 does not consider changes in the forage bromatological composition, the estimates of Y_m and CH_4 emissions were similar to that of the VL, which considers the dietary NDF, thus VL model would be more realistic whenever we would like to estimate CH_4 produced by ruminants grazing forages with different quality. Despite RIB used datasets from tropical conditions similar to our data, it also does not consider forage quality, a main drive for estimating CH_4.

Table 2. Methane conversion factor (Y_m) and enteric methane emission predicted with empirical models for beef cattle in livestock and livestock-forestry systems.

Model	Y_m (%GEI)	CH_4 (g/d)
Livestock		
IPCC1	6.5[c]	193.3[b]
IPCC2	8.3[a]	241.7[a]
VL	8.2[a]	243.7[a]
RIB	7.0[b]	207.9[b]
Livestock-forestry		
IPCC1	6.5[c]	191.6[b]
IPCC2	8.6[a]	239.7[a]
VL	8.5[a]	243.7[a]
RIB	7.0[b]	206.2[b]

IPCC1: IPCC (2006) – Tier 2; IPCC2: IPCC (2019) Tier 2; VL: van Lingen et al. (2019); RIB Ribeiro et al. (2020).
Letters compare models by the Kruskal-Wallis test ($P \leq 0.05$)

The gross methane emissions were similar between the systems because the animal performance was the same in the two systems (Carvalho et al., 2019). This demonstrates that with a well-managed monoculture livestock system it is possible to achieve results similar to an integrated system.
However, it is important to consider that the insertion of the forest component in the system increases the carbon sequestration capacity due to the fixation that occurs in aerial biomass and the root mass of trees (Nascimento et al., 2018), which none of the studied models we used give the necessary estimates. Also, the shading caused by the trees provides lesser temperatures to the soil, reducing the activity of microorganisms and consequently the decomposition of organic compounds, responsible for the carbon loss, mainly in the form of CO_2 (Hoosbeek et al., 2018). However, to be able to calculate the emissions of all components of the system, an analysis of life cycle assessment must be performed.

Conclusions

Empirical models estimated enteric CH_4 emissions that were similar for the livestock and livestock-forestry systems. The difference in estimated enteric CH_4 emissions between models is greater than the difference between production systems, which may limit their use in the current form. The estimation of CH_4 emission must be performed according to the forage quality and animal performance, as these parameters are the ones that most influence the emission.

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GROWTH PERFORMANCE AND IMPROVEMENT OF DIGESTIBILITY OF NEW PROMISING MUTANT LINES SORGHUM IN INDONESIA BY *IN VITRO* CONTINUOUS RUMEN CULTURE

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Introduction

Several livestock center areas in Indonesia have a dry climate, including in Sulawesi, East Nusa Tenggara and Yogyakarta. Appropriate forage management is necessary to anticipate this challenge. Sorghum (*Sorghum bicolor*) is suitable forage to cultivate in extreme environments. Forage sorghum is an important forage source in arid and semiarid regions. Sorghum is a perennial plant and use water more efficiently than corn. Sorghum as animal forage topic experiment has been widely studied in Indonesia. Moreover, the development of forage sorghum in Indonesia is mainly from mutation radiation technique by National Nuclear Energy Agency of Indonesia (BATAN).
In the last few years, BATAN has developed G5 and G8 as new mutant lines sorghum for ruminant forage. Based on leaf midrib and stem types, G5 is brown midrib (BMR) type, while G8 is green midrib (GMR) type. Growth performance and nutritive value for ruminant forage are affected by different types of sorghum. Therefore, this study aimed to determine the growth performance and digestibility of G5 and G8 as new promising mutant lines in Indonesia. These experiment is necessary to provide information about the performance of sorghum-based rations for ruminant that can be applied in dry areas in Indonesia.

Methods

The study was conducted in field laboratory, Center for Isotope and Radiation Application (CIRA), BATAN. Two varieties and two sorghum mutant lines were examined in our study. Numbu variety as national control, Pahat variety as mutant variety control, G5 and G8 as the new mutant lines. Each cultivars were planted in 20 x 60 cm planting area at 5 cm depth. Fertilizers added were urea, tri sodium phosphate and potassium chloride in a ratio of 2:3:2 (g/g/g) at 210 kg/ha for 1st fertilizer. For 2nd fertilizer application, urea was added for amount 140 kg/ha. Plant was harvested at hard dough stage (115 days after sowing).
Dry matter (DM) yield, plant height, stem diameter, leaf length, leaf width and stem:leaf:panicle ratio were observed for growth performance parameters. Nutrient and fiber composition also observed in this study. *The in vitro* digestibility was conducted by using Rumen Simulation Technique (RUSITEC)[8], [9]. Total mixed rations (TMR) based on sorghum forage and concentrate (60%:40% DM composition; 97 g/day crude protein and 481 g/day total digestibe nutrients) were observed in this study. Observation was recorded by 14 d. Dry matter digestibility (DMD), organic matter digestibility (OMD), total gas production and rumen fermentation products were analyzed in this study. The data was analyzed using randomized block design with five replication. Different among treatments were analyzed using Duncan Multipe Range Test (DMRT).

Results and Discussion

Except leaf ratio, there were significant difference ($p < 0.01$) in all parameters among these four sorghums (Table 1). G5 sorghum mutant lines had highest dry matter yield ($p < 0.01$), thus Numbu, as national variety, was tallest sorghum cultivar ($p < 0.01$). It is interesting to note that Pahat, as grain type sorghum, had highest panicle ratio and lowest stem ratio. High biomass is a major consideration in breeding program based on mutation radiation. Previous study reported that BMR sorghum types tended to produce higher biomass yield than conventional sorghum. However, it was not significantly different with staygreen/GMR types. In contrary, Li et al. demonstrated that there was no significant different in dry matter yield between BMR, GMR and white midrib (WMR) sorghum. The difference results between study maybe due to the differences of climate and organic material of the soil.

Table 1. Growth performance of new promising mutant lines sorghum in Indonesia

Parameters	Cultivars			
	Numbu*	Pahat**	G5	G8
Dry matter yield (ton ha^{-1})	25.21 ± 0.57^{a}	26.09 ± 0.26^{b}	26.88 ± 0.61^{c}	$25.28 \pm 1{,}03^{a}$
Plant height (cm)	305.44 ± 3.91^{c}	124.00 ± 3.91^{a}	237.22 ± 9.63^{b}	235.89 ± 3.33^{b}
Stem diameter (mm)	16.73 ± 0.86^{b}	22.97 ± 1.50^{d}	17.73 ± 1.36^{b}	14.83 ± 0.56^{a}
Leaf length (cm)	105.89 ± 2.20^{c}	93.67 ± 3.74^{b}	92.22 ± 3.63^{b}	83.78 ± 3.60^{a}
Leaf width (cm)	8.00 ± 0.23^{b}	8.48 ± 0.48^{b}	8.38 ± 0.52^{b}	7.08 ± 0.32^{a}
Ratio (%)				
Stem	63.10 ± 2.00^{b}	48.48 ± 3.03^{a}	60.60 ± 1.94^{b}	61.06 ± 2.01^{b}
Leaf	15.94 ± 1.29	17.23 ± 2.37	16.54 ± 2.32	17.66 ± 1.02
Panicle	20.97 ± 2.67^{a}	34.30 ± 2.87^{b}	22.86 ± 1.71^{a}	21.28 ± 0.99^{a}

*national variety in Indonesia
**variety in Indonesia from mutation breeding technique
Means with different superscripts within rows are different ($p < 0.01$)

Table 2. Nutrient fractions of new promising mutant lines sorghum (Wahyono et al. 2019)

Parameters	Cultivars			
	Numbu*	Pahat**	G5	G8
Organic matter	94.70 ± 0.53^{b}	89.66 ± 0.62^{a}	94.42 ± 0.44^{b}	93.80 ± 0.95^{b}
Crude protein	7.89 ± 0.17^{a}	8.32 ± 0.30^{b}	8.48 ± 0.26^{b}	9.19 ± 0.49^{c}
Ether extract	2.46 ± 0.38^{b}	2.11 ± 0.10^{a}	2.03 ± 0.12^{a}	2.10 ± 0.15^{a}
Non fiber carbohydrate	32.56 ± 0.93^{a}	34.54 ± 1.91^{bc}	38.79 ± 1.92^{d}	36.65 ± 2.18^{cd}
Neutral detergent fiber	52.78 ± 0.67^{b}	44.70 ± 1.53^{a}	45.11 ± 1.84^{a}	45.86 ± 1.37^{a}
Acid detergent fiber	31.93 ± 0.51^{d}	24.14 ± 0.91^{a}	26.14 ± 0.46^{b}	27.97 ± 0.93^{c}

*national variety in Indonesia
**variety in Indonesia from mutation breeding technique
Means with different superscripts within rows are different ($p < 0.01$)

Table 3. Average of digestibility of total mixed rations (TMR) based on new promising mutant lines sorghum by *in vitro* continuous rumen culture (n = 42; t = 14 d)

Parameters	TMR			SEM
	Pahat	G5	G8	
Dry matter digestibility (%)	45.20^{b}	45.21^{b}	42.42^{a}	0.366
Organic matter digestibility (%)	47.99^{b}	46.85^{b}	44.95^{a}	0.355
Total gas production (ml/15 g DM)	880.71	932.86	800.71	37.384
CO_2:CH_4 gas ratio	4.31^{a}	6.73^{b}	7.20^{b}	0.330
Total volatile fatty acids (mM)	116.71^{a}	135.43^{b}	121.07^{a}	2.826
N-ammonia (mg/100 ml)	7.74	9.01	8.22	0.289

*national variety in Indonesia
Means with different superscripts within rows are different ($p < 0.01$)

As reported in Table 2, G5, as BMR type had lowest neutral detergent fiber (NDF) and acid detergent fiber (ADF) values ($p < 0.01$). Furthermore, G5 also had highest non fiber carbohydrate (NFC) value ($p < 0.01$). Based on its characteristics, brown midrib sorghum has a lower lignin content than conventional sorghum This will affect high digestibility value, as shown in Table 3. Lignin is part of the ADF content. Acid detergent fiber had a negative correlation with forage digestibility. As GMR type, G8 contains highest CP content (Table 2) ($p < 0.01$). These characteristics will support the availability of protein derived from forage. The digestibility and rumen fermentation characteristics of TMR based on sorghum forage are presented in Table 3. Pahat and G5 showed highest dry matter and organic matter digestibility ($p < 0.01$). the low NDF and ADF fractions in G5 are the reason for the high digestibility values. Pahat variety has a high panicle ratio, which increases the non-structural carbohydrate fraction and improves digestibility [6].

Conclusion

Breeding program with radiation technique in Indonesia improves growth performance and digestibility of sorghum. As BMR type, G5 mutant lines produces high biomass yield and digestibility. G8 mutant lines contains high CP value. We can suggest both of them to be used as forage source in dry area in Indonesia.

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TITHONIA DIVERSIFOLIA AND TROPICAL GRASSES INTERCROPPING AS A SUSTAINABLE ALTERNATIVE FOR RUMINANTS

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Introduction

Tithonia diversifolia (Tithonia) is a shrub widespread in tropical and subtropical regions. Traditionally, all parts of the plant especially the leaves, are widely used by indigenous people for treating a wide spectrum of ailments and diseases ranging from topical application to oral administration (Tagne et al., 2018). Tithonia presents some characteristics of interest in ruminant production systems when compared with other common tropical forages; it has greater crude protein and phosphorus concentrations (Ribeiro et al., 2016) and nutritive value of Tithonia is maintained relatively constant throughout the dry season.
Tithonia is resistant to acid soils and has a low water demand (Calsavara et al., 2016). Despite the many agronomic benefits of including Tithonia in pastures for ruminants, the ideal dietary inclusion for optimizing animal performance should be clarified. The objective of this study was to describe the behavior of Tithonia for two yearlong focusing on its potential for intercropping with tropical grasses as a sustainable option in Southeastern Brazil ruminant production.

Material and Methods

The control of the agronomic behavior and performance of the Tithonia was carried out at the grazing area of the Animal Nutrition Laboratory, Center for Nuclear Energy in Agriculture, Piracicaba, São Paulo, Brazil for two yearlong. Tithonia was established in an 800 m^2 paddock (20 x 40 m^2), where 4 lines of Tithonia were planted longitudinally with 3 m space between rows and 1 m between plants, with an approximate density of about 2300 plants/ha. Therefore 3 plants from each line were randomly selected and cut periodically and their height (m), weight (kg) and residue height (m) were registered. The regrowth period between cuts ranged from 21 to 140 days and the climatic conditions data for dew point temperature calculation (Lawrence, 2005) were recorded from the Luiz de Queiroz College of Agriculture (ESALQ-USP) climate station in Piracicaba, Brazil (latitude 22°42′30″ S, longitude 47°38′30″W, elevation 546 m a.m.s.l.).
Tithonia samples were oven dried (55°C, 48 h), and ground through a Wiley mill to pass 1 mm screen for chemical analyses for quantifying dry matter (DM), ashes and crude protein (CP) according to AOAC (2011) procedures. For neutral detergent fiber (NDF) and acid detergent fiber (ADF) determination, methodologies of Van Soest et al. (1991), with adaptations (Mertens, 2002) were used in a Tecnal® fiber analyzer (Piracicaba – SP).

Results

Crude protein content showed a moderate negative correlation (r = - 0.59) with dew point temperatures, while there was a strong correlation (r = 0.61) between biomass production (tons of DM/ha) and dew

point temperatures. As expected, biomass production varied closely with climatic conditions, showing higher yields during wet seasons versus lower biomass production during dry season, but compensated by its higher CP content throughout the period. This could indicate an interesting potential for intercropping system using Tithonia with tropical grasses in these conditions, since usually topical grasses have poor nutritive and unsatisfactory performance during dry seasons (Figure 1). Our climatic data record showed that lowest dew point values were directly associated with lower temperatures and shortages of rain, usually from June to November (dry season), and higher dew point values were associated with higher temperatures and higher humidity, usually during from November to May (wet season).

No significant correlation was found between NDF content and dew point as well for regrowth period (Table 1), although higher NDF content can be observed (Figure 1) as the months go by, probably suggesting an aging process or a plant's response to the frequent grazing. A weak positive correlation was observed between ADF content and regrowth period, and a strong positive correlation between ADF and dew point temperature, suggesting that fiber content is more dependent of climatic conditions than the age of the plant.

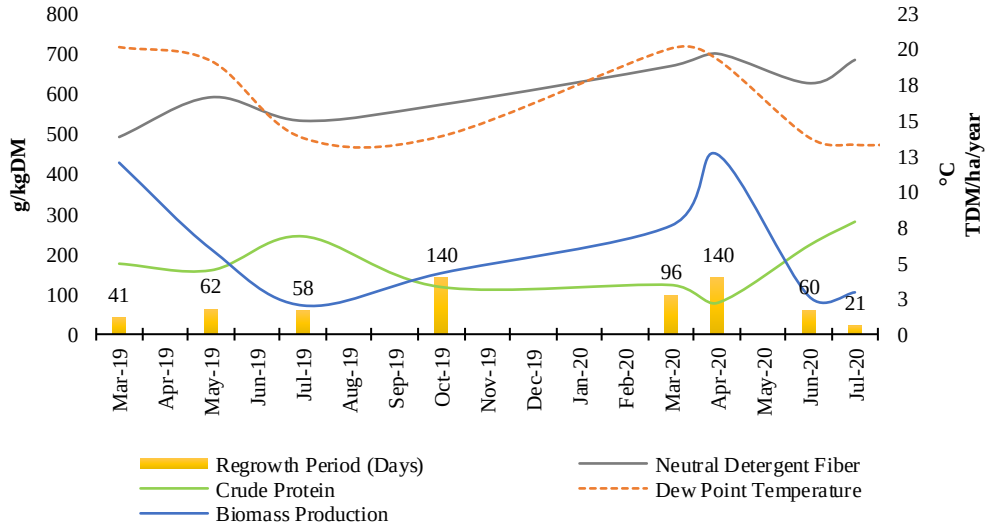

Figure 2. Nutritive composition and production behavior of Tithonia diversifolia in different ages and seasons in Southeastern Brazil.

Table 1. Pearson's correlations coefficients between climatic conditions, regrowth period and nutritional quality of *Tithonia diversifolia*.

Parameters	Dew point	Regrowth period
CP	-0.59***	-0.80***
NDF	-0.04	0.18
ADF	0.70***	0.30**
Ash	-0.69***	-0.63***
Biomass production	0.61***	0.54***

***: significant correlation at p-value < 0.001, **: significant correlation at p-value < 0.01, CP: crude protein, NDF: neutral detergent fiber, ADF: acid detergent fiber.

Shorter regrowth periods presented higher CP content which is clear based on the strong correlation observed for these parameters (Table 1), which is an important information for determining an optimal regrowth period for the system. During dry season, tropical grasses may have CP levels as low as less

than 70 g/kg, considered limiting for adequate ruminal fibrolytic activity and impaired microbial growth and reduced fiber degradation (Sampaio et al., 2010).
Tithonia showed a negative correlation between CP and dew point, it could be inferred that this plant is able to fulfill the nutritional needs of the animals fed on typical tropical grazing systems which are mostly dependent on tropical grasses, since Tithonia presented superior or equivalent nutritional value than that of these forages (Table 2) during most part of the year, especially during the dry seasons (Figure 1).

Conclusions

In Southeastern Brazil, Tithonia presented great potential for intercropping systems with tropical grasses for ruminant production, since it showed adequate nutritional value and good biomass production in seasons when grasses are not suitable for grazing by ruminants.

Table 2. Nutritive value of tropical grasses traditionally used in Southeastern Brazil according to season.

Grass forage	Season	Regrow[1]	CP	NDF	Reference
Brachiaria brizantha (Marandu)	Dry	27 d	88	703	Azar et al. (2011)
Brachiaria brizantha (Marandu)	Dry	35 d	137	623	Gerdes et al. (2000)
Brachiaria brizantha (Piatã)	Dry	28 d	73	742	Euclides et al. (2016)
Megathyrsus maximus (Tamani)	Dry	60-70 cm	165	649	Tesk et al. (2020)
Megathyrsus maximus (Tanzania)	Dry	35 d	153	663	Gerdes et al. (2000)
Megathyrsus maximus (Tamani)	Early wet	60-70 cm	85	700	Tesk et al. (2017)
Brachiaria brizantha (Marandu)	Wet	27 d	92	746	Azar et al. (2011)
Brachiaria brizantha (Marandu)	Wet	35 d	114	727	Gerdes et al. (2000)
Brachiaria brizantha (Piatã)	Wet	28 d	97	730	Euclides et al. (2016)
Megathyrsus maximus (Tamani)	Wet	60-70 cm	162	676	Tesk et al. (2020)
Megathyrsus maximus (Tanzania)	Wet	35 d	108	781	Gerdes et al. (2000)

[1] Regrowth period (days - d, sward height - cm); CP: crude protein (g/kg DM), NDF: neutral detergent fiber (g/kg DM).

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MULTITRAIT GENETIC EVALUATIONS OF AI BORN CROSSBRED OFFSPRING IN SRI LANKA FOR MILK AND CONSTITUENTS USING TEST DAY MODEL APPROACH

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Test day recording is essential to obtain data on milk component yield at field level where smallholder farmers have no recording equipment. A study was carried out on multiple trait estimation of genetic parameters and breeding values of dairy cattle on six traits (milk fat and protein yields, fat %, protein %, and electrical conductivity (EC) in milk) based on test day (TD) data in 3 districts of Sri Lanka. TD records were obtained with manual recording using Lactoscan analyzer by visiting all large Govt. farms (>150 cows) and smallholder farmers of 3 districts on monthly basis. After editing, a set of 2109 test day records with complete information on pedigree, birth date, parity, calving date, lactation length and parity of the animals with at least 3 TD data were used for Restricted Maximum Likelihood evaluations. A repeatability TD model (RPM) and a random regression model (RRM) based on Legendre polynomials (LP) were compared using Akaike's information criterion (AIC) and log likelihood ratio test (LRT). Herd-calving year-month group (fixed), TD group (random), and fixed LP regressions on age at calving and days in milk (both nested within parity) were used in both models. In addition REP model included animal and permanent environment (PE) effects while RRM included 3rd order random LPs for animal and PE effects.
Heritability estimates for the six traits under RPM were 0.28, 0.21, 0.28, 0.13, 0.38, and 0.002, respectively. EC showed negative genetic correlations with yield traits but positive estimates with constituent percentages. Sire rank correlations were significantly positive between the two models for all traits except for protein yield ($P<0.05$). The genetic correlation structure among traits and sire rankings show the need for implementing an index based selection to improve the economic traits simultaneously. With a superior fit (lower AIC and significant LRT ($P<0.05$)), random regression model can be recommended for future genetic evaluations of test day data at field level in Sri Lanka.

ADVANCES IN BIOTECHNOLOGIES FOR IMPROVING LIVESTOCK BREEDING AND FEEDING

IAEA-CN-281-110

DIVERSITY AND GENETIC ANALYSIS RELATED TO GASTRO – INTESTINAL PARASITIC RESISTANCE IN SHEEPS POPULATIONS FROM PERU AND SOUTH - AMERICA

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Gastro–intestinal parasitic infestations in small ruminants have severe consequences around the world specially in livestock farms in developing countries where productivity and profitability are seriously threatened by health system problems difficult to control only with anthelmintic drugs strategies. This study aimed to detect single nucleotide polymorphisms (SNPs) related to genes involved in pathways related to immune system associated with parasite resistance characteristics in Peruvian livestock, diversity analysis and the association of genotypes with host resistance characteristics against gastro–intestinal nematodes in the studied animals. A total of 119 SNPs were analyzed by endpoint allele discrimination PCR of DNA extracted from 52 blood samples from sheeps of both sex and Junin breed. Sampling was at the beginning of the dry season (May) and at the beginning of raining season (October). We analyzed the association of genotypes– phenotypes characteristics and physiological parameters: fecal egg count (FEC), body weight (BW), FAMACHA and packed cell volume (PCV) and the samples were classified in two candidate groups resistant and sensitive animals to gastro–intestinal nematodes. The diversity analysis was made with unrelated populations of different breeds all of them from South–America were as follows: Junin (Peru), Pampinta, Corriedale (Argentina), Cry. (Uruguay) and SAI (Brazil). Different diversity indices were calculated like genotype and allele frequency, expected heterozygosity and Hardy Weinberg equilibrium. The number of FEC were from 1430 to 4200 egg/g in sensitive animals and from 362 to 704 egg/g in resistant animals. In male animals the feces egg count increase during the rainfall as expected however, in female animals this indice decrease, it could be because the conditions of the pastures were different. Both males and females were grazing in different paddocks and the farm gave greater importance to males than to females and therefore males had better pastures especially in the rainy season. The average of PCV% was 40.43 (female animals) and 39,77 (males animals). The DNA extracted reached a purity of 1.85 A260/280 and a concentration of 31.32 ng/ul. 93 of 119 SNPs analyzed passed the quality allele controls and clustering in distinct genotypes plotted in X and Y axis respectively for each allele. Breeding programs focused on improving gastro–intestinal parasitic resistance in small ruminants could be an option to solve this problem and create a sustainable livestock system for developing countries.

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HAEMOCHUS CONTORTUS AND ITS HOST IMMUNITY

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Abstract

Interleukins (ILs) are related to innate and adaptive immune response of host against different diseases and parasites. In the current study, an association was checked between IL-α, IL-β, IL-3, IL-8, IL-32 and IL-33 gene single nucleotide polymorphism (SNPs; N=25) and resistance to gastrointestinal nematodes (GI), *Haemonchus contortus* (*H. contortus*) among different goat breeds; Yichang white (YCW), Hybrid yellow white (HYW), Nanjing yellow (NJY) and Enshi black goat (ENB). Ear tissue was collected for DNA extraction and fecal samples were collected for egg count (FEC). Association was determined under Chi-square (χ^2) between phenotypes, and SNPs by using PLINK software. Six SNPs in YCW, 4 in HYW and 3 SNPs in NJY had significant associations with FEC. YCW and HYW goat breeds had four while NJY had three missense mutations, which might be associated in with shifting gene function. The existence of these missense SNPs among three breeds suggests an association between SNPs and GI resistance. The findings of this study might be helpful for decreasing the parasitic infestation by making the immune system stronger and will be helpful to provoke further studies.

Keywords: Goat, Interleukins, *H. contortus*

Introduction

Goats are probably the first livestock to be domesticated, in the Fertile Crescent about 11000 years ago (Zeder, 2008). Around the world goats are reared for milk, meat, fiber and manure. In rural areas, they contribute to the livelihoods and well-being of their keepers and of local communities (Horcada, Ripoll et al. 2012).

The aim of this study was to identify single nucleotide polymorphisms (SNPs) in some candidate genes that are associated with immune function in goats which may help in the selection animals that are naturally resistant to nematode infections. We selected six functional candidate genes IL-α, IL-β, IL-3, IL-8, IL-32 and IL-33 which belong to the interleukin (IL) family and are signaling molecules of the innate and adaptive immune systems and may affect the control of *H. contortus*. The identification of resistance-associated genetic markers may contribute to the implementation of marker-assisted selection in animal breeding programs.

Material and Methods

Two hundred animals of four local goat breeds were selected from southern China. All goats were housed under uniform nutritional, managemental conditions and naturally exposed to *H. contortus*. The

McMaster (Ueno and Gonçalves 1998) technique was used for the counting of eggs. The genomic DNA kit (TianGen, Beijing, China) was used for the extraction of DNA from ear tissue. For detection of polymorphisms a DNA pool from 10 randomly selected DNA samples was used for PCR amplification.

Association studies, genetic models (genotypic, allelic, additive (Cochran–Armitage trend test), recessive and dominant) and haplotype association analysis for candidate genes were conducted by using FEC as the dependent variable. PLINK v1.06 software applications (Purcell *et al*., 2007) were used for the analysis.

Result and Discussion

In YCW goats, the SNPs in IL-α (3721), IL-β (3568) and IL-32 (1158, 2017, 7638 and 9375 were found significantly ($p<0.03$) associated with FEC (Table 1). In these goat breeds, all SNPs were present in exonic regions and missense substitutions were found in all except IL-32 (9375) and IL-β (3568), as shown in Table 1. Similarly, their allelic models and haplotypes were also associated with FEC.

Table 1: Association and comparison of significant SNPs of ILs with fecal egg count among different goat breeds

Yichang white					Hybrid yellow white					Nanjing yellow				
SNP	AM	H	χ^2	P	SNP	AM	H	χ^2	P	SNP	AM	H	χ^2	P
IL1β-3568	0.03*	TTG 0.002	4.6	0.031*	IL-8-2739	0.005*	GC,GG 0.008	7.8	0.005*	IL-3-146	0.01*		5.8	0.01*
ILα-3721	0.02*	TGC 0.01	5.3	0.021*	IL-33-63854	0.01*	GGT 0.02	5.7	0.01*	IL-α-10567	0.01*		5.9	0.01*
IL32-1158	0.02*	AAG,GGC 0.007,0.004	5.3	0.02*	IL-32-1158	0.004*	GAC 0.01	8.1	0.004*	IL-32-1158	0.003*	AGG 0.009	8.4	0.003*
IL32-2017	0.002*	AAG,GGC 0.007,0.004	9.4	0.002*	IL-32-7638	0.002*	GGG,GAC 0.01,0.05	9.2	0.002*					
IL32-7638	0.004*	GCA,AGG AAG,GGC 0.007,0.004	8.1	0.004*										
IL32-9375	0.012*	AGG,AGG 0.002,0.002	6.2	0.012*										

Note: * represent significance with (YCW $p<0.03$), (HWB $p<0.02$) and (NJY $p<0.01$) with FEC. Allelic model (AM), Haplotypes (H), Chi-square test (χ^2), Significance (P) and Single nucleotide polymorphism (SNP)

IL-8 (2739), IL-33 (63854) and IL-32 (1158 and 7638) SNPs, allelic models and haplotypes were found to be significant ($p<0.02$) with FEC in HYW goats. (Table 1). All the SNPs were present in exonic region and missense mutations, as shown in Table 1.

In NJY goats, IL-α (10567), IL-3 (146) and IL-32 (1158) SNPs were found to be significantly associated ($p<0.01$) with FEC (Table 1). These three SNPs were missense mutations (Table 1). The haplotype of IL-32 (1158) was found to be significant and the allelic models of the three SNPs were found to be significant with FEC.

Bressani *et al*. (2014) stated that an IL family (IL-2, IL-4, IL-13, and IFN-γ) was found to be significantly associated with resistance to GI and a missense mutation was found in the IFN-γ gene. The current study coincides with a previous study stating that IL SNPs were missense mutations and found to be significantly associated with FEC.

In the present study, IL-32 (1158) SNP has advantage over the others because it is found in three goat breeds and appeared to play significant role to enhance host immunity. In this study, YCW goats showed

6, HWB goat showed 4 and NJY goats showed 3 significant SNPs, respectively. In conclusion, YCW goat breed showed more significant missense SNPs than other breeds. Further investigation of the function of these genes, including their expression pattern, in large numbers of animals as well as the role of these mutations in vitro studies would improve understanding of their biological mechanisms in the control of GI.

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A GENOMIC ASSOCIATION STUDY OF GASTROINTESTINAL PARASITES IN GHEZEL SHEEP BREED

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Abstract:

This investigation is part of the International Atomic Energy Agency (IAEA) CRP projects on "Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity genetic resistance to nematodes in small ruminants". The present study was designed to identify association between a selected panel of SNPs in candidate genes and gastrointestinal nematode (GIN) infections. A total of 211 lambs were randomly selected in East Azerbaijan province, Iran. Nematode eggs were classified into four classes according to the species or group of species of origin. The sum of eggs in the four classes in each lamb's feces were reported as fecal egg count (FEC). FEC, FAMACHA test and packed cell volume (PCV) were measured Genotyping was performed at Seibesdorf Laboratory, Austria. Candidate genes selected for association were mostly involved in the KEGG-Pathways-Immune System. Preference was given to genes involved in innate immune system, particularly those coding for cell surface receptors involved in detection and binding of ligands, or PAMPs (Pathogen associated molecular patterns). This included all ten toll-like receptor genes, most of the Nod-like receptor, c type lectin binding receptor and RLR genes. Genes included in the MHC complex and cytokines (interferons, interleukins, etc.) were also selected. SNPs used in genotyping were those described in the literature. Association analysis was performed with the RepeatABEL package of R. A significant relationship was observed between SNPs in C-type lectin (CLEC1B) and NLRC4 genes and phenotypes of FEC, Strongyles and PCV.

Keywords: Fat-tail Sheep, Nematodes, SNPs, Association study.

Introduction

After China, Australia, India, Nigeria and Sudan, Iran ranks sixth in the world in the number of sheep with 40 million head. (FAO,2017). The number of sheep n in the world is 1.2 billion from which nearly 4% belong to Iran. Qizil or Ghezel, a fat-tailed sheep breed, is mainly reared in the North West regions of Iran and in the eastern and north-eastern parts of Turkey (as Morkaraman breed). The estimated population size of this breed is 17 million head (4 and 13 in Iran and Turkey, respectively) (Daskiran, 2014), respectively. Qizil sheep rearing in this area is mostly based on pasture and animals are challenged by gastrointestinal nematode infections (GIN). One of the strategies against GIN is anthelmintic treatment, but there are some reports about drug resistance of nematodes. Among GIN parasite control strategies, there is use of host genetic resistance. Some hosts have genetical mechanisms to resist GIN. There are three major reasons for selective breeding for parasite resistance: a) Helminthiasis, the disease caused by GIN is the most important livestock disease worldwide, b) Use of anthelmintic drugs is regarded as unsustainable due to emergence of multiple drug resistant parasites and c) Integrated parasite management strategies including selective breeding is an important long term

component objective to reduce the dependence on drugs for control of GIN. In the face of ongoing spread of anthelmintic resistance, there is increasing failure of existing control methods against GINs. McMahon et al., (2017) detected the reduced efficacy of benzimidazole, avermectins and moxidectin treatment in Northern Iceland. So, there is some issues about efficiency of existing anthelmintic methods for control of GIN. Drug resistance to Levamisole and Albandazole in sheep flocks of Iran have been reported as 66 and 27%, respectively. Moreover, nowadays the environmental problems associated with chemical control of parasites is notably important for consumers (Charlier et al., 2017). Breeding for genetic resistance of the host is among complementary investigated solutions to anthelmintic use (Pretson et al., 2014). The selective breeding for resistant animals is one of the successful strategies against GIN. The most utilized trait that has been used in classic selection of sheep is FEC. However, the use of FEC as an effective trait in sheep breeding programs has some limitations. One of the limitations is the necessity of animal infection by GIN and thereafter recording the FEC. So, interest in marker assisted selection for genetic resistance to GIN is increasing (Atlija et al., 2016). This investigation is part of the Coordinated Research Projects sponsored by IAEA on "Genetic Variation on the Control of Resistance to Infectious Diseases in Small Ruminants for Improving Animal Productivity genetic resistance to nematodes in small ruminants". The present study was designed to identify association between GIN and a selected panel of SNPs in candidate genes.

Material and methods

This study was conducted when animals were potentially at the highest risk of becoming contaminated with GINs. In total n=211 were selected among weaned lambs (age 4-6 months) in 10 flocks (nearly n=20 per flock) in East Azerbaijan province, Iran. The sum of eggs in the four classes in each lamb's feces were reported as FEC. Body weight, FEC, FAMACHA test and PCV were measured twice at one-week intervals. Nematode eggs were classified into four classes according to the species or group of species of origin: 1- EPGO: Strongyles including *Haemonchus contortus*, *Teladorsagia circumcincta*, *Ostertagia occidentalis*, *Trichostrongylus axei*, *colubriformis*, *vitrinus*, *and rugatus*, 2- EPGN: Nematodirus spp., 3- EPGT: Trichuris sp. and 4- EPGM: *Marshallagia marshalli*. FAMACHA is an ordinal trait with five levels. In the analysis, FAMACHA test was considered as a threshold trait, so scores of 1 and 2-5 recoded as 1 and 2, respectively. The phenotypic data were submitted to Box–Cox transformation to reach a nearly normal distribution. Candidate genes selected for association were mostly involved in the KEGG-Pathways-Immune System. Preference was given to genes involved in the innate immune system, particularly those coding for cell surface receptors involved in detection and binding of ligands, or PAMPs. This included all ten toll-like receptor genes, most of the Nod-like receptor, c-type lectin binding receptor and RLR genes. Genes included in the MHC complex and cytokines (interferons, interleukins, etc.) were also selected. A panel of eight sheep belonging to Asian and European breeds was used for targeted sequencing and SNP discovery. Hence, gene selection was based mostly on the information on genes and not on mutations. Most or almost all SNPs used for genotyping were newly identified on a panel of unrelated animals from different geographic locations from the breeds investigated by CRP. Genotyping was performed at Animal Production and Health Laboratory, Seibersdorf, Austria. The Identical by descent matrix was created based on the results obtained using PLINK 1.7. The results of genomic association study (GAS) were corrected for multiple comparison by FDR with normal and FLAT kinships. We examined two methods of analysis including with and without Flat kinship. Following descriptive statistics, the PROC GLM of SAS was used to identify the significant fixed effects. Phenotypic data included the traits with repeated measurements. GAS analysis was performed by RepeatABEL package of R. The statistical model was : $Y_{ijklm}= BW + S_i + T_j + F_k + SNP_l + e_{ijklm}$ Where y=each observation, BW=body weight as covariate, S=Sex (i=1,2), T=time (j=1,2), F=flock (1,…,10), SNP=SNP marker (l=1,…,156). A molecular relationship matrix was included in the model.

Results and Discussion

Descriptive statistics of phenotypic traits are shown in Table 1. The robustness was evaluated by bootstrapping kinship data and testing a flat kinship matrix. We present the results achieved by the flat kinship matrix as it yielded more significant tests. Significant SNPs for more than one trait showed that genes of CLEC1B-261-AG and NLRC4-918-AG control three phenotypes: FEC, PCV and EPGO. We corrected the results of GAS for multiple comparisons by FDR with normal and FLAT kinships. Only EPGT remained significate with normal kinship, while with the FLAT kinship the number of significant SNP increased dramatically among studied phenotypes (Figure 1). The GAS identified 39 genes located on 19 chromosomes. The number of significant SNPs (n=118) associated with the traits of EPGO, FEC, EPGN, EPGM, EPGT and PCV were 72, 16, 14, 9, 5 and 2, respectively. Some of genes had relationships with more than one trait. For example, C-type lectin domain family 1(CLEC1B) and NLR family, CARD domain containing 4 (NLRC4) had significant associations with PCV, EPGO and FEC. On the contrary, we found no significant SNP for FAMACHA. Figures 2 and 3 show the number of significant identified SNPs by chromosome number and gene symbols, respectively.

MacKinnon et al., (2015) showed evidence of greater parasite resistance in hair sheep breeds. They showed the high resistance of hair breeds is related to the immune system. Qizil are more similar to hair sheep than wool sheep from the viewpoint of fleece characteristics. Dominik (2005) suggested the combination of two methods of gene mapping and candidate genes to detect the genetic mechanism of GIN. Genetic selection for resistance to GIN cannot be utilized as a single method and it should used with anthelmintics (Moreno et al., 2017). Bhuiyan et al. (2017) investigated differential transcriptional outcomes for genes in goats. They reported BCL2, CD96, ITGA4, and ST6GAL1 as up-regulated genes. Considering the RNA-Seq samples in an experimental infection by Teladorsagia circumcincta, Chitneedi et al., (2020) reported the regions showing the highest variant density located on OAR6, OAR11, OAR14, OAR17, OAR20 and OAR22. The SNP in this study provide useful information for GIN genetic resistance prediction in extensive-sheep production systems. Qizil sheep breed have potentialfor the study of genetic resistance to GIN in natural infection situations. Before utilization of identified SNP in selective breeding programs against GIN, they should be defined in a suitable genetic-epidemiology model. Genes controlling the innate immunity system give resistance against a wide spectrum of parasites (Bishop et al., 2010) and we thus suggest to emphasize SNP identified from such genes for future study.

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Table 1: Descriptive statistics of phenotypic traits

Variable	N	Mean	SD	Minimum	Maximum
Body weight, Kg	379	28.29	5.09	16.30	39.00
PCV, %	351	32.44	3.36	18.00	41.00
FEC	319	159.71	190.99	25.00	1530.00
EPGN	351	99.41	103.87	25.00	830.00
EPGM	351	49.46	48.07	25.00	340.00
EPGT	319	26.75	9.11	25.00	112.50
EPGO	351	71.14	99.32	25.00	637.50

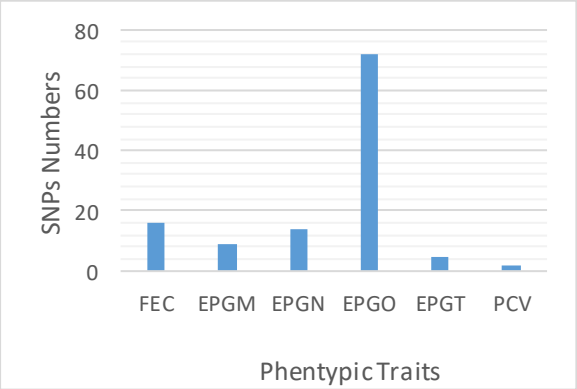

Figure 1: Frequency of SNPs by phenotypic traits.

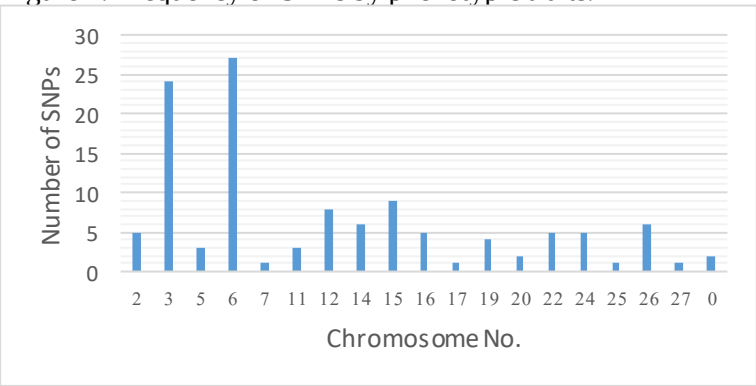

Figure 2 Number of significant identified SNPs by chromosome number. For abbreviations see the material and methods.

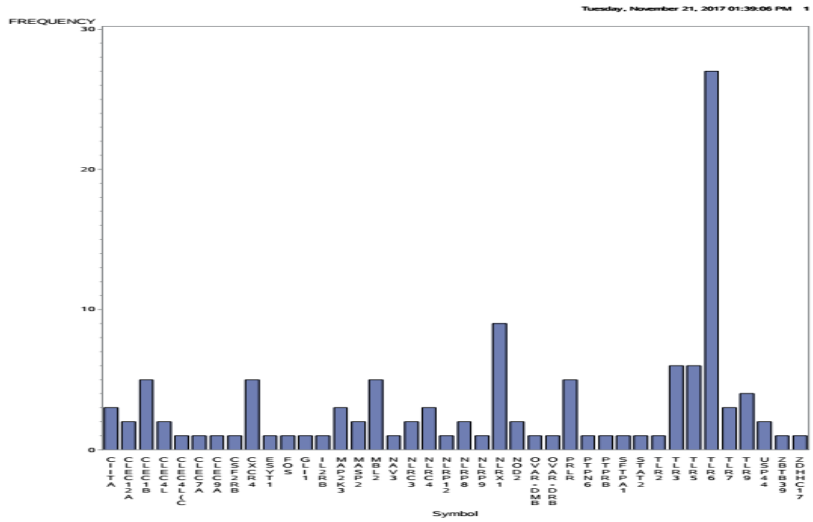

Figure 3 Frequency of Gene Symbols

IAEA-CN-281-151

ARTIFICIAL INSEMINATION MONITORING, GENOMIC SELECTION OF BULLS AND BREEDING EVALUATION IN TUNISIA

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A total of 668 cows were used in this study, they belong to OTD (Tunisian Office of that State Lands) and distributed in 6 farms located at different area in Tunisia. Dairy checks were be carried out (number = 18) and the criteria to be determined were Quantity, Quality, SCC and Mastitis. Several milk production was between 5000 and 7000 kg (n=328) and it was evaluated depending the father of the female 26 father that have more than 10 daughters). Reproductive parameters were saved from two sources of information (Computer Software and Individual Card) those data allowed the determination of fertility and fecundity criteria(Calving Interval, Calving First AI Interval, Calving Conception Interval) Those parameters were analyzed according to father of female factor. Blood sampling for DNA extraction was established. A total of 186 exactly samples were realized from 186 cows according to their father. The number of females per father is variable and generally is more than 10 daughters per male. EBCs will be estimated and traditional and imported EBVs for sires will be compared

IAEA-CN-281-159

PERFORMANCE OF MECHERI LAMBS INTROGRESSED WITH FECB GENE IN TROPICAL CLIMATIC CONDITIONS OF TAMIL NADU, INDIA

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Mecheri sheep is one among the recognized breeds of sheep of Tamil Nadu, India and are reared mainly for meat and the primary by-product is skin (1). They are known for their skin quality and higher dressing percentage and better adaptability to the harsh climatic conditions. To improve fecundity in Mecheri sheep, a genetic improvement programme on introgression of FecB gene has been implemented. The genetic introgression of FecB gene was made in the year 2013 and the production of F_1 animals was achieved through crossing purebred Mecheri sheep (n=87) with crossbred rams (using six rams carrying homozygous (BB) genotype). The F_1 crossbred Mecheri ewes were again mated with the foundation stock rams to produce F_2 crossbred.

Data on performance of 236 Mecheri crossbred lambs (131 males and 105 females), maintained at Mecheri Sheep Research Station (MSRS), Pottaneri, Salem, Tamil Nadu, India were collected over a period of four years (2013- 2016). The body weights of crossbred lambs at different ages i.e., at birth, weaning (three months), 6, 9 and 12 months were collected from the birth and growth registers maintained in the farm. The data were classified according to period, season, type of birth, generation, parity of dam and sex and analysed using least-squares analysis of variance (2). In addition, the data on mortality, causes of death and disposal particulars have been collected and analysed using standard statistical procedures.

The overall least-squares means for body weight of crossbred Mecheri lambs at birth, three, six, nine and 12 months of age were 2.296 ± 0.04, 9.60 ± 0.23,13.72 ± 0.32, 15.62 ± 0.32 and 18.95 ± 0.37 kg respectively (Table 1). The study on non-genetic factors on body weight at different ages revealed that the body weight observed in F_1 generation were higher than those observed in F_2 generations in all the age groups and were significant ($p<0.05$) at birth and three months of age.

The sex of the lamb had a significant ($p<0.05$) effect on body weight at six, nine and 12 months of age. Comparison of the least-squares means for lamb weights at different ages showed that the difference between male and female lambs increased from 0.043 kg at birth to 4.41 kg at 12 months of age. The difference in body weight between male and female lambs with the advancement of age might be due to the increasing differences in the endocrine system between males and females. These sex differences are consistent with results from other investigations (3; 4).

Table 1. Least-squares means (±SE) for body weight of Mecheri crossbred lambs

Effects	N	Birth	Three months	Six months	Nine months	12 months
Overall	**236**	2.296 ± 0.04 (236)	9.60 ± 0.23 (161)	13.72 ± 0.32 (109)	15.62 ± 0.32 (95)	18.95 ± 0.37 (63)
Generation		*	*			
F1	199	2.420 ± 0.05[b] (199)	10.022 ± 0.25[b] (141)	13.82 ± 0.33 (104)	15.75 ± 0.34 (90)	19.07 ± 0.37 (62)

F2	37	1.883 ± 0.08[a] (37)	7.97± 0.54[a] (20)	12.57 ± 1.27 (5)	14.42 ± 1.19 (5)	16.30 ± 0.00 (1)
Sex				*	*	*
Male	131	2.317 ± 0.05 (131)	9.80 ± 0.32 (78)	14.60 ± 0.52[b] (38)	16.12 ± 0.59[b] (27)	22.40 ± 0.78[b] (14)
Female	105	2.274 ± 0.06 (105)	9.40 ± 0.32 (83)	13.11 ± 0.41[a] (71)	15.41 ± 0.39[a] (68)	17.99 ± 0.42[a] (49)
Type of birth		**				
Single	203	2.471 ± 0.05[a] (203)	9.75 ± 0.26 (135)	13.98 ± 0.36 (95)	15.93 ± 0.35 (83)	19.36 ± 0.40 (55)
Twin	33	1.926 ± 0.07[b] (33)	9.16 ± 0.47 (26)	12.97 ± 0.72 (16)	14.55 ± 0.80 (12)	17.48 ± 0.91 (8)
Year						
2013	33	2.559 ± 0.11 (33)	9.31 ± 0.50 (31)	14.87 ± 0.59 (31)	16.40 ± 0.60 (30)	19.90 ± 0.54 (27)
2014	83	2.433 ± 0.08 (83)	10.03 ± 0.35 (81)	12.88 ± 0.47 (57)	15.21 ± 0.47 (46)	18.25 ± 0.53 (33)
2015	49	2.184 ± 0.07 (49)	9.91± 0.48 (31)	13.44 ± 0.71 (20)	15.31 ± 0.68 (19)	17.60 ± 1.27 (3)
2016	71	2.136 ± 0.07 (71)	8.85 ± 0.56 (18)	17.30 ± 0.00 (1)	-	-
Season		**				
Off season	64	2.049 ± 0.07[a] (64)	8.93 ± 0.44 (38)	13.34 ± 0.81 (16)	14.87 ± 0.86 (11)	19.73 ± 0.90 (16)
Main Season	172	2.438 ± 0.05[b] (172)	9.90 ± 0.26 (123)	13.79 ± 0.35 (93)	15.73 ± 0.35 (84)	18.92 ± 0.38 (57)
Parity		**				
First	104	2.018 ± 0.06[a] (104)	8.94 ± 0.36 (70)	13.03 ± 0.54 (48)	15.41 ± 0.55 (42)	17.62 ± 0.62 (28)
Second	70	2.224 ± 0.08[ab] (70)	8.88± 0.51 (47)	13.57 ± 0.62 (31)	15.14 ± 0.58 (28)	17.36 ± 0.85 (19)
Third	38	2.434 ± 0.09[ab] (38)	9.91 ± 0.53 (26)	14.61 ± 0.78 (18)	15.43 ± 0.76 (16)	19.53 ± 1.03 (10)
Fourth	8	2.808 ± 0.18[b] (8)	10.67 ± 0.88 (6)	14.45 ± 1.53 (4)	16.95 ± 1.43 (3)	21.02 ± 1.10 (2)
Fifth	8	2.625 ± 0.17[ab] (8)	10.07 ± 0.78 (6)	14.11 ± 1.06 (4)	14.63 ± 1.09 (3)	19.91 ± 1.03 (2)
Sixth	8	2.588 ± 0.17[b] (8)	11.42 ± 0.87 (6)	13.40 ± 1.14 (4)	18.05 ± 1.35 (3)	20.25 ± 1.27 (2)

Figures in parentheses are number of observation. * (P<0.05); ** (P<0.01).

The lambs born as single had higher body weight than those born as twins and weight was significantly (p<0.05) different at birth. The parity had highly significant (p<0.01) effect on body weight at birth alone. In general, the body weight increased with the advancement of parity and the lambs born in 3rd and 4th parities had higher body weight at different ages. The significant difference in parity of the dam on the birth weight of lambs was also reported earlier (5) in Mecheri sheep.

The Overall least-squares mean for body weight gain from birth to weaning, weaning to six months and six month to 12 months of age were 81.62 ± 6.77, 43.10 ± 7.33 and29.01 ± 6.48 g respectively. In general, the efficiency of growth measured in terms of the gain in body weight per kg of initial weight decreased with advancing age. The reduction in growth efficiency during post-weaning development as against the pre-weaning stage indicated that the maximum growth rate had occurred during the pre-

weaning stage.

A total of 39 crossbred lambs died at various ages and the percentage of lambs died due to acute bloat, enteritis, plant poisoning, pneumonia, debility, poor birth weight, hooked tooth, toxaemia, hepatitis, impaction due to polythene bag obstruction, intestinal rupture and enterotoxaemia 5.1, 12.8, 5.1, 2.6, 2.6, 28.2, 17.9, 5.1, 2.6, 5.1, 2.6 and 10.3% respectively and the highest mortality was observed due to poor birth weight. A total of 109 lambs were sold during different age groups and majority of them were sold by slaughter sale (69.7%) and the remaining (30.3 %) were sold for breeding purpose.

The tupping (based on total ewes allowed), lambing (based on total animals mated)and twinning percentages observed (based on total ewes lambed) in Mecheri crossbred ewes carrying FecB gene (both homozygous and heterozygous) were 68.49, 78.04 and 32.25 per cent respectively and the average litter size at lambing was 1.32.

In general, introduction of crossbred Mecheri lambs to the farmer's field conditions revealed leastpreference with the local farmers due to the deterioration of the skin and meat qualities and hence, the crossbred lambs fetched lower prices, when compared to the purebred animals. Under the low-input production system, the survivability of the crossbred lambs was low due to poor birth weight and lesser mothering ability of the ewes due to poor milk yield. As a result, the crossbreeding programme has been discontinued and pure breeding is recommended, both in station and in the field for further genetic improvement. Selective breeding of the Mecheri sheep with conventional as well as molecular markers have been suggested for faster genetic improvement. In the future, before starting any new genetic programme, a thorough analysis of the consumer preference towards rearing of the crossbred sheep in its native breeding tract is needed.

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DEVELOPMENT OF A HIGHLY RESISTANT OVINE RACE TO GASTROINTESTINAL PARASITES (PGI), "PRELIMINARY STUDY"

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Abstract
In this study, with the data obtained to date, it was determined that gastrointestinal parasites play an important role in sheep productivity and whose resistance will depend on the breed (Pure, Cross animals or Creoles), and that the productive and reproductive capacities be maintained at In spite of the parasitic load and with the use of the lowest degree of dewormer, and instead of these dewormers, food supplements should be used to promote resistance characteristics.

Keywords: Gastrointestinal parasitic, FAMACHA, body condition, phenotypic and genotypic

Introduction

The sheep population in México represents about 8.9 million animals and 8.7 million goats (SIAP, 2017), while the introduction of meat products has decreased by 74%, but this does not meet the needs of the domestic market, despite this meat imports remain at 34% (Desdémona, 2019). The growth of the sheep population has been maintained although in a very sustainable way. However, technification in sheep production has not been enough; There are mainly reproductive and productive problems, despite the great variety of pure species that have been introduced; parasitic diseases constitute the main biological factor that has prevented the emergence of this species in production as a staple food of the population. The high dose of dewormer that is administered to animals has produced resistance to many gastrointestinal parasites (GIP) mainly from *Haemonchus contortus, Trichostrongylus spp* and *Strongyloides spp* (Caicedo *et al.*, 2018), it can be added that parasitic diseases constitute the a disease that produces more economic damage (Alba-Hurtado and Muñoz-Guzmán, 2013) and that ***Haemonchus contortus*** constitutes the gastrointestinal parasite that causes the most alterations in sheep worldwide (Gasser *et a*l., 2016). The objective of this study was to detect the prevalence of Gastrointestinal parasites (GIP) in sheep from different ecological zones in the State of Puebla and Tlaxcala-México.

Materials and methods

Animals: 620 male and female sheep of different races (Pure, Cross animals and Creoles) were used, the predominant race was the Katahdin and Dorper and the same from different zoogeographic areas of two States (Puebla and Tlaxcala).
Phenotypic characteristics: to detect the effect of parasites, three parameters were used to detect the phenotypic characteristics of each animal (FAMACHA)
Parasitic Load (Eggs/grams): determined by animal during one year of sampling, the FAMACHA method was used (it measures the degree of anemia of an animal, when it is infested by the parasite

Haemonchus contortus); The parasitic load was measured by the number of eggs detected per grams of feces in the modified Mac Master Chamber. The feces were subjected to a saturated NaCl concentration (30 ml per-1 gram of feces).
Statistical analysis of the data obtained: The data were correlated with Excel 2010 and Stata-15 and for the analysis of averages the ANOVA.

Results

The FAMACHA data obtained were 3,166 ± 0.915, correspond to the values in Creole sheep, these animals do not show significant damage, the females have normal estrous cycles and their young are kept in ideal conditions, the important thing is to deworm, despite the load parasitic to ***Haemonchus contortus*** and ***Strongylus*** *spp* (hpg) does not decrease, despite the use of medications, while FAMACHA values of 3,016 ± 0.5 in the Dorper breed, these animals are more susceptible to *Haemonchus contortus*, but animals are dewormed in time they fall into metabolic problems and die; In the Katahdin breed, the FAMACHA value obtained was 2.13 ± 0.44; the parasite load of this breed is very low; hematocrit values are kept low in Creole animals and in animals of the Dorper breed ($p < 0.05$), while in the Katahdin race the values fluctuate based on the type of parasite; On the other hand, many independent animals of the breed are extremely susceptible to the ruminal parasite called ***Oesophogastomum*** *spp*. Several herds presented this parasite during the beginning of the rainy season. According to data that were obtained in a sampling year.
The data shows that the parasite most often is ***Haemonchus contortus***, however sheep die from having degrees of conjugate parasitic load that is with other types of parasites.

Discussion

The values found in this work are consistent with Castells (2009) and Goldberg (2010), in which they found *Haemonchus contortus* as GIN mostly primordial in their sampling and the damage it causes to animals is significant, despite this, it is important to correlate these phenotypic data found (parasitc load-FAMACHA) with the analysis and genotypic characteristics of each animal (Dlamini *et al.*, 2019), in order to detect the reason for parasitic resistance to the different gastrointestinal parasites (GIP), in each breed of animal studied (pure animals, crossed animals and animals creole).

Conclusions

There are selected animals that showed resistance to *Haemonchus contortus*. Deworming should be supervised by trained personnel and monitor and strengthen the immune system of each animal by administering multivitamins to animals to improve their health status and the development of a breed resistant to gastrointestinal parasites (GIP), through the comparison of phenotypic and genotypic information of each animal.

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BREEDING FOR SHEEP PARASITE RESISTANCE IN EXTENSIVE PRODUCTION SYSTEMS: FROM PHENOTYPE TO GENOTYPE

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Worldwide, gastrointestinal parasites (GIP) generate numerous productive and economic losses in sheep production and Uruguay does not escape to this problem. Due to the aggravating situation of anthelmintic resistance to all drugs available in the market, the use of non-chemical alternative strategies is essential to address the problem of GIP. For this reason, commercial producers who raise their sheep in temperate and subtropical areas under extensive systems and the breeders (that provide genetics), would like to consider genetic resistance to GIP in their selection objective. In Uruguay, genetic evaluations of wool and meat quality and production traits are routinely carried out by the Uruguayan Secretariat of Wool (SUL) and the National Institute of Agricultural Research (INIA) (www.geneticaovina.com.uy). Since the beginning in 1994, genetic evaluations of the Merino and Corriedale breeds have included Fecal Egg Count per gram (FEC) as the selection criterion. The national approach has been to contribute to the selection of genetically resistant animals within an integrated control of parasites. However, as it is a difficult characteristic to record, different strategies have been included to augment genetic improvement of resistance to GIP, which are described below.

Support for recording and new criteria

Given the reluctance of producers to collect data, different projects had been working to support the beginning of recording. As an example, we can mention INIA and Inter-American Bank financing for the development of recording for Corriedale (2002) and Australian Merino (2004). More recently, coordination and registration are being done directly on Corriedale (2018) and Merilin (2020) stud flocks, within an INIA project in conjunction with the breed societies. Currently, the genetic evaluation is consolidated in the Merino breed and growing in Corriedale. Table 1 reports the information in the national database (SULAR) within the genetic evaluation system (INIA-SUL) collected in the last four years, in Table 2 the total data within the system.

Table 1: Number of animals with FEC registration, according to birth year

Breed	2014	2015	2016	2017
Corriedale	1211	1079	1301	1451
Merino	726	1298	1400	1397
Total	**882**	**2435**	**2796**	**3133**

**FEC: Fecal egg count post weaning.*
PCV: Packed cell volume.
BCS: Body condition score

Table 2: Total data in SULAR related to GIP resistance

Breed	Trait*	N	mean	sd
Corriedale	BCS at FEC	1394	2.74	0.6
	FAMACHA	3077	2.28	0.8
	FEC	21772	1530.07	2319.66
	PCV	2495	35.27	4.75
Merino	BCS at FEC	230	2.81	0.39
	FAMACHA	1590	2.11	0.85

	FEC	25700	1279.08	1967.7
	PCV	1356	32.05	5.41

Estimated FEC heritability in lambs is moderate, with values ranging from 0.2 to 0.4 in agreement with the international studies (e.g. review by Safari et al., 2005), which would allow genetic progress for this trait. Due to the described efforts in registration, it has been possible to estimate the FEC heritability for the main breeds in production systems under natural infestations. These estimates are between 0.15 ± 0.01 for Merino (Ciappesoni et al, 2013) and 0.21 ± 0.02 for Corriedale (Castells, 2009). Studies have also been conducted to relate the nematode resistance in periparturient ewes and post-weaning lambs in Uruguayan Merino sheep (Goldberg et al., 2012). Currently, the study of the spring rise phenomenon continues in different breeds and production systems (Del Pino et al., 2019). Furthermore, different studies have been carried out looking for new, easy-to-measure or complementary selection criteria such as: FAMACHA © (Ciappesoni & Goldberg 2018), fecal occult blood test (FOB) (Rodriguez et al., 2015), control of IgA levels (Escribano et al., 2019) and dag score (RUMIAR project, INIA).

Generation of resistance selection nucleus

As a strategy for the generation of genetically resistance breeding stock and the dissemination of genetic tools (i.e. EBV), selection nuclei are in place in experimental stations: (1) Corriedale: divergent selection lines by FEC EBV (from 2000, SUL Cerro Colorado); (2) Merino: selection by FEC EBV and production (since 2015 FAgro-Udelar - San Antonio); (3) Corriedale: selection by FEC EBV and production (since 2017 INIA Glencoe).

Development and use of molecular tools

Initially, many countries aimed to use genetic markers to identify alleles associated with resistance to GIP and to select for breeding young animals carrying these alleles. Marker association studies were also carried out in Uruguay using microsatellite molecular markers (STRs) in the Corriedale (Nicolini, 2006) and Merino (Ciappesoni et al., 2010) breeds. A low-density panel of 507 SNPs associated with GIP resistance and useful for parentage testing (Peraza et al., 2019) was also developed in Uruguay. Nowadays, the genomic selection is the preferred approach, particularly for expensive or difficult to measure traits such as FEC. By using single nucleotide polymorphism (SNP) information (i.e. SNP type molecular marker panels of different density) together with phenotypic and pedigree information, it is possible to increase EBV accuracy in young animals. Currently, several countries are implementing genomic selection in sheep, such as New Zealand, Australia and France. In Uruguay, particularly in the Merino breed, the reference population is being developed with aim of publishing the first genomic EBV for FEC in 2020. Currently DNA samples of more than 8000 Merino animals with FEC phenotypes are stored in the National DNA Bank of INIA. In addition, there are currently more than 1200 animals genotyped with different arrays (15k and 50k), with 3000 animals planned to be reached in 2021.
Additionally, SNP panels of different densities have been useful for breed genetic characterization and population structure analysis. Several studies have been carried out with the Uruguayan Merino, Corriedale and Creole breeds (Grasso et al., 2014), Australian Merino and other related breeds (Vera et al., 2019; Ceccobelli et al., 2019). Likewise, mechanisms involved in genetic resistance to GIP in Corriedale were investigated using RNA sequencing (Peraza et al., 2016). We plan to include selection signature studies in Corriedale breed using divergent selection lines.
In summary, several tools to improve the genetic resistance of sheep to GIP have been developed and further research is ongoing with the aim of increasing their effectiveness and contributing to greater economic benefits and more sustainable production systems. Research and innovation initiatives have been made possible due to the coordinated work of research and knowledge transfer institutions, with the support of breeders' associations.

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APPLICATION OF GENOMIC TOOLS FOR GENETIC IMPROVEMENT OF CROSSBRED FRIESIAN CATTLE IN BANGLADESH

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Background

Productive and reproductive performances are crucial factors determining the profitability of dairy farming. In Bangladesh, limited growth in dairy farming is due to poor genetics of the cattle. Artificial insemination (AI) has been practiced as a breeding tool to improve genetics of adopted indigenous cattle by using semen of exotic bulls from temperate countries since 1950s. AI activities in Bangladesh have resulted in production of more than 3 million crossbred cattle in the country. However, the impact of AI on increasing milk production remained far below the expectations of stakeholders. About 6.5 million AIs were performed in cattle in Bangladesh in 2018-19 among which 4.13 million were performed by the Department of Livestock Services (DLS) of Government of Bangladesh (Annual Report, 2018-2019). Currently AI services can cover about 50% of breedable cows in Bangladesh. The post-AI conception rate in smallholder cows was reported to be 51% (Siddiqui et al., 2013). Among others, the conception rates varied widely according to the skill of the AI technicians (Siddiqui et al., 2013). There is lack of proper recording system in AI programme. Moreover, there is no identification of cattle at farmers' level and absence of record keeping on performance traits. Poor heat detection was also indentified as an important limiting factor for conception. In a study using progesterone RIA, Shamsuddin et al. (2006) observed that oestrus was accurately detected in 30% of cases. Another 30% of cows were detected as in oestrus when they were not (false positive) and 40% cows remained undetected when they were in oestrus (false negative). Poor oestrous detection has also been reported in buffaloes, where the cyclicity was evaluated by determining progesterone in milk by using ELISA (Banu et al., 2012). Considering the above mentioned facts, it is important to indentify the best dam and sire with respect to performance traits using genomic tools for production of improved crossbred Friesian cattle in the country.

Objective

To determine the parental admixture of genome and their performances in crossbred Friesian cattle in selected population bred by AI in Bangladesh.

Methodologies

A total of 1000 phenotypically looked crossbred Friesian cows from public and private commercial farms and 25 crossbred Friesian bulls routinely used for AI by Central Cattle Breeding and Dairy Farm (CCBDF), Savar, Dhaka, Bangladesh were selected. Ear tags were used in all selected cows of private farms for animal identification and no tag was used in public farms as it was routinely used there. Phenotypic data on productive and reproductive performances were collected from all selected cows at 1-2 months interval using questionnaire. Single blood sample (~5 ml) from each selected cow and 25 bull were collected for DNA extraction. Extracted DNA samples were stored at -20°C or -80°C until analysis for admixture in laboratory of IAEA using 60K SNP. The data are supposed be analyzed using appropriate statistical methods.

Results

Collection of production and reproduction performance related data have been going on from 1000 crossbred Friesian cows. Blood samples from 846 crossbred cows and 25 breeding bulls have been collected for DNA extraction. DNA samples have been extracted from 25 bulls and 139 cows. DNA samples are supposed to be extracted from all selected cows and sent to IAEA laboratory for analysis of admixture of genome by June 2020.

Conclusions

Cattle identification system will be in place and performance data of 1000 crossbred Friesian cows will be available in Bangladesh. Gene bank of phenotype recorded cattle will be established and genetic tool(s) for testing parentage and admixture level will be available. The research work is in progress.

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RUNS OF HOMOZYGOSITY REVEAL MODERATE LEVELS OF INBREEDING IN LOCAL CATTLE POPULATIONS IN SOUTHWESTERN BURKINA FASO

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Introduction

Inbreeding refers to mating of parents who share one or more ancestors (Curik et al., 2014). Understanding and control of inbreeding are key factors of genetic improvement strategies because increasing inbreeding reduces genetic variation and leads to inbreeding depression (Ferenčaković et al., 2013a). Three community-based cattle breeding programs are being implemented in the southwest of Burkina Faso and aim to increase body size and improve trypanotolerance. In this area, reproduction is characterized by uncontrolled mating. According to responses of cattle owners, young males from the own herd are a main source of breeding bulls, making the systems potentially highly susceptible to inbreeding depression (Ouédraogo et al., 2020).
The goal of this study is to estimate the levels of inbreeding of the breeding populations using runs of homozygosity (ROH) derived from medium density single nucleotide polymorphism (SNP) markers. ROH provide reliable indicators of inbreeding level in systems in which pedigree recording is missing (Ferenčaković et al., 2011, 2013a, 2013b).

Material and methods

A total of 658 animals were genotyped using the Illumina Bovine SNP 50K bovine BeadChip, featuring 53,714 SNP. These animals were part of breeding programs implemented in three production systems in the area: sedentary pure Baoulé system (SPB), sedentary mixed breed (SMB) and transhumant Zebu and crossbred systems (TZC) (Ouédraogo et al., 2020). Quality control was performed using Plink. 1.9 (Chang et al., 2015). After quality control, the total genotyping rate in remaining samples was 0.97729. A total of 990 SNPs were removed due to missing genotype data and 3,407 SNPs due to disequilibrium with the Hardy-Weinberg exact test ($p<0.1e-6$). After filtering, 38,207 SNPs and 631 animals were included in the analysis. Among the 631 animals, 343 came from SPB, 156 from SMB and 132 from TZC systems, respectively.

R code (version 3.5.2) and functions were used to perform the summary statistics and cgaTOH (Zhang et al., 2013) was applied to compute the genomic inbreeding coefficients. The genomic inbreeding coefficient of each individual was calculated as $F_{ROH}=\sum L_{ROH}/L_{AUTOSOME}$ where L_{ROH} is the total length of all ROH in the genome of an individual, where the regions contain the minimum specified length of segments containing successive homozygous SNPs, and $L_{AUTOSOME}$ refers to the specified length of the autosomal genome covered by SNPs on the chip (Curik et al. 2014). Differences of inbreeding levels between production systems were tested with Kruskall-Wallis, pairwise comparisons were performed with Wilcoxon tests.

Results and discussion

ROH at different length categories (>1Mb, >2Mb, >4Mb, >8Mb, >16Mb) were analyzed for the three populations to detect autozygous segments over the whole genome. Figure 1 describes the average length of ROH at different categories for ROH segments >2Mb and >8Mb. The length decreased substantially with increasing minimum ROH length, which is in agreement with expectation and previous findings in Fleckvieh, Holstein, Polish Red, Limousin and Simmental cattle (Ferenčaković et al., 2011; Szmatola et al., 2016), while within length categories, differences between production systems were small.

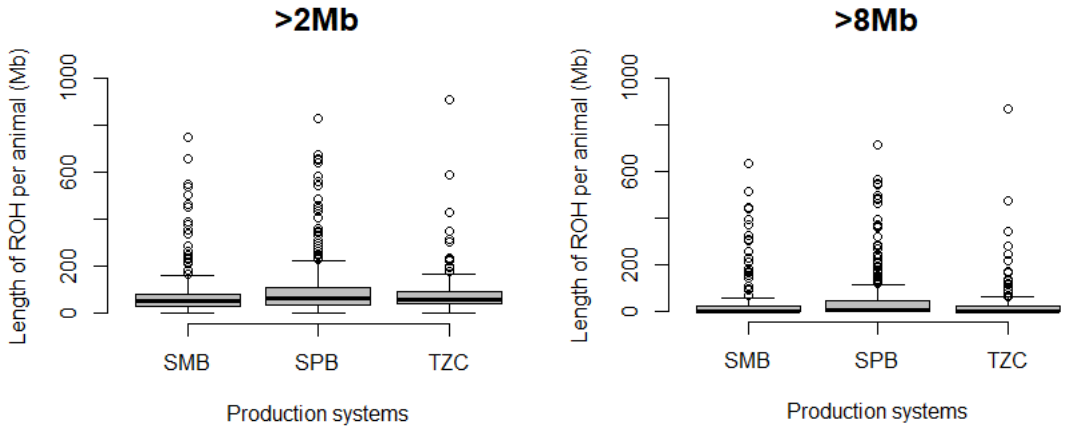

Figure 1. Box and Whisker plot of total length of ROH for >2Mb and >8Mb length categories (SPB: Sedentary pure Baoulé, SMB: Sedentary mixed breed, TZC: Transhumant Zebu and crossbred)

The genomic inbreeding coefficients of individual animals at different ROH lengths are presented in Table 1. Genomic inbreeding coefficients (F_{ROH}) ranged from 0% to 40.40%. Comparing to well managed European cattle breeds, F_{ROH} in this study were lower than observed in Brown Swiss and Holstein, close to what was reported for Fleckvieh, Norwegian Red and Tyrol Grey and higher than found in Polish Red, Limousin and Simmental (Ferenčaković et al., 2013a, 2013b; Szmatola et al., 2016).

Table 1. Genomic inbreed coefficients

	SPB		SMB		TZC	
	Mean (SD)	Range	**Mean** (SD)	Range	**Mean** (SD)	Range
F_{ROH1}	**0.106** (0.056)[a]	0.014-0.375	**0.093** (0.056)[b]	0.009-0.361	**0.098** (0.045)[c]	0.020-0.404
F_{ROH2}	**0.042 (**0.05)	0.000-0.332	**0.039** (0.051)	0.000-0.301	**0.037** (0.043)	0.001-0.364
F_{ROH4}	**0.027** (0.048)[a]	0.000-0.311	**0.024** (0.049)[b]	0.000-0.277	**0.020** (0.043)[ab]	0.000-0.357

F_{ROH8}	**0.021** (0.043)[a]	0.000-0.287	**0.019** (0.045)[b]	0.000-0.255	**0.014** (0.040)[b]	0.000-0.349
F_{ROH16}	**0.016** (0.037)	0.000-0.252	**0.015** (0.039)	0.000-0.252	**0.011** (0.036)	0.000-0.341

SPB: Sedentary pure Baoulé, SMB: Sedentary mixed breed, TZC: Transhumant Zebu and crossbred, SD: Standard Deviation.
[a],b,c: FROH not sharing superscripts within the same row indicate significant difference at $P < 0.05$. FROH were not significantly different in rows without superscripts.

Production systems were significantly different ($P<0.05$) for F_{ROH1}, F_{ROH4} and F_{ROH8}, yet, differences were quite small. This was not according to expectation, as lowest levels of inbreeding were observed in herds of transhumant farmers who claimed to only use bulls raised in their own herds. The moderate levels of inbreeding in this area despite the use of bulls from the herds could be due to a relatively short time of bull use, limiting mating of related animals. Indeed, only one animal each (i.e. <1%) in the three systems was likely a product of parent-offspring or full sib mating ($F_{ROH8}>0.25$) and 3-5% were likely products of half sib mating. A similar study in African goats showed a substantially lower inbreeding coefficients ($F_{ROH2}=0.037$, average over a range of populations) contrary to what was expected in populations in which breeding is not controlled (Nandolo et al., 2019).

Conclusion

This study was performed to assess the inbreeding levels of cattle in different breeding programs in Burkina Faso. Our results indicate moderate levels of inbreeding, comparable to some well managed European dairy and beef cattle breeds. While the management system with frequent use of bulls derived from the own herd, according to farmer reports, would suggest high levels of inbreeding, animals that were likely products of parent-offspring or full sib mating were very rare. Yet, in the context of breeding programs, attention should be paid to inbreeding levels. Upon implementation of community-based breeding programs, farmers have been encouraged to exchange selected bulls to minimize the mating of related animals.

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GENOME-WIDE ASSOCIATION STUDY OF TRYPANOSOME PREVALENCE IN BAOULE CATTLE OF BURKINA FASO

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Introduction

The sequence of the bovine (*Bos taurus*) genome has been available for some time now (Bovine Genome Sequencing and Analysis Consortium, 2009). With the advent of high throughput genotyping technologies, the discovery of cattle single nucleotide polymorphisms (SNPs) and the development of commercial cattle SNP chips with many thousands of polymorphic markers have become straightforward. SNP chips can be used for genome wide association studies (GWAS) to find SNPs that are associated with traits of interest. The main purpose of a GWAS is to identify chromosome regions that harbor the gene(s) that contribute to the phenotypic variation of a trait, which then could serve as putative regions for further studies (Sahana et al., 2010) .
In this study we focus on the discovery of regions affecting trypanosomosis in Baoule cattle of Burkina Faso. The Baoule cattle have existed in tsetse challenged zones for a long time and acquired an immunology phenomenon (trypanotolerance) that has a genetic basis. These animals have a capacity to rid themselves of trypanosome parasites and maintain low parasitemia. (Agyemang, 2005; Naessens et al, 2002).

Materials and Methods

The trypanosomosis status (positive and negative) was recorded for 387 pure Baoule animals from the Southwest of Burkina Faso, using indirect Elisa (Desquesnes et al., 2003). The genotype data from the Illumina Bovine SNP50 BeadChip were available for these animals. Quality control of the data was performed with PLINK 1.9 (Chang and al, 2015). The dataset was cleaned using standard quality control to exclude non-autosomal SNPs as well as SNPs with minor allele frequency lower than 0.01, those with a call rate of <95% and those that deviated from Hardy Weinberg equilibrium with Fisher's exact test with P-value < 10E-6. After applying the quality control criteria, 34,346 SNPs and 343 animals were left for the analysis.
Single-SNP associations were performed using GEMMA software (Xiang and Mattew, 2012). GEMMA implements the Genome-wide Efficient Mixed Model Association algorithm for a standard linear mixed model and some of its close relatives for GWAS. It fits a univariate linear mixed model

for marker association tests with a single phenotype to account for population stratification and sample structure.

Results and Discussion

In the single-SNP analyses, 6 SNPs showed significant (-log10 *p*-value = 5) associations with trypanosomosis status (Figure 1) on chromosomes (CHR) 8, 9, 16, 22, and 24, four of which were significant after Bonferroni correction (p-value < 1.45×10^{-6}), located on CHR16, CHR22, CHR8 and CHR9 (Table 1).

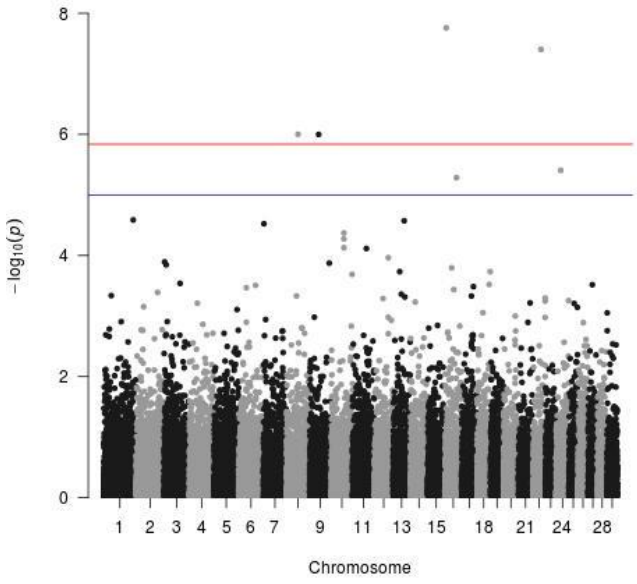

Figure 1: Manhattan plot for the Trypanosomosis status in the Baoule cattle
The upper solid line is the Bonferroni threshold, the lower solid line an indicative threshold at -log10 (*p*-value) = 5.

Table 1 SNPs with genome wide significant effects for trypanosomosis status

Chromosome	Name	Position (bp)	P-value
16	BTA-109095-no-rs	6452389	1.74e-08
22	BovineHD2200011407	39725145	3.95e-08
8	BovineHD0800017070	56636956	9.97e-07
9	BovineHD0900012349	44431079	1.01e-06
24	Hapmap38329-BTA-57629	22454666	3.91e-06
16	UA-IFASA-4552	56715642	5.17e-06

These results identify chromosome regions that might harbor the gene(s) that contribute to the trypanosome prevalence in Baoule cattle. GWAS for trypanosome infection status and level of parasitemia for groups of artificially infected African taurine, Zebu and their crosses was performed by Hanotte et al. (2003). The signals in that study overlap with the signals found in the current study. We also compared our GWAS signals with a range of studies on selection signatures in African cattle (Dayo et al., 2009, Gautier et al., 2009, Smetko et al., 2013, Tijjani, 2019), where trypanosomis is considered a very strong driver of selection. The second strongest signal in our study, within the protein tyrosine

phosphatase receptor type G (PTPRG) on CHR 22 (Mb 39.0-39.7), was considered an indicator of tropical adaptation in African Zebu cattle (Tijjani, 2019).

Conclusion

In the present study, SNP associations indicated potential regions related to trypanosome prevalence in six regions of the genome in Baoule cattle. The signals were not very strong, potentially because of the small sample size. Yet, Silbermayr et al., (2013) found that Baoule cattle and Baoule x Zebu crosses in the region have substantially smaller trypanosome infection rates compared to pure local Zebu cattle. Therefore, genetic control of infection status is likely. A GWAS for trypanosome infection status including Baoule, Zebu and their crosses in the South West of Burkina Faso is under way.

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THE STUDY OF GENETIC CONTROLS ON LIVESTOCK PRODUCTION TRAITS TO ENHANCE REPRODUCTIVE EFFICACY IN THAILAND

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Sixty years ago, in Thailand, there were no dairy cattle breeds that could produce dairy products as efficiently as western breeds. The Royal Thai Government has since introduced suitable dairy breeds to serve domestic demand for dairy products. The Tropical Holstein (TH) was developed, and is now the main dairy cattle population in Thailand. The TH breed is the combination of Holstein Friesian and the Thai native cattle breed and is built to extract the fittest traits for climate and environment as well as the management systems in Thailand. The TH breed has been developed through genetic selection methods including physical performance testing, genetic evaluation (eg. Genomic Estimated Breeding Values: GEBV), and molecular genetic approaches. Numerous studies of interested genes have been conducted to improve production efficacies of the breed. The two main types of genetic research were the identification of genetic diseases, and the study of production trait genes. For examples, in genetic disease identification, the genotyping of the CD18 gene was studied to find Bovine Leukocyte Adhesion Deficiency (BLAD) which is the genetic disease that causes immuno-deficiency and infertility in dairy cattle. Likewise, the SLC 35A3 gene was studied to detect Complex Vertebral Malformation (CVM) which is the genetic disease responsible for malformation of calves and still-borns, particularly in Holstein Friesian cattle. These studies give an advantage to animal selections by culling out genetic disease carriers from the clusters. Another example for productive trait genes, the DGAT1 gene was found to have an association with milk protein production in TH cows. The genotyping of the Butyrophillin (BTN) gene can identify associations with higher averages of fat in the milk yield. The study of productive trait genes benefits in delivering alternative means of animal selection and reducing cost and time. All in all, the study of genetic control helps improve the efficacies of animal selection and reproduction technology to enhance overall livestock production in Thailand.

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VALIDATION OF CATTLE FERTILITY MANAGEMENT TECHNOLOGIES WITH PARTICULAR REFERENCE TO COW-SIDE PROGESTERONE TESTS

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The IAEA Animal Production and Health section has a long track record of researching cattle fertility and that of other species through the application of diagnostics. This began with the application of radioimmunoassay technology to hormone measurement, in particular progesterone. Over the years, newer techniques such as ELISAs were developed which greatly facilitated research and practical application particularly in LMICs. However even these remined largely laboratory based. More recently the introduction of lateral flow technology has enabled the development of more rapid 'pen-side' tests which represents another step forward in the ability to manage farm animal fertility more pragmatically. The major purpose of the present experiments was to validate such a test, the P4 Rapid cow-side test (Ridgeway Science, UK) and to evaluate its use under tropical smallholder farm conditions.

Reproductive failure remains one of the main reasons for culling in dairy herds worldwide. Prolonged inter-calving periods occasioned by failure or delay in conceiving reduce the lifetime productivity of the cows and increases the herd replacement rate. As important, in breed improvement schemes where premium value semen (e. g. sexed semen) is being used, the cost of pregnancy failure is high and therefore it is critically important to maximise the chances of a successful pregnancy at insemination. The ability of the *postpartum* cow to return to cyclicity, express oestrus, conceive when inseminated, and carry the pregnancy to term within a prescribed period, assures a cow's productivity and its chances of remaining in the herd. One of the key activities in the management of the *postpartum* dairy cow is oestrus detection. Successful conception requires that oestrus is detected accurately and cows that are identified to be in oestrus are inseminated at the right time, in relation to ovulation.

The goal of the present work was to evaluate and compare the degree of agreement, effectiveness and robustness of three cow-side oestrus detection tools (ODTs) in three production and climatic conditions. The three ODTs selected for investigation were CowAlert®, (IceRobotics, UK), a cow activity monitoring platform, Estrotect™ (Rockway Inc, USA), a scratch card-based mount detector and, P4 Rapid (Ridgeway Sciences, UK), a lateral flow technique based on milk progesterone. Three experiments were carried out, one each in a large-scale farm in a temperate region, a large-scale farm in the tropics and small-holder farms in the tropics. The purpose of the different production environments was to understand how the effectiveness and robustness of the ODTs changed with each production environment. The expectation was that at the end of the study, recommendations would be made on which of the tested ODT was best suited for that production environment.

The first experiment was carried out Scotland's Rural College's (SRUC) Dairy Research and Innovation Centre in Dumfries, UK as the large-scale farm in a temperate region. The three ODTs were evaluated for their degree of agreement, effectiveness and robustness. Degree of agreement was evaluated by Cochran's Q test, the effectiveness evaluated through sensitivity analysis while the robustness was evaluated by carrying out the sensitivity analysis on groups of cows categorised according to their body condition and locomotion scores, milk yield (kg), weight (kg), days in milk and parity. The ODTs were applied concurrently to the cows to allow for accurate comparison. There was not consistent agreement

between the three ODTs in their ability to detect oestrus. P4 Rapid had the highest effectiveness as measured by the sensitivity (0.86), in detecting oestrus followed by CowAlert® (0.50) then Estrotect™ (0.31). The body condition and locomotion scores, milk yield and weight did not influence the effectiveness of the ODTs. For all three ODTs there was a statistically significant difference in their effectiveness when grouped in their different parities and between P4 Rapid and both CowAlert® and Estrotect™ when the cows were grouped in their days in milk. A closer examination at the influence of parity on the effectiveness showed that P4 Rapid was statistically significantly different to Estrotect™ in all parities recorded but only statistically significantly different to CowAlert® in cows from parity four and higher. For the days in milk, there was a statistically significant difference in the effectiveness between P4 Rapid and both CowAlert® and Estrotect™ in cows up to 60 days in milk. For this production system P4 Rapid was deemed the most effective and robust while Estrotect™ was found to be the least effective.

The second experiment followed the same experimental design as the first and was carried out at the College of Agriculture and Veterinary Sciences (CAVS), University of Nairobi (UON) dairy farm in Kenya. This served as the large-scale farm in a tropical environment. In this experiment. P4 Rapid had the highest sensitivity (0.98). However in this experiment both CowAlert® and Estrotect™ had a sensitivity of less than 30%. In deviation from the first experiment, only the parity had an influence on the effectiveness of the ODT. For this production system P4 Rapid was deemed the most effective and robust while CowAlert® was found to be the least effective. However, there were certain drawbacks associated with the use of Estrotect™ as it allowed for potentially infective ticks to burrow under the strip, providing a haven for them against acaricides. While detecting oestrus is an important management activity, it is prudent that it is not done at the expense of cow health.

The third experiment was conducted in the tropics with 34 small-holder farms in Kiambu county Kenya. The experimental design was modified somewhat based on the findings from the first two experiments. With these small-holder farms, the first objective was to evaluate the reproductive status monitoring practices in use, with a focus on oestrus detection and pregnancy diagnosis. All the farms sampled used visual observation to detect signs of oestrus prior to insemination and their absence (non-return to oestrus) as an indicator of conception. None of the farms sampled had used an ODT prior to this study. The second objective was to evaluate the performance of P4 Rapid and CowAlert® in detecting oestrus both at insemination and as an indicator of non-conception up to 24 days following insemination when compared with visual oestrous detection. P4 Rapid was found to be the most effective in detecting oestrus during insemination and non-oestrus up to 24 days post insemination.

In conclusion, P4 Rapid was consistently the most sensitive in detecting an oestrus event in the three production systems tested. This is considered likely because P4 Rapid is a direct measure of an intrinsic parameter, progesterone. Progesterone concentration has a well characterised pattern during oestrous cycle progression and has been used successfully to identify ovulation and thus signifying that oestrus occurred. However, both CowAlert® and Estrotect™ occur in response to elevated systemic levels of oestrogen and thus are not direct measures of an intrinsic parameter. Increased activity can also occur outside oestrus for the case of CowAlert® and for Estrotect a scratched strip is not an indicator of mounting activity.

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ALLELIC AND GENOTYPIC FREQUENCIES OF rs29004488 AND rs29004508 POLYMORPHISMS OF THE LEPTIN GENE, IN BREEDING BULLS OF THE CARORA BREED

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Background

Leptin is a protein of 16 Kda, constituted by 146 amino acids, highly conserved, with homology between 84 - 97% in cattle (Bos taurus), mice (Mus musculus), humans, pigs (Sus scrofa) and sheep (Ovis aries) (Bidwell *et al.*, 1997; Dyer *et al.*, 1997; Ji *et al.*, 1998). Is secreting mainly by adipose tissue, transporting to the brain where it stimulates factors that participate in biological functions such as satiety, regulation of the neuroendocrine system, energy expenditure, hematopoiesis, angiogenesis, puberty, and reproduction; also participates in adaptability and immune function (Agarwal *et al.*, 2009; Guerra *et al.*, 2005; Hatami-Baroogh *et al.*, 2010). This hormone regulates its expression through feedback mechanisms, as the number of adipocytes rises, the serum concentration of Leptin increases. (Auwerx & Staels, 1998; Houseknecht *et al.*, 1998).

This hormone is encoding by the Leptin gene (*LEP*), located on chromosome 4, region q32 in bovines (*Bos taurus-Bos indicus*) (Stone *et al.*, 1996). It contains more than 15,000 base pairs (bp) and consists of 2 introns and 3 exons (Coleman, 1978). The coding regions are located in exons 2 and 3 and show several polymorphisms associated with characteristics of economic interest in cattle, such as fat content in the carcass, food consumption, live weight, energy balance, fertility, milk production, and protein yield in milk (Buchanan *et al.*, 2002; Haegeman *et al.*, 2000; Lagonigro *et al.*, 2003; Liefers *et al.*, 2002; Liefers *et al.*, 2005). Among the nucleotide polymorphisms (SNPs) identified and evaluated in genetic association analysis are rs29004488 and rs29004508 located in exon 2 and 3 respectively (Buchanan *et al.*, 2002; Komisarek & Antkowiak, 2007).

The above studies highlight the importance of the *LEP* gene in lipid metabolism and growth of domestic animals, as well as in regulating the productive performance of herds; the latter has generated great interest for the evaluation of *LEP* as a candidate gene for genetic association studies that allow predicting the productive and reproductive behavior of cattle. In Venezuela, Salazar *et al.* (2015) and Vásquez *et al.* (2012) made some reports on Carora and Criollo Limonero cattle; however, the distribution of the above-mentioned *LEP* polymorphisms in the Carora herds is unknown.

The Carora breed constitutes a valuable animal genetic resource for the country. It is a synthetic breed, native to Carora, Lara state, Venezuela. It was formed by crosses between the Amarillo Quebrada Arriba Creole cattle and the Brown Swiss breed, which made it a breed adapted to the tropical climate and with good milk production (ASOCRICA, 2002). Its formation process began in the 30s, with semen from Brown Swiss bulls from Europe and North America, and then continued with the use of mixed-breed bulls to maintain its adaptation to the tropical environment (Cerutti *et al.*, 2006). It has favorable traits for meekness, strength, vigor, and good reproduction, also, natural selection favored specific genes of the Creole, which gives it rusticity, the capacity to adapt to the climate and to take advantage of tropical forages. It has established itself as a tropical dairy breed, a result of the genetic improvement program implemented by the Carora Cattle Breeders Association (ASOCRICA) and the Carora Artificial Insemination Center (CIAC)(Cerutti *et al.*, 2006).

This research constitutes the first approach for the characterization of the *LEP* gene in Carora cattle and will allow considering the genetic potential of the breed for the production of milk or meat, with added value characteristics such as fat in milk or fatty acids from the carcass, as well. like some reproductive

traits. Based on the above, the objective of this study was to characterize the polymorphisms rs29004488 and rs29004508 of the *LEP* gene, in a selected herd of breeding bulls of the Carora breed.

Methods

The samples were collected from 43 Carora breed bulls belonging to the CIAC breeding stock, located in Carora, Pedro León Torres municipality, Lara state, Venezuela, born between 2002 and 2013. The DNA was obtained from saline precipitation methodologies (De La Rosa *et al.*, 2013; Salazar *et al.*, 2012). Genotyping was performed using a Tetra Primer ARMS PCR (Ye *et al.*, 2001), and the amplification products were verified in horizontal electrophoresis on agarose gels 1.5 %. Using the POPGENE 1.32 program, the allelic and genotypic frequencies, the observed (Ho) and expected (He) heterozygosity were calculated and the Hardy-Weinberg equilibrium was tested.

Results

For the SNP rs29004488, two alleles (C and T) and three genotypes (CC / CT / TT) were observed; the frequency of the T allele was 0.59, while that of the C allele was 0.41. The genotype frequencies were 0.14 (CC), 0.53 (CT) and 0.33 (TT). About the polymorphism rs29004508, it presented two alleles (C and T) and two genotypes (CC / CT); the TT genotype was not observed. The allelic frequency was 0.94 for C and 0.06 for T. The genotype frequencies were 0.88 (CC) and 0.12 (TC). No deviations from Hardy-Weinberg equilibrium were observed in any of the evaluated polymorphisms. The observed heterozygosity (Ho) values were 0.535 (rs29004488) and 0.119 (rs29004508), while the He values were 0.483 and 0.112 for rs29004488 and rs29004508, respectively.

Conclusions

The evaluation of the H-W Balance indicated the existence of equilibrium in the population, as well as gene stability. The heterozygosity observed showed the presence of a moderate diversity in the SNP rs29004488 and a low diversity in the SNP rs29004508. The frequencies of the alleles and genotypes evaluated could confirm that these animals can be used in intensive dairy systems, as well as dual purpose. However, it is necessary to increase the sample base to make a more accurate estimate of the allelic and genotypic frequencies of these polymorphisms in the Carora breed.

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COMPARISON OF BREEDING VALUES' ACCURACY USING BLUP AND SS-GBLUP METHODOLOGY IN PERUVIAN ALPACAS

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Introduction

The textile industry places exceptionally high-quality demands on alpaca fiber to be competitive on the international market. Therefore, reducing the fiber diameter and reducing the percentage of medullation are economically important breeding goals to reduce the itching factor (Gutiérrez et al., 2009). To date, no national breeding program has been established in Peru, but there are several local genetic improvement initiatives carried out (Gutiérrez et al., 2018). Best Linear Unbiased Prediction model (BLUP) is the standard method for estimating breeding values. Recently, a SNP-chip for alpacas has been developed, and possible use in genomic selection is discussed (Calderon et al., 2020).
This study aims to evaluate the possible advantage of including genomic information in estimating breeding values. Therefore, accuracies of estimated breeding values for three different fiber traits (fiber diameter (FD), standard deviation (SD), and percent of medullation (PM)) using two different methods, namely BLUP and ssGBLUP, are compared.

Material and methods

The data were obtained from the PacoPro v5.10 software from the Pacomarca scientific research experimental station, which contains pedigree information from 1992 to 2020 and phenotypic data collected from 2001 to 2019. The fiber traits were FD and SD described by Gutiérrez et al. (2009) and PM described by Cruz et al. (2019). Table 1 provides an overview of the number of observations for each trait.

Table 1. Traits and number of observations recorded

		Total records		
	Animals (n)	FD	SD	PM
Full pedigree	7,012			
Animal with records	6,889	24,169	24,169	8,386
Genotyped animals	431	2,774	2,774	1,767

FD=fiber diameter, SD=standard deviation of fiber diameter, PM=percentage of medullation

Genotyping data of 431 animals from the project N° 028-2016-INIA-PNIA/UPMSI/IE of the National Agrarian University - La Molina was used. The quality control was carried out using the R language. All SNPs with a genotyping rate lower than 95% and minor allelic frequency (MAF) ≤ 0.05 were removed, leaving 60,624 SNP markers.
Two methods to determine the accuracy of breeding values of three fiber traits were used, a traditional BLUP method with phenotypic data and pedigree-based relationship matrix (A), and ss-GBLUP based on a combined matrix (H) constructed from a matrix A and a genomic relationship matrix (G).

The model fitted for FD, SD and PM was: $y = Xb + Zu + Wp + e$

where **y** is the vector of observations, **b** is the vector of fixed effects, **u** is the vector representing the additive genetic effects, **p** corresponds to the vector of permanent environments, and **e** is the vector of residuals; **X**, **Z**, and **W** are the incidence matrices for respectively fixed, genetic and permanent effects. The fixed effects included: coat color (9 levels), combined effects sex and physiological state of lactation (3 levels), year of recording as a contemporary group (19 levels), and age as a linear and quadratic covariate. Breeding values (EBV) were estimated using BLUPF90 family of programs as RENUM, REML and BLUP (Misztal et al., 2015). The computing procedures for genetic evaluation, including phenotypic, full pedigree, and genomic information, where a numerator relationship matrix (**A**) can be modified to a matrix (**H**) that includes both pedigree-based relationships and differences between pedigree-based and genomic-based relationships, were applied (Misztal et al., 2009).
The calculation of the accuracy of the genetic and genomic values was carried out in the following steps: First, the de-regressed mean of the phenotypic data was calculated, adjusting for the effects of the contemporary group, age, coat color, and the combination of the effects of the sex and physiological status of the females, for this the *lm* function of the R package was used. Second, 10 times random samples of 100 animals were taken from the 431 animals. For each run the phenotypic information was dispensed from each animal. Third, the prediction of genetic accuracy was calculated as a correlation between the predicted genetic value estimated by the BLUP methodology and the de-regressed mean, assuming it as the true value. Fourth, the prediction of genomic accuracy was calculated as a correlation between the predicted genomic value estimated by the ss-GBLUP methodology and the de-regressed mean, assuming the later as the true value.

Results and discussion

Table 2 shows the accuracy (scale 0 to 1) of estimates of 10 runs for the three fiber traits using BLUP and ss-GBLUP method. The BLUP method's average accuracy estimates were 0.452, 0.351, and 0.371 for FD, SD, and PM, respectively. The average accuracy estimates using ss-GBLUP method were 0.490, 0.436, and 0.465 for FD, SD, and PM, respectively. The differences between BLUP and ss-GBLUP methods were 9.8%, 29.8%, and 29.1% for FD, SD, and PM, respectively, in favor of the method using additional information from molecular markers.

Table 2. Accuracy of the genetic values (BLUP) and genomic values (ss-GBLUP) and the difference of accuracy between both methods in alpacas

BLUP	p1	p2	p3	p4	p5	p6	p7	p8	p9	p10	Mean
FD	0.547	0.408	0.450	0.507	0.291	0.475	0.584	0.474	0.367	0.422	0.452
SD	0.463	0.382	0.204	0.310	0.376	0.381	0.376	0.402	0.291	0.321	0.351
PM	0.327	0.385	0.332	0.504	0.352	0.282	0.375	0.579	0.230	0.343	0.371
ss-GBLUP											
FD	0.577	0.495	0.508	0.523	0.386	0.472	0.575	0.479	0.407	0.474	0.490
SD	0.518	0.470	0.451	0.355	0.432	0.447	0.417	0.418	0.404	0.454	0.436
PM	0.459	0.522	0.512	0.515	0.462	0.399	0.397	0.572	0.315	0.495	0.465
Difference between BLUP- ssGBLUP (%)											
FD	5.3	21.5	12.9	3.2	33.0	-0.7	-1.6	1.1	11.1	12.3	9.8
SD	12.0	22.9	121.0	14.3	14.9	17.5	10.6	3.9	39.0	41.4	29.8
PM	40.5	35.7	53.9	2.2	31.0	41.6	5.8	-1.2	37.3	44.0	29.1

BLUP= Best linear Unbiased Prediction methodology, ssGBLUP= single step genomic best linear unbiased prediction methodology, FD= fiber diameter, SD= standard deviations, PM= percentage of medullation, p1-p10: runs.

Our results are in line with studies for other livestock species. For carcass traits in sheep an increase of accuracy of 33.3% was reported (Daetwyler et al., 2012). While in dairy sheep an increase of 47.98% was found (Legarra et al., 2014), only an improvement of 5-7% was described in dairy goats (Teissier et al., 2018). The use of genomic information (ss-GBLUP) generates greater accuracy than the traditional BLUP since genomic relationships are more accurate than relationships based on pedigree (Meuwissen et al., 2016), and it can detect small genetic variations since it can integrate the phenotypic, genomic and pedigree information (Gao et al., 2019). Genomic selection is considerably more precise than the traditional BLUP methodology, especially for a low heritability trait (Calus et al., 2008) such as percentage of medullation.

Conclusion

The study could demonstrate that the additional use of genomic information for breeding value estimation increases the accuracy. This result is particularly interesting for traits with low heritability. However, to implement genomic selection on a larger scale, the necessary conditions, such as animal identification, comprehensive field data collection of production and reproduction traits, must first be created. In addition, the number of genotyped animals has to be increased to take full advantage of the technology.

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APPLICATION OF IMPROVED TECHNOLOGIES FOR SUSTAINABLE LIVESTOCK PRODUCTIVITY: THE WAY FORWARD

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SUSTAINABLE ANIMAL PRODUCTION IN PAKISTAN BY USING NUCLEAR AND RELATED TECHNIQUES; PAST EXPERIENCES AND FUTURE PROSPECTS

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Background

At present, the livestock subsector has surpassed the crop subsector as the biggest contributor to value added in agriculture in Pakistan. It contributed 60.5 percent to the overall agriculture and 11.2 percent to the national GDP during 2018-19 (Economic Survey 2018-19). The sector is also an important source of foreign exchange earnings contributing around 3.1% to the total exports, in addition to generating 35-40% of income for over 8 million rural families while provides them food security and nutrition by supplementing high value protein. Sustainable animal production in Pakistan is crucial for socio-economic uplifting of a large proportion of the population due to its role in the national economy, public food supply balance and healthy lifestyles. The Nuclear Institute for Agriculture and Biology (NIAB), with national and international support, especially from IAEA (RAS/05/030; RAS/05/035; RAS/05/044 and IAEA project 4173 RB), has introduced multiple initiatives to improve livestock productivity through better nutrition and breeding strategies, including adoption of reproductive aids like estrus synchronization and progesterone radioimmunoassay (RIA) to improve the outcome of artificial insemination. At the same time, efforts are being made to safeguard the national herd from trans-boundary animal diseases through efficient diagnostics and quality vaccines. Another area for sustainable animal production is NIAB capacity strengthening through IAEA collaboration for food safety regarding residues of veterinary drugs and pesticides in animal-based products. All these initiatives are helpful in achieving sustainable animal production through better nutrition, improved breeding, ensuring livestock health and by providing analytical services to promote export of animal-based products.

Past Experiences for use of nuclear and related techniques

I. Animal Production

Feed supplementation strategies for optimum animal production

To decrease production losses due to nutritional deficiencies in livestock, NIAB, with the help of IAEA, developed and tested Urea Molasses Multinutrient Blocks (UMMB), which contain all the essential nutrients in appropriate proportions to boost rumen fermentation. Extensive feeding trials were performed on animals at 100 small farms, and the use of "NIAB Feed Block" increased milk yield significantly with positive effects on health and reproduction of animals (Khanum et al., 2011). This technology is recommended to farmers to prepare and use the NIAB Feed Block to improve the health of their animals, increase meat & milk production and hence their income. During severe drought and adverse climate conditions these blocks help save farmers from economic losses by ensuring optimum milk and meat productivity (Fig.1). Other supplementation strategies like fibre degrading enzymes have also been found to be successful to improve livestock productivity (Hussain et al., 2014).

Nutritional evaluation of various feedstuffs using in vitro gas method

Nutritional quality of non-conventional feed resources has been determined by using the*in vitro* gas method. The feedstuffs having different digestibility showed significant differences in the rate and amount of gas production, metabolizable energy (ME) and digestibility of organic matter. Predicted metabolizable energy values were very low in feedstuffs having high fiber and low protein content. Among non-conventional feedstuffs, tree leaves such as *Acacia ampliceps*, *Acacia nilotica*, *Sesbaniaaculeata*, *Leptochloafusca* and *Prosopis juliflora* were found to have potential as fodders. Extensive use of the *in vitro* gas method proved its potential as a tool to evaluate various ruminant feeds for their energy component (Khanum *et al.*, 2007).

Fig. 1. Nili-Ravi Buffalo licking UMMB

Manure management as a tool for sustainable agriculture

To decrease nutrient losses to farmyard manure (FYM) in open heaps, storing manure by covered means resulted in an improvement in the nutrient profile (NPK) of manures and the soil on subsequent application. Fresh fodder yield was 28, 32 and 35.2 metric tons per hectare, respectively for the control, uncovered and covered manure plots (Table-1).

Table 1. Effect of manure storage on maize fodder yield

Chemical Composition		Experimental Plots		
FYM Treatments		Nil FYM	Uncovered FYM	Covered FYM
Plant Height (m)		1.58 ± 0.09[c]	1.67 ± 0.1[b]	1.79 ± 0.02 [a]
Fodder Yield	Tons/hectare	28[c]	32.0[b]	35.2[a]
	DM Yield/hectare	3.9[c]	4.49[b]	5.44[a]

Conserving the environment by mitigating methane emission

Methane emission from livestock was reduced by balanced feeding and providingfeed supplements. Through IAEA project RAS/05/044, balanced feeding to Nili-Ravi Buffalo resulted in a 10.8% reduction in methane emission compared with the non-supplemented control (278g compared with 312 g methane per animal per day).

Optimum animal productivity by exploiting seasonal forages

There is a need to optimize forage yield with respect to maturity stage since forage is offered on a fresh basis by the cut and carry system in Pakistan. Work is in progress for optimum yield and nutritional quality of Rabi and Kharif forages. This process will help decision making about an optimum forage yield stage, which can further be utilized for forage preserved through making hay and silage.

Animal Reproduction

Use of Radioimmunoassay to improve reproductive efficiency

Owing to the technological benefits offered like precision, sensitivity and efficiency, the RIA technique was adapted and applied for hormonal analysis of indigenous livestock breeds. Baseline data for progesterone, estradiol and other important hormones have been generated (Khanum *et al.*,2008) and the information generated is being utilized for improvement of reproduction in buffalo and cattle.

Adaptation of estrus synchronization techniques for breeding

Medroxy Progesterone Acetate impregnated sponges (MAP: 60mg) have been developed at NIAB. These intravaginal sponges are being used in buffalo, goat, camel and sheep for estrus synchronization followed by hormonal profile by RIA, resulting in better reproductive efficiency of the animals (Khanum *et al.*, 2006). Strategies for better nutrition of the animals, especially antioxidant supplementation, and pregnancy follow-up by ultrasonography are also being used for better reproduction of the animals.

Future Prospects for sustainable animal production using nuclear and related techniques

Nuclear and related techniques have an edge over conventional ones since these are more directed to achieve a targeted objective like sustainable animal production and are more specific and efficient. In continuation of already taken initiatives related to sustainable animal production in the area of nutrition and reproduction, in the future, NIABIAEA TC Project Pak 5052 for the cycle 2020-2021entitled "Improve Livestock Productivity Using Nuclear and Related Techniques by Exploiting Indigenous Feed Resources while Reducing the Enteric Greenhouse Gas Emission" is directly related to sustainable animal production. Facilities developed through this project will be sustainable for better livestock production in Pakistan, as it is part of a major national research programme at NIAB. The obtained results from this project will be adopted at the national level to improve livestock feed, enhance reproduction of animals and decrease methane emission. Modified feed supplementation strategies will be highly sustainable since these can earn more profit for the end-users, which will ultimately result in better socio-economic impact.
Improved livestock productivity with positive climate impact would be achieved by establishing an effective programme that uses nuclear and other related techniques for screening the nutritive value of locally available feeds. Similarly, estimation of microbial nitrogen and biomass production using the15N-tracer technique and assessing the hormonal profile by RIA will be carried out. Some of the favoring factors for sustainable animal production in Pakistan include human lifestyles, historical and religious bonds with animals and provision of social and financial security by keeping the animals in rural households. Animals are also a means of recreational events like horse dancing, camel racing, and buffalo fights etc. alongwith a means of draft power for traction, especially in a time when the prices of fuel are rising day by day. Govt projects like "Save & Fattening of Calf" (Rs.5.3 Billions) and "Backyard Poultry Programme" (Rs.0.3 Billions) will further help achieve better animal productivity.

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GOAT IMPROVEMENT THROUGH USE OF GENOMIC TOOLS IN LOW INPUT SYSTEMS

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I Background

Goats are an important source of livelihoods to people in sub-Saharan Africa, where they are mostly raised in low input production systems. They provide food and nutrition to many households (Rege et al., 2011) and are an essential component of socio-cultural systems. Goats are a valuable resource as moving banks and as a ready source of cash in case of unexpected events, such as funerals, and for routine purposes such as payment of school fees. They are used as a versatile coping mechanism by resource-poor farmers living in regions with unpredictable climates (Jones and Thornton, 2009). This is due to a lot of advantages that goats have over other species, not least of which is that they are highly adapted to a wide range of production environments (Kosgey and Okeyo, 2007). They are hardy and can survive well with minimal human intervention in almost all climates in tropical and subtropical regions. They withstand droughts better than larger ruminants such as cattle (Lebbie, 2004), and can be easily moved in case of floods and other crises (Peacock, 2005).

II. Potential of goats to meet rising demand for livestock and livestock products

With the rising demand for more livestock products as a result of increasing human population and greater disposable incomes (Delgado et al., 2010) , goats fit nicely into the agenda towards achieving higher production. Not surprisingly, goats are among the most populous livestock species in low input systems in Asia and Africa, which have the largest goat populations in the world (459 and 235 million, respectively, as of 2013; Skapetas and Bampidis, 2016). In countries like Malawi, the goat populations have been growing at an almost exponential rate in recent years, reflecting their growing importance to people's livelihoods. Improving goat production in low input systems is an effective step towards poverty alleviation. Many non-governmental organizations (NGOs) are implementing development activities that include goat rearing to help people "graduate" from poverty. This calls for measures to tackle the many challenges hampering the improvement of goat production in the systems.

III. Need for genetic improvement of goats

Some of the challenges to goat production include high prevalence of diseases and parasites, limited feed availability and poor marketing (Gwaze et al., 2009). The root cause of some of the challenges is climate change, which is leading to lower crop yields and reduced access to water. The direct effect of these changes is low animal productivity. There is a need to breed for more adaptability and resilience to these climatic shocks, which are becoming more frequent and of far more dire consequences in low input goat production systems. This requires fast-tracking the genetic improvement.

IV. Challenges of genetic improvement in goats

The major challenge of genetic improvement in goats under low input systems is lack of breeding structures and programmes. There is little or no recording, which means it is not easy to carry out meaningful genetic evaluation. Several goat improvement initiatives are being implemented in low input systems in Africa. The interventions include community-based breeding programmes (CBBP) in Ethiopia, Uganda and Malawi (Abegaz, 2014; Nandolo et al., 2016b, 2016a). These programmes include improvement of recording routines as a core activity. However, the recording is done with heavy involvement of enumerators, not just by the farmers themselves. This is leading to a lot of deficiencies in the records, especially in the identification of sires, which would be greatly improved if the farmers were more involved in the recording exercises. Getting the farmers more involved in the recording exercises requires demonstrating the benefits of recording as a catalyst for genetic improvement, and this is not easy if the records being collected in the demonstration phase are deficient in the first place. This is a potential risk to the survival of the CBBPs and similar goat genetic improvement interventions. Use of genomic tools can be harnessed to solve this problem.

V. The role of use of genomic tools in facilitating genetic improvement of goats

Recent advances in genotyping technologies offer more opportunities for knowing about the genetic potential of animals (Hanotte et al., 2010) even in the absence of extensive performance and pedigree data, which is currently the situation in the emerging CBBPs and similar genetic improvement interventions in low input systems. Strategic genotyping of goats can be done to provide "proof of concept" to demonstrate the practicality of genetic improvement of goats using the traditional goat genetic improvement approaches (using performance and pedigree data).

The most promising avenues for achieving this are (i) marker-assisted selection and (ii) parentage testing for breeding candidates. In the long term, the cost of genotyping animals may be too high for the farmers, but there is need to facilitate genetic improvement to encourage the farmers to participate more in the goat breeding programme activities, especially in animal recording.

Moreover, having genetically improved animals can motivate farmers to improve their management practices (Peacock, 2005), and if there is demonstrable superiority in the performance of the improved animals over the unimproved ones, it is likely to motivate farmers to sustain the improvements in management, thereby countering the effects of the climatic and other shocks and consequently leading to improved productivity.

VI. Conclusion

Long term goat genetic improvement requires that we should put in place effective structures for goat pedigree and performance data recording and management, considering that "phenotype is king" (Ackerman et al., 2016; Ackerman, 2015) in animal breeding. However, it is also imperative that genomic tools be harnessed to effectively facilitate the realization of meaningful goat genetic improvement in the shortest time possible in order to put up a case for establishing and consolidating long term goat breeding programmes.

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LIVESTOCK TECHNOLOGIES FROM PILOT TO SCALE: STRATEGIES AND PRACTICAL APPROACHES

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Introduction

Scaling refers to progression of innovations (technologies or practices) successful in certain contexts to wider areas covering large number of users or beneficiaries to receive more or less the same advantages of the new technology or practice (Management Systems International, 2014). Scaling can be (1) scaling up /out (scaling innovation, say from 10 villages to 10,000 villages) (3) functional scaling, which is scaling with an additional component to the innovation (e.g. micro credit) to overcome a weakness (lack of credit facility) identified during piloting (Management Systems International, 2014). There is yet another scaling known as scaling deep, which means transforming social-cultural norms and practices and attitudes through awareness raising and capacity building (Lennart Woltering, 2019). Sometimes scaling happens autonomously without any deliberate effort (for example, spread of maize (*Zea mays*) to all corners of African continent) and sometimes with determined and planned approaches (e.g. promoting the use of high yielding rice varieties in India during the Green Revolution). Over the past 50 years there has been significant investments in research aimed at addressing livestock issues through development of new technologies/practices. But uptake of these technologies has often been poor, and many never scaled beyond the lab or experiment /pilot stage. This brief explores various reasons for no/low adoption and scaling of innovations, particularly technologies related to improvements in small-holder based livestock systems, channelized through different pathways. Alternative pathways towards greater/better scaling success are also presented/proposed with examples.

Adoption and scaling – constraints and outlook

In the normal research to practice continuum – consisting of research, development of proof of concept, field-based pilots and scaling for impact – the movement of an innovation from pilot to scale is generally found to be stuck, especially in the smallholder context. Some technologies are inconvenient, risky, not cost-effective or otherwise ill-suited to the needs of poor livestock keepers. Quite often technologies or practices are developed top-down or based on researcher's interest to promote their pet technologies with no consideration on the context in which it is to be applied. In most cases the external inputs required for the innovations coming out of research are not available and unaffordable, especially in remote areas and poor communities. For instance, the use of energy-dense concentrate feeds for fattening pigs, though a proven technology, is difficult to adopt by smallholder farmers as this feed is not found supplied to rural areas on a regular basis (institutional or value chain constraint) and there is lack of availability of credit facility (financial constraint) to buy feed on a daily basis. Another example is preventing heavy economic loss due to animal diseases by protecting them through vaccination. Though vaccination is a proven and widely used technology, availability of vaccine, its cost, quality, maintenance of cold chain in remote locations (infrastructure) and availability of skilled persons (human power) are factors deciding adoption of the technology. Some technologies (e.g., forage production) demand biophysical resources such as land and water and some others (e.g. fiber fortification, feed chopping) require additional labor. The villag women who are already loaded with household and farm responsibilities will find it difficult to spare additional time to adopt labor intensive practices. Lack of

conducive policy also plays a key role in adoption and scaling of promising innovations (IFAD, 2015). For instance, in Cambodia the price of pork has been declining over the last three years, at least in part because of competition from cheaper slaughter pigs being imported from Vietnam It is estimated that every day about 3000 pigs are entering Cambodian market from Vietnam. Now Vietnamese pigs are available in the Cambodian market at a lower price compared to domestic pigs. Domestic producers are not able to compete at this price. Similar is the case in Bangladesh where the importation of cheap milk powder (to reconstitute and supply whole milk to consumers) seriously affects domestic milk production. Dairy farmers now increasingly show less interest to invest in genetics, feed and health control, no matter how outstanding the technology is.

In short, for any technology there is a 'technology' component and an 'enabling' part. The 'technology' part will be exciting, and scientists generally do not pay enough attention to the determinants of technology scaling (Figure 1). In pilots, the enabling environment – or absence therefore – is not a factor considered seriously. Rather, a pilot is conducted under somewhat artificial conditions rather than the real-life situation a prospective user or beneficiary might face. The saying, "pilots never fail and never scale" thus comes as no surprise. Therefore, one has to be very careful, particularly in the smallholder context, where innovations around social and institutional mechanisms are critical. If we try to simply scale out the technology without scaling the processes and improving the enabling environment that led to the success of the technology in a particular context tends not to work (Alan, 2010). One has to be particularly careful around innovations intended for use by small holders as they may be particularly dependent on enabling factors also to be present for an innovation to work for them. Therefore, researchers/innovators must be mindful of developing innovations that will work in the real life context or work with stakeholders that can change the context.

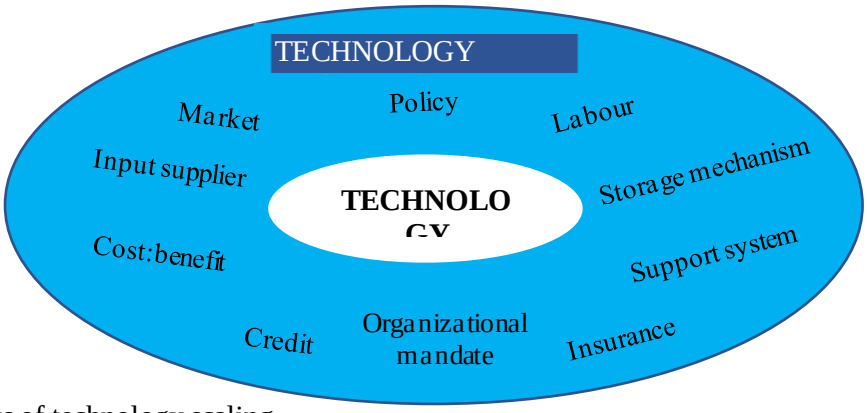

Figure 1: Determinants of technology scaling

Scaling pathways and strategic approaches

In pilots, if the technology /innovation showed significant noticeable impact and is economically viable, then it can be considered for scaling. Scaling can be attempted through different institutional pathways such as government (public sector institutions), private, development organizations, farmer-based organizations or a combination of these four organizational types. The appropriate pathway depends on a number of factors including outreach capacity, organizational mission and motive of the organization(s) that take up the scaling task.

This can be illustrated with a few examples.

In one of the pilot projects of the Feed the Future Innovation Lab for Livestock System in Cambodia, the project recommendation was to supplement the local diet of pigs with soybean meal, which is estimated to increase the profit by US$ 10 per pig and improve the economic competitiveness of pig production. This is a sound technical solution. But when the pig feed stores in the area were surveyed it was found that only five stores out of 81 stocked soybean meal and there are policy constraints to allowing importation of soybeans. It shows that the technology cannot be scaled unless someone works

with the policy makers and feed traders to make soybean meal more readily available in feed stores at a reasonable price and link micro-credit agencies with smallholder pig farmers. Scaling will be much easier if these agencies (feed dealers, micro-credit agencies) are identified and involved in the research process right from the beginning.

Another example is from Nepal, where one of the pilot projects on mastitis control revealed that the food safety quality of milk at the farm level was very poor and hence resulted in 15 recommendations (e.g., personal hygiene, udder washing, sanitization of milk pails, removal of first few strips of milk etc.) to improve the same. Some of the technological determinants of scaling these practices are awareness creation, input supply, economic incentive for better quality milk, and enforcement of regulations. Providing these enabling conditions would result in higher likelihood of scaling the technical recommendations - the potential scaling agencies in this context are the milk cooperatives (awareness creation), private and government sector dairies (quality-based pricing) and the Department of Food Technology and Quality Control (law enforcement) - these agencies, if identified at the beginning of the research and involved as partners, would be more readily positioned to begin mainstreaming the research results.

Funding research – the non-negotiables for scaling

Various examples, including the above show that identifying the right potential scaling agencies and involving them as partners right at the beginning of research is an effective measure to make scaling and mainstreaming of research outputs more likely, which can impact masses. Further, involvement of policy makers from relevant ministries /departments right from prioritization of the research agenda and connecting them throughout the research would have immense value in scaling as government has the mandate, human power, wide official network of institutions, financial resources and wider mass outreach. Therefore, the research funders should make it mandatory to involve potential scaling agencies in the research they are funding. This can motivate the researchers to do 'research with the end in mind'.

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POULTRY AID (PA) – SUSTAINABLE CAPACITY DEVELOPMENT IN POULTRY TECHNOLOGY, PRODUCTION AND HEALTH TO IMPROVE LIVELIHOODS IN ETHIOPIA

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Synopsis

The East African poultry sector is very important for food security and livelihoods and employs many people - particularly women. The sector is becoming more popular, since land holdings are reduced and most people are turning to poultry, which requires limited space. The high incidence and prevalence of infectious diseases in the region are, however, undermining the possibility of optimal benefits from keeping poultry. Frequent outbreaks of diseases often wipe out entire flocks, and there are no established control programs against most of these diseases. The situation is worsened by the scarcity of well-trained and skilled personnel, who are needed to offer technical advice for disease management and control well as to improve productivity[1].

Poultry Aid (PA) is a project launched in partnership between University of Veterinary Medicine, Vienna (Austria)[2], Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) (Ethiopia)[3] and the A.L.P.H.A (African Livestock Productivity and Health Advancement) initiative of the incorporated global animal health company, Zoetis (Belgium)[4]. The project focuses on building the capacity of the Ethiopian partner institution to combat poultry diseases in East Africa through tailored training of staff and delivery of sustainable diagnostic laboratory services beyond the duration of grant funding. It is a pioneering endeavour that mainly aims to address the issues of poultry medicine and management in Ethiopia through the establishment of a Centre of Excellence for Poultry Medicine and a poultry diagnostic laboratory. The project also envisages not only the improvement of poultry disease diagnosis in the region, but also the establishment of appropriate curricula for enhanced capacity development in the sector. As an outcome, a Centre of Excellence for Poultry Medicine will be established in which relevant activities will be agglomerated. Therefore, the project will support various activities including human capacity building, institutional development and knowledge transfer, sustainability planning, among others. With this, Poultry Aid supports to establish a viable poultry sector which can significantly contribute to ensuring food security and improved nutrition.

As the project aims at alleviating challenges of poultry health and productivity, it is planned to:

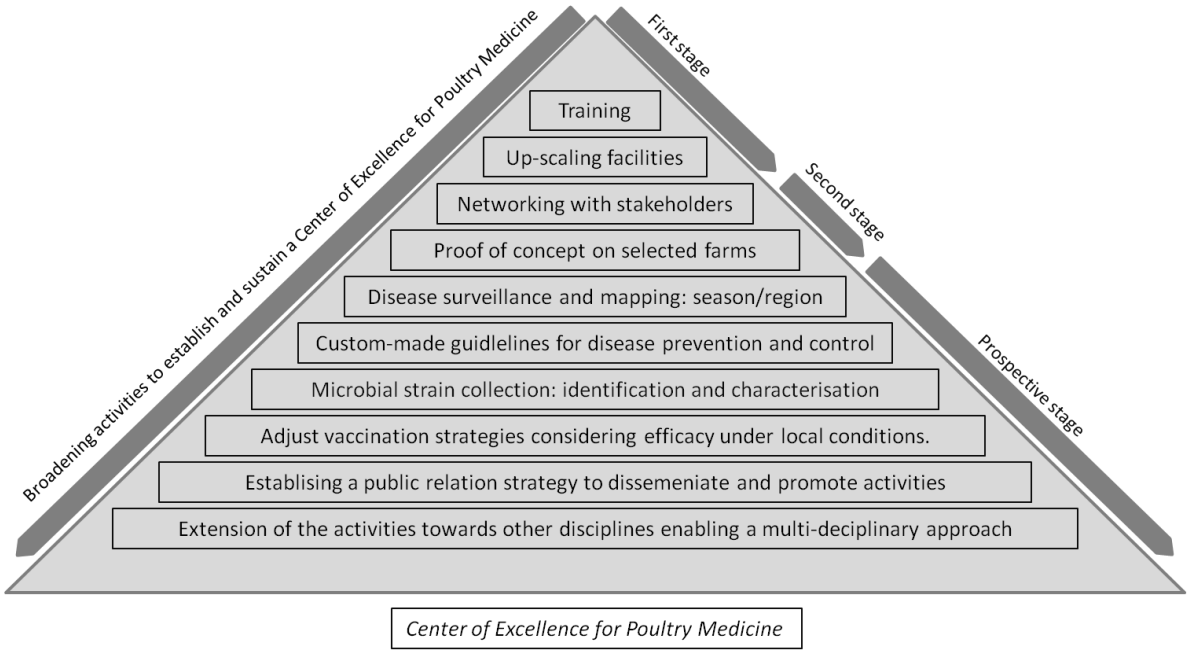
1. Enhance staff capacity at JUCAVM in poultry medicine and production with specifics to skills of field, clinical and laboratory investigation of poultry diseases;

2. Assist in utilizing the existent and new resources of JUCAVM to up-scale to a functional Center of Excellence for Poultry Medicine, with a strong focus on diagnosis and research on poultry diseases and sustainable service provision to support small-scale poultry farmers.

Envisaged outputs are:

1. Academically strengthened institution of higher education with increased competence in poultry medicine,
2. Empowered East African community availed with packages for: a) Improvement of poultry health through early diagnosis, b) Disease prevention via prophylactic action, and c) Poultry health policy development and implementation by accumulating and disseminating state of the art knowledge and procedures in the Centre of Excellence for Poultry Medicine.

Scheme of subjects as in-plan and prospects for PA:



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INTEGRATING TECHNOLOGIES FOR THE SUSTAINABLE CONTROL OF GASTROINTESTINAL PARASITES IN SHEEP. THE ARGENTINIAN CASE.

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Introduction

Most smallholders in tropical and subtropical areas practice small ruminant production in natural grasslands, but infection with gastro-intestinal parasites (GIP) causes serious losses and restrictions to farmers. In the northeast region of Argentina there are 37,288 sheep breeders (31.6% of the national total) and 2.1 million (14.2% of the total) sheep grow under conditions of high temperature and humidity. These conditions favour the development of *Haemochus sp*, which is the parasite that most affects sheep throughout the year. Although the losses in productive traits are difficult to specify, estimated percentages of decrease in body weight, wool yield and pregnancies are 10, 15, and 10%, respectively. Even death can occur in severe infections. For many years, the common practice to control GIP was by chemicals. But as parasites are becoming increasingly resistant to these drugs, which also leave residues in food and pastures, today other alternatives are necessary in an integrated control programme against this huge threat to national wool and meat industries. The main objective of this study was to find genetic variation underlying parasite resistance in sheep to be used in breeding programs. To accomplish ths, two specific objectives were posed: 1.- the collection of pedigree, phenotype data and DNA samples in sheep breeds; 2.-the joint analysis of phenotypic and genotypic data.

Materials and methods

Animals, protocol, phenotypes and genotypes

Artificial challenges were performed on 1025 Corriedale lambs of both sexes, from 20 half-sib families, with complete pedigree information for three generations. The animals were born between 2010 and 2019. The flocks studied belonged to two INTA experimental stations located in the northeast of Argentina. Briefly, the protocol used was as follows: animals were weaned 90 days after birth and kept on field until they were 4 to 5 months of age. At that time, lambs were separated by sex, dewormed, and moved to a corral. Lambs were artificially challenged via the rumen with 5,000 infective third-stage larvae (L3) of *Haemonchus contortus* (85%), *Cooperia spp* (9%), and *Trichostrongylus spp* (8%), derived from local sheep feces. At days 0, 28, 35 and 42 post-challenge, body weight (BW0, BW28, BW35 and BW42, respectively), faecal egg count (FEC0, FEC28, FEC35 and FEC42, respectively), packed cell volume (PCV0, PCV28, PCV35 and PCV42, respectively) and FAMACHA scores (FAMACHA0, FAMACHA 28, FAMACHA 35 and FAMACHA 42, respectively) were determined.

Later, animals were dewormed and bred in extensive systems under common flock management. One-year-old lambs were sheared and 12 wool related traits were recorded. Furthermore, the BW was recorded every 45 days throughout one year. An alternative and reduced protocol used only to segregate the most susceptible and resistant animals within flocks was developed for breeders.
Genomic DNA was obtained from blood samples using commercial kits following the manufacturers' protocols. A total of 173 SNPs belonging to 77 candidate genes for immune response from every ovine chromosome except for chromosomes 4, 9, 10, 18, 21, 23, and Y were genotyped on 624 animals. Candidate genes and markers were selected as described in Periasamy *et al*. 2014. Genotyping was performed by competitive allele specific PCR (KASPar) assays based on FRET chemistry (KBiosciences, LGC Genomics, UK). Cycling conditions for each assay were those recommended by the manufacturer. BioRad CFX96 (BioRad, USA) software was utilized for genotype calling. A quality control (QC) check of the genotypes was performed using PLINK v1.9 software (Chang *et al*., 2015). QC consisted of excluding samples with call rate <95%, and removing from the analysis SNPs with call rate <95% and minor allele frequency (MAF) <0.05. The latter parameter was determined using SNP frequencies on founder animals in order to avoid bias due to inbreeding. No SNP was highly deviated from Hardy-Weinberg equilibrium (all SNPs showed p-values for HWE exact test > 1.10^{-3}).

Statistical analysis

FEC was not normally distributed, consequently, observed values of FEC were log transformed, LNFEC = ln (FEC + 250), and used instead. Repeated measures analyses were conducted, using mixed model procedures of the SAS 200 software package (SAS Inst. Inc.,Cary, NC) to identify effects and covariates that contributed significantly to the variation of BW, LNFEC, PCV and FAMACHA© index. These models included lamb's BW at the beginning of the trial and days from the challenge (time) as covariates; farm, year of trial, and sex as fixed effects; and ram as a random effect. Based on the selected models, univariate fixed regression animal models were used to estimate additive genetic variance (estimated breeding value, EBV) and permanent variance within trial for BW, LNFEC, PCV and FAMACHA© index. Phenotypic and genetic correlations were estimated using bivariate regression animal models. Restricted maximum likelihood (REML) estimates were obtained with the EM algorithm using the WOMBAT software (Meyer 2007).
After genotype QC, 624 lambs and 149 SNPs remained in the dataset. Association analyses between each individual SNP and the EBVs for FEC were carried out in PLINK v1.9 using linear models. Additive linear models were fitted and adjusted for significant effects among sex, the combined effect of month and year of birth, litter size, year trial, farm, challenge age, PCV0, BW0 and the first two principal components derived from a principal component analysis performed using PLINK v1.9. To account for the risk of false positives due to the multiple testing problem, p-values were adjusted by Bonferroni correction. Corrected p-values < 0.05 were accepted to represent a proof of significant associations with the character under study.

Results and discussion

Estimated heritabilities, genetic and phenotypic correlations for BW, LNFEC, PCV and FAMACHA© index are shown in Table 1.

Trait	BW	LNFEC	PCV	FAMACHA© index
BW	**0.38**	-0.36	0.04	-0.37
LNFEC	-0.23	**0.21**	-0.49	0.30

Table 1.	PCV	0.15	-0.38	**0.18**	-0.36
	FAMACHA© index	-0.25	0.78	-0.41	**0.18**

Heretabilities[a] and genetic and phenotypic correlations
[a]Heritabilities on the diagonal, genetic correlations above the diagonal and phenotypic correlations below the diagonal.

On average, heritability had a standard error of 0.06, genetic correlations 0.15 and phenotypic correlations 0.03. The minimum and maximum EBV, estimated with an accuracy of ≥ 0.7, were as follows: BW –2.4 kg to +4.2 kg; LNFEC -1462 to +2469; PCV +4.06 % to –2.3 % and FAMACHA© index –0.38 to + 0.50 units. The FAMACHA© index shows a good positive phenotypic correlation with the FEC so it can be used as indicator of parasitism with *Haemonchus* sp.
The values of heritability for BW and LNFEC are similar to those reported in the bibliography by (Goldberg *et al* 2018). The phenotypic and genetic negative correlations between BW and LNFEC allow animals to be selected for both characters simultaneously with a positive response.
Seven SNPs showed significant p-values < 0.05 when testing association to EBVs for FEC. Among those significant associated markers we found OLADRA1_479_CT on OAR20, four SNPs on OAR3 (CLEC8A_532_CT, CLEC12A_567_CT, IL2RB_180_TC, and CLEC12A_440_CT), a SNP on OAR6 (TLR10_292_CG) and a SNP on OAR 12 (MASP2_104_CT) (Table 2).

Table 2. SNPs associated to EBVs for FEC.

SNP	Position (OAR:bp) [1]	Candidate gene	Bonferroni-corrected p-value
OLADRA1_479_CT	20:25775019	OLA-DRA	3.39 e-7
CLEC8A_532_CT	3:204456657	OLR1	7.521 e-4
CLEC12A_567_CT	3:204592787	CLEC12A	0.001723
IL2RB_180_TC	3:180362559	IL2RB	0.002201
CLEC12A_440_CT	3:204592660	CLEC12A	0.002255
TLR10_292_CG	6:57993319	TLR10	0.002699
MASP2_104_CT	12:40735932	MASP2	0.003702

[1]Chromosome and base pairs positions refer to OAR v3.1 assembly.

Final remarks

The protocol used for 10 years in more than 1000 challenged lambs showed to be robust because:
.- objective data were obtained from at least 17 phenotypic traits that allowed us to estimate variability, heritability and the genetic correlations between traits and to demonstrate the potential to improve parasite resistance without negatively affecting body weight
; .- none of the challenged animals was affected; neither for growth nor health;
.- the artificial challenge with L3 allows us in a short time, 35-40 days, to obtain data on resistance to GIP
.- it allowed us to adapt a protocol for breeders to segregate animals in a short time
.- the SNPs found associated with FEC of the candidate genes encourage to use molecular technologies and genomic information in animal breeding for parasite resistance
The immediate future for the control of the GIP will be based on an integrated flock management that minimizes the application of chemical products, the resistance of parasites to drugs and contamination of pastures while maximizing the productivity.

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CURRENT STATUS AND FUTURE TRENDS IN THE RESEARCH OF POULTRY MEAT AND EGGS IN CROATIA

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In recent years the topics of greatest scientific interest of the poultry researchers in Croatia have been improving quality of poultry products by dietary supplementation of functional ingredients in animal feed as well as application of novel technologies like high hydrostatic pressure processing, high intensity ultrasound and vacuum freezing; and the impact of these technologies on the quality of poultry meat and eggs. Also, the safety of poultry products has been studied from various aspects, ranging from incidence of *Salmonella*, *Gallibacterium anatis*, *E. coli* and *Campylobacter* in layer poultry flocks to antimicrobial resistance of bacteria in the food chain. Additionally, the incidence of keel bone damage in laying hens and possibilities for reducing it have been studied. Due to emerging occurrence of white striping and woody breast myopathies, new research will focus on finding accurate assessment and detection methods and prevention strategies, since a fast growth rate and high breast yield of broilers are related to these defects. The quality traits of poultry meat with these muscle abnormalities are low. Also, sustainable meat production is essential because of the impact on the environment and poultry meat production has much lower carbon footprint than ruminant meat. Therefore, we can expect further increases in poultry meat production. Consequently, researchers should focus on additional improvement of poultry products as well as on utilization of poultry by-products and waste management.

International Symposium on Sustainable Animal Production And Health
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ESTIMATING CARBON FOOTPRINT FOR MILK PRODUCED UP TO FARM GATE AT CATTLE FARMS IN KURUNEGALA DISTRICT, SRI LANKA

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Abstract

In recent years, the dairy sector is received much attention regarding its environmental impacts, especially for greenhouse gas (GHG) emission. Carbon footprint is the term that has been used to quantify the total GHG emission associated with a product or a process. In the Sri Lankan context, the contribution of GHG emission from the dairy sector is not much studied yet. To address this issue, Kurunegala district was selected as the study area of this research due to the prevalence of the highest dairy cattle population in the country. Ten farms were randomly selected to represent both intensive and semi-intensive farming systems in the area. The carbon footprint of milk production was analysed up to the farm gate for two management systems based on the conceptual framework of Life Cycle Assessment. Data collected from individual farm level were used to calculate the carbon footprint in each system. Dry matter intake, methane emission factors for enteric fermentation and direct nitrogen emission from the soil were the most attributed parameters in GHG emission. The calculated average carbon footprint for 1kg of fat and protein corrected milk for the year 2017 was 1.09±0.17 kgCO_2e for intensive farms and 3.95±0.72 kgCO_2e for semi-intensive farms. Methane from enteric fermentation was the main source of the carbon footprint in both systems (74% for semi-intensive and 77% for intensive), followed by emissions from application of nitrogen either as synthetic fertilizer or organic manure. This study concluded that there is a significant difference between the carbon footprint of the semi-intensive system and intensive system due to the changes in feed ration and the manure management system.

Introduction

Climate change has become a global issue requiring a global response. Greenhouse gas (GHG) emission from the human and human-related activities is a significant driver of climatic change. On a global level, agriculture is estimated to be the fourth largest contributor of GHG after energy supply, industry, and forestry (IPCC, 2007). Moreover, animal production is perceived to be much more harmful to global warming than other food production systems. A report issued by the Food and Agriculture Organization (FAO, 2010) identified livestock as being responsible for 18% of the world's total GHG emissions through the emissions of greenhouse gases such as carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O). These contribute to total greenhouse gas emissions at rates of 5-10%, 50% and 27-38%, respectively. In recent years the livestock sector has received much attention regarding its environmental impact. As a result of this, a need has developed for expressing the total GHG emission associated with a product or service. A term that has come to represent this quantification is called carbon footprint (CF). The CF of a product is the sum of the net GHG emitted throughout its life cycle within a set of system boundaries and in relation to a defined amount of a specified product (IDF, 2010). The objectives of this study were to estimate the CF of dairy cattle production in Kurunegala, Sri Lanka up to farm gate and to identify the most obvious areas with potential for mitigating the GHG emissions.

Materials and Method

System descriptions

The study was conducted in Kurunegala district. In the study area, cattle are reared under two management systems named as semi-intensive and intensive. Under the intensive system cattle are kept in the shed for 24 hours while supplying feed (zero grazing). Under the semi-intensive system, calves and heifers below one year are not allowed to graze and other animal are allowed to graze for eight to ten hours per day.

Data collection

Ten farms were selected randomly. A detailed questionnaire was prepared to collect farm data and the information needed for the CF estimation. The information collected through the questionnaire was implemented in an Excel sheet to perform the calculations of GHG emissions. The IPCC (2006) estimation method was chosen to calculate the CH_4 and N_2O emissions from animals, manure management, and crop cultivation. GHG emissions derived from farm fuel and electricity consumption were estimated using published standard coefficients adaptable to the national conditions. Within the 3 tiers suggested by IPCC (2006), tier 1 and tier 2 were used to calculate the GHG emission associated with enteric fermentation, manure management, and crop cultivation.

Methodology for carbon footprint

This study was conducted according to the principles of Life Cycle Assessment (LCA) for estimating CF. It is an environmental assessment tool standardized according to ISO 14040 (ISO, 2006a) and 14044 (ISO, 2006b). ‘‘A common carbon footprint approach for dairy’’ (IDF, 2015), the guidelines developed by International Dairy Federation was used as a guide for this study.

Results and Discussion

The calculated average carbon footprint for 1 kg of fat and protein corrected milk for the year 2017 was 1.09±0.17 $kgCO_2e$ for intensive farms and 3.95±0.72 $kgCO_2e$ for semi-intensive farms. As shown in Figure 1, relative contribution from CH_4 is highest for both semi-intensive (79%) and intensive (73%) systems, while N_2O contributed 23% from the semi-intensive system and 10% from the intensive system. As shown in Figure 1, contribution of CO_2 on GHGs was higher in intensive dairy cattle system (11%) than the semi-intensive dairy cattle system (4%). The GHG emission associated with different activities in milk production is shown in the Figure 2.

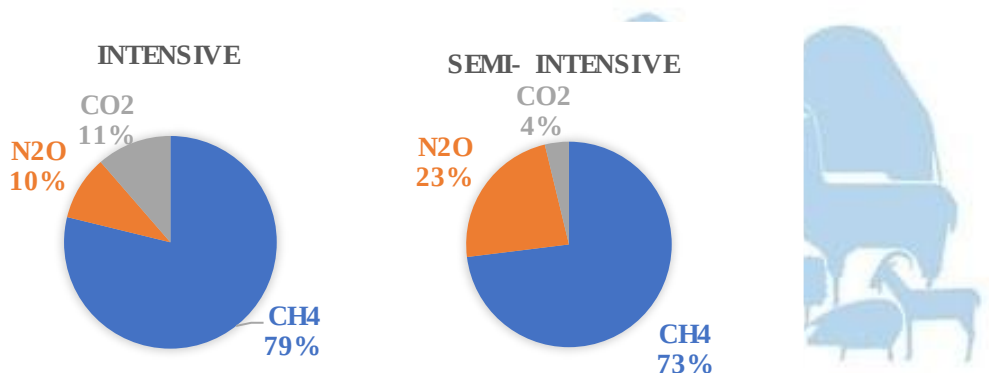

Figure 1: Percentage of GHG emission from intensive and semi- intensive dairy cattle farms in Kurunegala district

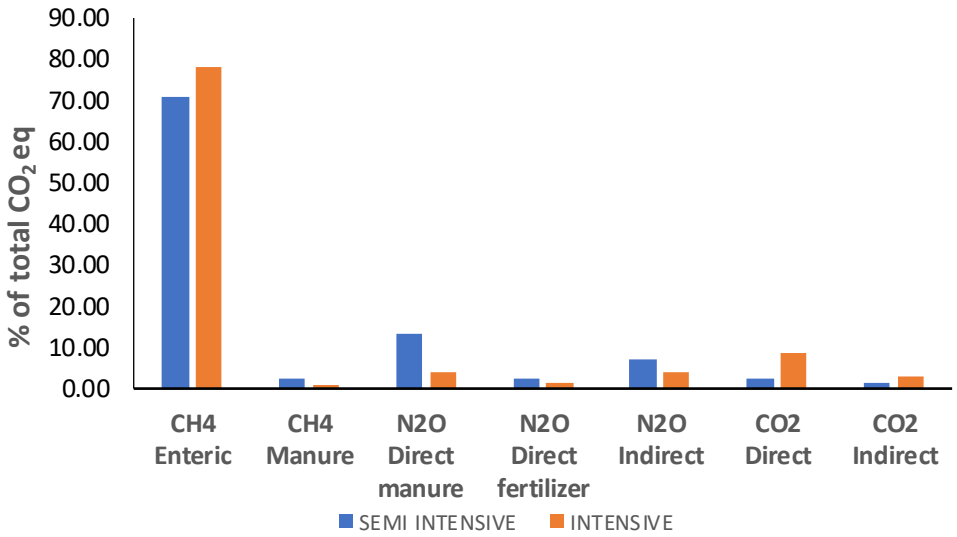

Figure 2: Contribution of different sources of GHG to milk carbon footprint up to farm gate in Kurunegala district

Conclusion

The CF of milk production up to farm gate in Kurunegala district, Sri Lanka varied according to the farming system. The intensive dairy cattle farms had a lower CF than did semi-intensive dairy cattle farms in Kurunegala district. Emission of CH_4 from enteric fermentation was the major contributor of CF in both systems. Improving feed digestibility could help to reduce enteric CH_4 emissions while increasing the productivity of the animals.

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MITIGATION OF HEAT STRESS INSIDE CONFINED LIVESTOCK BUILDINGS CAUSED BY GLOBAL WARMING

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Introduction

Pig and poultry production occurs predominantly inside livestock confinement buildings, which are often referred to as industrial systems (Gerber et al., 2013). These systems are characterised by a mechanical ventilation system, high stocking density, and insulated buildings. The increase of heat stress in recent decades for farm animals due to the anthropogenic warming is well known, especially in livestock confinement buildings. Mikovits et al. (2019) showed that for Central Europe the indoor climate of livestock confinement systems is more sensitive to heat stress compared to the outdoor situation, with increasing trends observed in the frequency and duration of heat stress over the past three decades. This calls for adaptation measures (AM) to reduce the existing, as well as the upcoming, increase in heat stress and its impact on welfare, health and productivity.
The AMs can be divided into two groups. The first group modifies the sensible and latent heat balance of the building by cooling the inlet air, reducing the sensible and latent heat release, and modifying the thermal properties of the building. The second group influences the immediate thermal vicinity of the animals. Examples for such AMs are floor cooling (conductive cooling) (Bull et al., 1997; Cabezon et al., 2017; Silva et al., 2009), higher air velocity at the animal level to increase the convective heat release (wind-chill effect) (e.g., by tunnel ventilation, booster fans, hybrid ventilation systems (Zhang and Bjerg, 2017)), radiative cooling by a cooled cover of the laying zone (Pang et al., 2010), cooled drinking water (Jeon et al., 2006; Renaudeau et al., 2012), wallows (Bracke, 2011; de Mello et al., 2017), or water baths (Huynh et al., 2006). The AMs of the first group can be evaluated by simulation models at the housing level (Schauberger et al., 2019), whereas the second group needs models at the animal level, which describe the heat release and the thermal regulation for individual animals.

Material and Methods

In our investigation, we selected a steady state simulation model of the indoor climate (Mikovits et al., 2019; Schauberger et al., 2000) and applied it to a typical livestock building for growing-fattening pigs in Central Europe since 1981. On the basis of such model calculations, the multi-decadal temporal trend of the thermal climate inside livestock buildings can be calculated. The results from a period of 37 years, particularly for extreme years, should indicate which of the AMs will be appropriate for reducing heat stress for growing-fattening pigs under future climate conditions.

Results and Discussion

Results are shown in Figure 1. Details on all seven AMs are discussed in detail in Schauberger et al. (2019). For the AMs, three different energy saving air preparation systems were investigated (Vitt et al., 2017): the direct evaporative cooling by cooling pads (CP), an indirect evaporative cooling by the combination of cooling pads with a regenerative heat exchanger (CPHE), and an earth-air heat exchanger (EAHE). Two further AMs modify the management of the livestock building by a reduction of the stocking density (SD) to 80% and 60% of the design values and corresponding heat release of the livestock during the summer period and by a change in the diurnal variation of the animal activity due to a shift of the feeding and resting times by half a day (SHIFT). Another measure affects the design value of the ventilation system by doubling the maximum volume flow rate (VENT). The temporal trend of a reference building (REF) without an AM is used as a baseline.
The AMs without air treatment (SHIFT, SD80%, SD60%, and VENT) lie close to REF and show limited effectiveness. The two air treatment scenarios with adiabatic cooling (CP and CPHE) show better performance. The best performance by far was reached by EAHE.
The relative linear trend of exceedance frequency P_X and exceedance area A_X is positive for all heat stress parameters, showing a mean relative annual change for the indoor climate of 1.3% (P_T).

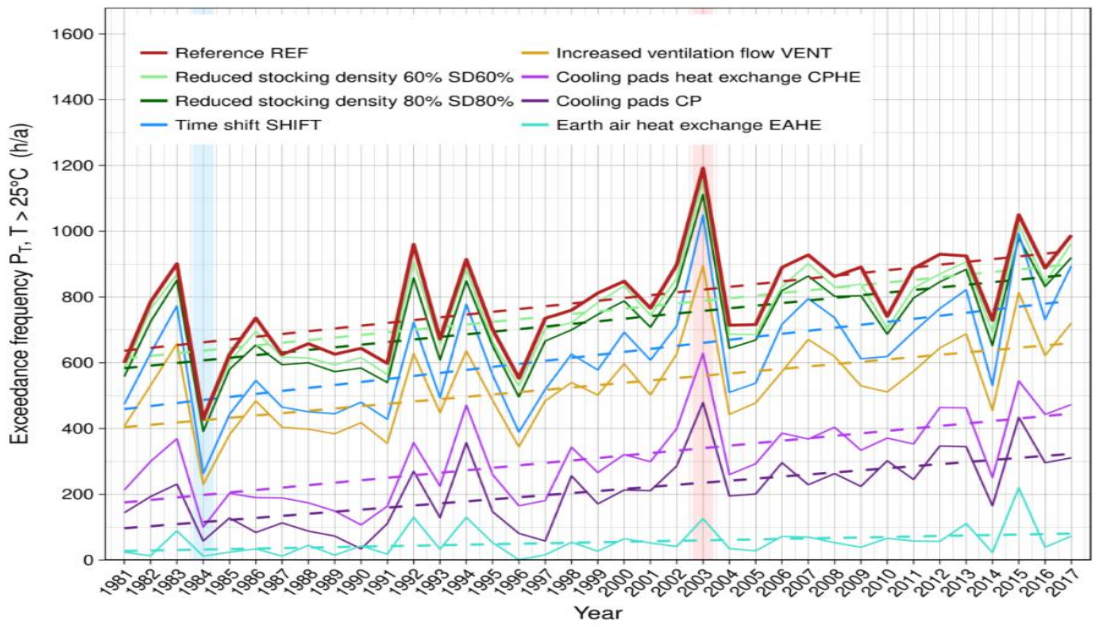

Fig. 1: Annual exceedance frequency PT for the threshold of the indoor air temperature XT = 25°C determined for the conventional reference system REF and the seven adaptation measures AM: reduced stocking densities SD80% and SD60%, diurnal shift of the activity pattern SHIFT, doubling of the summer ventilation rate VENT, the cooling pads plus heat exchanger CPHE, cooling pads CP, and earth-air heat exchanger EAHE. The linear regressions are shown by dashed lines.
The performances of the seven AMs were analysed by the reduction factor *R*, which is related to the heat stress parameters of REF (Schauberger et al., 2019). The AMs were ranked in ascending order, according to the reduction factor *R*, as a measure of the performance. The weakest performance was calculated for the reduction of the stocking densities SD80% and SD60%, with a reduction factor of *R*

below 10%. The reduction factors for SHIFT and VENT are between 23% (P_T) and 40% (A_T). The highest heat stress reduction of more than 50% for most of the heat stress parameters was found for the air treatment devices (CP, CPHE, and EAHE).
The linear slope of the temporal trend *k* was used to evaluate the resilience against global warming of the livestock system Fig. 1. The resilience can be determined twofold, first in relation to the outdoor situation and second in comparison to REF. A lower resilience of the livestock system results in a steeper slope of the heat stress parameter for a certain AM compared to the outdoor situation or to REF. For the four constant thresholds, the resilience of REF and for all AMs without air treatment (SD80%, SD60%, SHIFT, and VENT) shows a steeper slope (lower resilience) compared to the outside situation. The three AMs with air treatment (CPHE, CP, and EAHE) show a lower slope, which means that these systems increase the resilience for the livestock system in comparison to REF and are able to compensate the impact of global warming. The cooling efficacy of the three air treatment devices is high enough to keep the temperature inside the livestock system lower compared to outside, even though the animals are causing a high sensible heat load. All seven AMs improve the resilience of the livestock system against global warming. Nevertheless, the three air preparation AMs show a much better performance, compared to the other AMs.
The advantages of the presented modelling approach in comparison to measurements are manifold: (1) the model can be applied to other sites by the use of corresponding meteorological datasets, (2) near future scenarios can be assessed by the extrapolation of the linear trend in a long time series (e.g., 1981 to 2017) as robust predictions (Hendry and Pretis, 2016), (3) future climate scenarios can be calculated by datasets on an hourly basis (e.g.van Leuken et al., 2016), (4) case studies can be performed for combinations of AMs to optimise the indoor climate by the use of heat stress parameters as a cost function, (5) optimisation of the design values (e.g., for the EAHE) can help improve the efficacy relative to the climatic situation for a certain site, (6) future developments of system parameters can be considered (e.g., market demand of heavier pigs at slaughter), and (7) heat stress can be quantified in comparison to qualitative assessments (e.g. Derner et al., 2017).
Global warming has negatively impacted livestock kept in confined buildings during the last three decades and will do so in the future according to the trend analysis in this study. Robust measures of heat stress inside livestock buildings can only be quantified by a simulation model of the indoor climate over longer time periods. Compared to the outdoor rearing of farm animals, the indoor situation shows a lower resilience. By the use of adaptive measures, heat stress can be reduced, and resilience can be increased. Energy-saving air preparation devices especially can reduce heat stress in the range up to 100%. Other measures for adaptation, such as the reduction of the stocking density and the shift of the activity pattern of the animals to night-time, are less effective. The selection of appropriate adaptation measures, in addition to improving animal welfare, can also be seen as a contribution to strengthen the economic resilience of farmers (Schauberger et al., 2020).

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INTRODUCTION OF REPRODUCTIVE TECHNOLOGIES TO FACILITATE THE COMMUNITY BASED GOAT BREEDING PROGRAMME IN MALAWI

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Introduction

In Malawi, like in the rest of the Southern African Development Community (SADC) region, goats are increasingly becoming important livestock and are reared by more than 60% of farming households (Banda & Sichinga 2001, SADC 2013). Goats, with their small size, short generation interval and little investment requirement for production, have demonstrated an exponential growth over the past decade with an annual increase of 10% and registering a population of 9 million in 2019 (MoAIWD, 2019). The Government's aim and goal on small ruminants is to increase numbers and improve productivity of the indigenous breeds by practicing improved standards of management and breeding, proper housing, proper feeding, disease and parasite control (DAHLD, 2006). The upward trend of goats is resultant to the improved livestock production technologies initiated by both the Government and Non-Governmental Organization (NGO) sectors. Goats also naturally flourish in environments that do not favour cattle and sheep. The current situation of growing trend requires strategies to harness increased productivity and sustainability in order to economically support livelihoods of the goat farming communities. One such strategy is the Community Based Breeding Programme (CBBP), a program under the African Goat Improvement Network project (AGIN - www.ars.usda.gov/office-of-international-research-programs/ftf-livestock-improvement) and locally facilitated by the Lilongwe University of Agriculture and Natural Resources. The CBBP aims to genetically improve goats through selection from within the breeds and communities (Nandolo et al, 2016). Local or indigenous goats represent over 90% of the population and flourish in rural communities, but are lowly productive. There is an increasing necessity to produce animals with high genetic potential to increase the productivity of each animal unit instead of increasing the number of animal herds to achieve efficient and high quality production. To achieve this goal, there is a need to introduce assisted reproductive technologies in the current breeding system.

The Community Based Breeding Programme

The Community Based Breeding Programme (CBBP) is a breeding strategy that engages the community to primarily select breeding males from the community breeding populations to improve productivity and numbers of the targeted species. The aim is to improve performance of animals adapted to local climate and production systems with the help of researchers, extension support systems and development practitioners. CBBPs have been implemented in other regions, including CBBP for dairy goats in Mexico and for sheep and goats in Ethiopia (Abegaz *et al.*, 2014). Results have demonstrated that it is an effective approach in genetic improvement and conservation of indigenous farm animal genetic resources (AnGRs).
The program is seen to enhance benefits farmers get from their animals. In addition, CBBP improve capacity of local communities in management, conservation and utilization of the AnGRs. The CBBP operates in 6 communities in Malawi with two sites in Shire Valley agricultural development district (ADD), two in Lilongwe ADD and two in Mzuzu ADD; which represent the South, Central and Northern regions, respectively. The plan is to up-scale and out-scale to other localities and species. In

these communities, the CBBP establishes open nucleus breeding centers that are run and managed by communities. Researchers and extensionists come in as facilitators. As such, CBBP utilizes both scientific and community traditional practices and indigenous knowledge in determining suitable candidates for breeding. The breeding structure requires establishing mating that identifies both bucks and does for pedigree recording. The goal is to set up a reference population to enable researchers to track pedigree records and link performance of individual animal to their progeny and relatives. Regularly, on set dates, communities congregate their goats for selection as guided by the facilitators and extension agents. Based on the agreed numbers as needed by the community, the best bucks that have demonstrated fast growth, higher birth weights and good phenotypic characteristics for breeding are selected. The bucks left out can either be sold to other communities for breeding purposes or to butchers. They are also sometimes castrated if not fit for breeding and grown out further. All male animals that are born in these populations are required to be tagged and weighed. The bucks are then weighed again at 2, 4, and 6 months of age. To facilitate accurate data recording and management, the CBBP assigns two village enumerators for each site who are trained and to do data collection according to a preplanned protocol. A data management clerk is also assigned and trained to receive data from the enumerators and enter the data into a program called DREMS.
Selection of breeding bucks is done at the community-flock level. Individual flocks from different households are treated as one large community-flock. The farmers with guidance from the club committee and extension workers develop bylaws to guide management and sharing of the selected bucks. The community agrees to use the selected bucks for one year in the breeding groups and then have them swapped to other groups for another year. The bucks are therefore in use for a maximum of two years, after which they are sold. The unselected bucks, including those initially selected, but eventually used for breeding are sold for income.

Use and Expected Achievements of Assisted Reproductive Technologies in Cbbp

The operationalization of CBBP in Malawi is in selected locations and so far, limited to goat production communities. The CBBP principle can easily be applied to other AnGR, like sheep, chickens, cattle and pigs. CBBP is accepted amongst participating communities as a useful tool for breeding programs where the best results depend on mating the best with the best. The introduction of assisted reproductive technologies in CBBP would therefore be a key strategy to sustainably and extensively utilizing quality genetic material for improved stock productivity and farm benefits. The principle behind this is that reproduction is directly affected by various management related factors that can be manipulated to cause positive changes in reproductive performance. These factors for example include those which influence the fertility, the fecundity, the kid survival and the interkidding period coupled with reproduction improvement programs, as well as pasture improvement and technical assistance (Vivanco, 1985). Reproductive processes in animals offer numerous advantages in livestock production. Nevertheless, these emerging techniques should be judicially supplemented with good practices in animal health, nutrition and management at the level of stake holders.Manipulation and improvement of health, production and reproductive performance of any livestock species will facilitate the production and dissemination of superior germplasm, thereby enhancing the overall productivity (Choudhary *et al.*, 2016). Assisted reproductive technologies should therefore be adopted to overcome the increasing costs of maintaining animals in the midst of growing human population. There is a need to continue deploying incentives to improve these techniques (Shelton, 1990). Furthermore, the use of reproductive technologies will enhance to spread the genetics of superior bucks within and outside the CBBP communities, facilitate pedigree determination and mitigate inbreeding through managed matings.

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IMPROVEMENT OF CATTLE BREEDING IN TOGO

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Background

In Togo, breeding appears to be the main activity for 1.6% of the active agricultural population. The contribution of the livestock sub-sector represents 16.56% of agricultural GDP and 6.73% of national GDP. At national level, 6.2% of agricultural households practice cattle breeding (FAO, 2017). This extensive type of breeding is characterized by (i) low animal performance in terms of milk productivity and carcass yield and (ii) high herd mobility. Animal feed is mainly fodder taken from natural pastures, resulting in high herd mobility. Thus, each year, conflicts of varying magnitude are recorded between Fulani agropastoralists and the local populations regarding access to resources (pastures and water points) or damage caused by animals. To alleviate the problem of herd mobility and improve cattle production, the Togolese State, through the Ministry of Agriculture, Animal Production and Fisheries, undertook in 2020 the development of a model consisting of the creation of areas of Developed Areas Cattle Production (DACP) and the implementation of milk, meat and fodder value chains. This project is part of the national development plan (NDP) and aims to transform and develop cattle breeding and transhumance in Togo and to ensure their contribution to the rural and national economy.

Methodology

In each prefecture, where clusters of livestock farmers are identified, around the existing pools of cattle herds, one to three Developed Areas for Cattle Production (DACP) will be set up, each with a minimum area of 500 ha with a precise livestock feeding model. Each DACP will allow to set up two models of breeding: meat model, and Mini-dairy model. In meat model cattle will be produced in DACP and Private Domain for Cattle Production (PDoCP). The males from birth (140, 000 head per year) will be fattened for 2 or 3 years to reach 200 to 300 kg to be slaughtered. Lean animals are purchased from transhumants or from neighboring countries (100 000 head for a target weight of 300 kg and 220, 000 head for a target weight of 200 kg) and then fattened in these areas developed to reach the target weight. In Mini-dairy model it will be necessary to promote the installation of fifty (50) mini-dairies each capable of processing 1,000 liters per day. These mini-dairies will be responsible the collection of raw milk around the Developed Areas for Cattle Production (DACP) and Private Domain for Cattle Production (PDoCP).

Expected outcomes

The meat model allowed to organize the beef sector in order to produce, process and market good-quality beef to meet needs and limit imports, which are synonymous with a loss of foreign currency for the country. The mini-dairy model produces 18,000 tons of local milk annually to meet the annual needs of the Togolese population estimated at 15,000 tons and to export the surplus.

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THE EFFECT OF CROSSING BETWEEN THE PUREBRED YEMENI SHEEP AND THE IMPROVED AWASSI SHEEP USING ARTIFICIAL INSEMINATION TECHNIQUE ON GROWTH PERFORMANCE OF CROSS PRODUCED

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Abstract

This study aimed to evaluate the growth performance of hybrids resulting from the process of crossbreeding purebred Yemeni sheep strain with improved Awassi males (½Awassi and, ¾Awassi crossbred) using the artificial insemination technique. The data obtained from the records of the Regional Research Station in the central highlands of Yemen were for 8 consecutive years. The measurements identified were genotype group, the weight of dam at lambing, sex, year and season on the birth weight (BW), weaning weight (WW), average daily weight gain (ADG), and survival rate at birth to weaning of lambs. The results showed that Average live weights of lambs at birth and weaning were 2.98 ±0.06 kg and 14.34±0.47kg respectively. Variation among genotype groups were significant ($P<0.001$) at birth and weaning weight. Birth weight and weaning weight of crossbreed lambs showed a higher than purebred Yemeni lambs as well as the ¾Awassi lambs was significantly ($P<0.05$) a higher than ½Awassi and purebred Yemeni lambs. The superiority of crossbreed lambs (½Awassi, ¾Awassi) was over the purebred of Yemeni sheep from birth to weaning ranged between 24% and 33%. Likewise, the daily gains in ¾Awassi and ½Awassi genotype lambs were significantly ($P<0.05$) higher than Yemeni sheep, it was 144.15, 132, and 109 gm/day respectively. Genotypes of lambs have a significant effect ($P<0.05$) on the survival rate of lambs since the survival rate of purebred Yemeni sheep lambs was higher at the early stages of life while the crossbred lambs showed superiority in survival rate with advancing age. Results indicating that the ½Awassi and ¾Awassi crossbred sheep showed better growth performance than purebred Yemeni sheep, which may presumably indicate that Awassi breed could be an appropriate breed for crossbreeding to improve Yemeni breeds ewe's productivity.

Keywords: Yemeni sheep, purebred, growth performance, crossbred

Introduction

Sheep contribute meat, wool and skin as well as manure and serve as a sole or subsidiary source of income to the livelihood for a large number of small and marginal farmers and landless laborers in Yemen. There are 11 traditional recognized sheep breed in Yemen of which five are wool and six are hair types (Wilson, 2003). All sheep breeds in the country are indigenous and it has fat tail although the conformation of the tail varies considerably (Hasanain *et al.*, 1994). According to the 2019Agricultural census, there were 8.8 million sheep in Yemen, which is about 45.4% of the total livestock population in Yemen. These local breeds, despite their lower growth rates and less prolificacy, they are characterized by the ability to survive and reproduce normally in the range without supplementary feeding (Albial and Singh,2013). Since Awassi sheep (important meat breeds of sheep in Syrian) have better growth rates than purebred Yemeni sheep, a cross breeding programme has been conducted by improved Awassi males using the artificial insemination technique for improving the body weight of purebred Yemeni sheep. Hence, this study aimed to evaluate the growth performance of hybrids resulting from the process of crossbreeding purebred Yemeni sheep strain with improved Awassi males (½Awassi and, ¾Awassi crossbred) at different ages.

Methods and materials

Flock management practices

The genotype of sheep involved in this study were purebreds of Yemeni sheep and hybrids resulting from the process of crossbreeding purebred Yemeni sheep strain with improved Awassi males (½Awassi and, ¾Awassi genotypes) using the artificial insemination technique by corporation with the Arab Center for the Studies of Arid Zones and Dry Lands (ACSAD). These flocks were raised at Regional Research Station in the central highlands, Dhamar, Yemen. The management system used at the station was intended to be similar to the traditional system of sheep husbandry in the area, in terms of grazing and supplementation. All ewes were grazed together during the day for about 6-8 hours. Then, they were housed in covered pens with free access to grass hay, water and mineral lick blocks. Also a supplementary concentrate ration of 250-500 g/head/day, depending on season and physiological status, was fed to animals in the morning before grazing. A controlled mating scheme was used with three mating periods over two years. After lambing each ewe was put separately with their lamb into a lambing pen for about 2 to 7 days. Ewes and their lambs were weighed and ear-tagged after 24 hours from parturition. The weaning of lambs took place at an average of 91±8 days of age.

Statistical analysis

The data utilized in this study were obtained from weights of 552, 502 records of lambs at birth and at weaning, respectively, through eight years period (2013-2020). Some factors affecting the growth performance of lambs, like genotype, lamb sex, and weight of dams at lambing, season lambing and year lambing were investigated using least-square procedure (SAS, 2003). Duncan test was utilized for determining differences among subgroups means. Analysis was performed according to the following linear model:

$$\mathbf{Y_{ijlmdr} = \mu + B_i + S_j + A_l + C_d + G_r + e_{ijlmdg}}$$

Where: Y_{ijlmcr} = studied trait, μ = overall mean, B_i = fixed effect of the i^{th} genotype (i = Purebred Yemeni sheep, ½ Awassi and ¾Awassi), S_j = fixed effect of the j^{th} sex of lamb (j = male and female), A_l = fixed effect of the l^{th} weight of dam at lambing (l = 1, 2, and 3) where 1 = ≤25kg, 2= 24- 34kg and 3=≥35kg, C= fixed effect of d^{th} season of lambing (d=summer, autumn and winter), G= fixed of r^{th} year of lambing (r= 2013 to 2020), e_{ijlm}= represents the random error associated with each observation.

Result and discussion

Growth performance of lambs

Least-squares means and their standard errors of body weights of purebred Yemeni lambs, ½Awasi, and ¾Awassi crossbred lambs are presented in Table (1). Average live weights of lambs at birth and weaning were 2.98 ±0.06 kg and 14.34±0.47kg respectively. Variation among genotype groups were significant (P<0.001) at birth and weaning weight. Birth weight and weaning weight of crossbreed lambs showed a higher than purebred Yemeni lambs as well as the ¾Awassi lambs was significantly (P<0.05) a higher than ½Awassi and purebred Yemeni lambs. The superiority of crossbreed lambs (½Awassi, ¾Awassi) was over the purebred of Yemeni sheep from birth to weaning ranged between 24% to 33%. Better indicators of the crossbreds compared with the pure lambs were observed due to the significant genetic and geographical difference of exotic sire breed (Awassi) and probably due to the suggested influence of heterosis. Similar results were reported by Dawson *et al.* (2002) and Momani Shaker *et al.* (2002), who found that crossbreds had faster growth and higher live weight at birth and weaning as compared with purebred lambs. Males were significantly (P<0.05) heavier than female at birth and weaning weight. The levels of advantages of male over female lambs were similar (3-7%) at birth and weaning weight. The size of the ewe which was reflected by dam weight during lambing had also significant effect (P< 0.01) on birth weight of the lamb born in all genotypes. The results obtained

from this study show that lamb's birth and weaning weight tended to increase as the live weight of dam increased from ≥24kg to more than 35kg. This result is in agreement with the findings of Dixit *et al.* (2001) who reported that an increase in the dams' weight at lambing by 1 kg resulted in a significant increase in body weight at birth by 29g. Birth weight was affected by the year of lambing but there was no significant by lambing season whereas the lambs born in summer and autumn had higher for body weight than those born in winter due to the Differences in availability the pasture and fodder during the seasons. This finding was in agreement with those reported by Albial and Singh (2013) for White Boni sheep and by Thiruvenkadan *et al*. (2009) for Mecheri and their crossbred lambs.

Average daily gain (ADG)

Average daily gain of cross lambs was higher than the pure lambs during birth to weaning. The two crossbred lambs had significantly (P<0.05) higher than purebred Yemeni lambs. It was 144.15gm/day, 132gm/day, and 109gm/day for ¾Awassi, ½Awassi and purebred Yemeni lambs respectively. Similar findings of the effect of genotype on average daily gain were reported by Marzouk and Mousa (1998) and Thiruvenkadan *et al*. (2009). Male lambs had significantly (P<0.05) higher for average daily gain compared to females (130.49vs. 119.45 g/d) from birth to weaning. These results are in agreement with Abbas et al., 2010; Marzouk and Mousa (1998) who found that the effect of sex on daily gain was not significant. Weight of dam at lambing was significant (P<0.05) effected on growth rate at birth to weaning. Similar positive relationships has been reported by Dixit *et al.* (2001) and Barbar *et al.* (2004). This may be attributed to the high and significant correlation exist between the pre-weaning average daily gain of the lambs and the amount of milk produced by their dams which depend on their weights and nutrition during the last period of pregnancy and during suckling. The year lambing was no affected on ADG but the season was affected significantly (P<0.05). The growth of lambs during seasons of summer and autumn born was faster than winter born. This may be due to availability of good quality fodder for the dams during the rainy season (summer and autumn) than winter season. These results in present study were confirmed by prior reports of the scientists Albial and Singh (2013).

Lambs survival rate

Results for survival rate from birth to weaning of age are presented in Table (2). Genotypes of lambs have a significant effect (P<0.05) on the survival rate of lambs since the survival rate of purebred Yemeni sheep lambs was higher at the early stages of life while the crossbred lambs showed superiority in survival rate with advancing age and no significant at weaning by genotype. The higher survival rate of Yemeni purebred lambs during the birth of age could be due to their adaptation to the local conditions than exotic genotypes. In contrast, crossbred lambs had maintained their high survival rate after birth to weaning of age, the higher survival rate during this period of crossbred lambs may be due to their superiority in growth rate during the advancement period. Effect of genotype on survival rate is consistent with results from other studies (Morsy, 2002 who reported that breed group had insignificant effect on survival rate. On the contrary, Hamdon, (1996) stated that breed group had a significant effect on survival rate, especially at early ages.

Conclusion

Suitable choice of mutton and prolific breeds and correct method of crossbreeding with the use of excellent performance of the indigenous sheep breed will significantly contribute to an increase in mutton production. It is clear from the results that the ½Awassi and, ¾Awassi crossbred sheep showed better growth performance than purebred Yemeni sheep which may presumably indicate the effect of hybrid vigour in first or second generation crosses on 50% or 75% Awassi blood compared with pure Yemeni sheep. It is concluded that Awassi breed could be an appropriate breed for crossbreeding to improve Yemeni breeds ewe's productivity and early lamb growth under Yemen conditions system.

Table 1: Least-squire means ± standard error of some factors affecting the growth performance of purebred and crossbred lambs

Fixed effect	Birth weight (kg)		Weaning weight (kg)		Average Daily Gains (gm)	
	n[1]	Mean± SE	n	Mean± SE	n	Mean± SE
Overall mean	552	2.98 ±0.06	502	14.34±0.47	**486**	**124.87**±4.73
Genetic group		***		***		***
Purebred (Yemeni sheep)	299	2.49±0.06 [c]	252	12.45±0.45 [c]	251	110.31± 4.74 [c]
½Awassi	65	3.43±0.06 [b]	63	15.56±0.52 [b]	60	133.20±5.59 [b]
¾Awassi	188	3.61±0.07 [a]	187	16.47±0.44 [a]	175	142.89±4.67 [a]
Sex		***		***		***
Male	271	3.08±0.06 [a]	246	14.99±0.44 [a]	239	130.49±4.72 [a]
Female	281	2.96±0.06 [b]	256	13.88±0.44 [b]	247	119.45±4.64 [b]
Weight of Dam		***		***		***
≤25kg	147	2.58±0.07 [c]	124	12.80±0.47 [c]	123	114.67±5.02 [b]
24- 34kg	264	2.94±0.06 [b]	243	13.99±0.45 [b]	233	119.79±4.80 [b]
≥35kg	141	3.63±0.07 [a]	135	17.00±0.46 [a]	130	143.62±4.93 [a]
Season of birth		n.s		***		***
Summer	286	3.02±0.06 [a]	255	14.84±0.44 [a]	255	131.10±4.64 [a]
Autumn	150	2.91±0.07 [a]	131	14.34±0.48 [a]	130	126.45±5.10 [a]
Winter	116	2.99±0.07 [a]	116	13.23±0.46 [b]	101	107.09±4.98 [b]
Year of birth		***		n.s		n.s
Period1 (2013 - 2016)	280	3.04±0.06 [a]	242	14.53±0.44 [a]	241	124.08±4.67 [a]
Period2 (2017- 2020)	272	2.92±0.06 [b]	260	14.13±0.44 [a]	245	125.64±4.71 [a]

n.s: Non-significant, * Significant (P< 0.05), ** Significant (P < 0.01), *** Significant (P < 0.001).

[a-c] Means with different letters in each subclass within a column differ significantly at (P < 0.05). [1] n Means number of records.

Table 2: Effecting of genotype on survival rate of purebred and crossbred lambs

Fixed effect	Survival rate%			
	n	At birth	n	At weaning
Overall mean	594	93.78	577	95.85
Genetic group		*		**n.s**
Purebred (Yemeni sheep)	324	97.52[a]	309	94.50
½Awassi	69	93.76[b]	69	98.56
¾Awassi	201	91.67[b]	199	96.99

[a-c] Means with different letters in each subclass within a column differ significantly at (P < 0.05).

[1] n Means number of records.

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AQUACULTURE OF *HETEROTIS NILOTICUS* IN SUB-SAHARIAN AFRICA: POTENTIALS AND PERSPECTIVES

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Abstract

The study was carried out during the months of October 2019 to July 2020. The goal was to carry out a literature review on aquaculture of *Heterotis niloticus* in order to contribute to a better knowledge of the breeding of commonly named *African bonytongue* in Africa. This review, based on 41 published papers starting from 1980 to 2020 concerning common fish and *Heterotis niloticus* farming research in Africa. The most farmed fish species documented were *Oreochromis niloticus, Clarias gariepinus, Cyprinus carpio* and less documented is *Heterotis niloticus*. *Heterotis niloticus* is microphagous and omnivorous species. The standard and total length of male and female are statistically close but their body weight is different according to gender. The fingerlings of *H. niloticus* grows rapidly with a diet 36% of protein and 6% of lipids. In Africa, the Specific Growth Rate (SGR) of *Heterotis niloticus* varies between 3.22g/day to 4g/day. The gonadosomatic index (GSI) varies to 0.003% -0.6% and to 0.2%-2.2% respectively for male and female, which is correlated with period of spawning. The aquaculture of *Heterotis niloticus* has huge potentials in high fish demanding environment and therefore needs accurate data.

Keywords: Aquaculture, *Heterotis niloticus,* Sub-Saharian Africa, constraints, opportunities.

Introduction

In 2017 for instance, world fish production from fishery was 92.5 million tonnes, compared to 80.1 million tonnes from Aquaculture (FAO, 2019). In Africa, fish production represents 3.64 % of world fish production. This production is very low and insufficient for Africa population which is estimated at 1.3 billion; because fish production is insufficient, its consumption is less than 20.3 kg per capita (FAO, 2018). To complete this demand, local supply depends on frozen fish imports in many countries. Aquaculture is most often done by species introduction in local production systems. All over the world, the introduction of new fish species in the fresh waters is often undertaken with the aim of improving the ichtyological production (Economidis *et al.*, 2000). Since this introduction, *H. niloticus* was reared by fish farmers in Africa, because of its omnivorous diet, good meat quality and relative high commercial value. The aquaculture of these resources is very important for scientist, fishers, fish farmers and consumers. However, its use for aquaculture is limited by the difficulty in the massive production of fingerlings (Monentcham, 2009). In Africa, many authors studied this species on various aspects such as reproduction, feeding, biology and ecology. The current review aims at giving insight to aquaculture of *Heterotis niloticus* in Africa for better research approach.

Methodology

This study was based on wide consultation of 41 published papers starting from 1980 to 2020 to different study on *Heterotis niloticus* in Africa and America. PubMed, Google Scholar and African Journals Online databases were used to search articles published in English and French on *Heterotis niloticus* in Africa. No limit on publication dates was set. Literature search started on October 1st 2019, with an update on July 31th, 2020. Reference list of relevant articles were checked for additional titles for inclusion in the review. Software R was used to analyse some data using Chi-square to separate mean.

Results

Biology of Heterotis niloticus

Heterotis niloticus is the only african species of Osteoglossidae family. African bonytongue is a large freshwater fish native to the Sahelo-soudanese region in northwest Africa. It is found in rivers and lakes of Nilo-soudanian area, Central and West Africa (Moreau, 1982). This species lives at 21.50-30.10°C water temperature, and where oxygen is at 4.8-7.78 mg/l (6.20±2.10 mg/l of water), pH 6.70-7.25 (Monentcham, 2010).

Spatial distribution of Heterotis niloticus

African bonytongue been introduced in many rivers, lakes and trough aquaculture. *Heterotis niloticus* is found in West, East and Central Africa and in America. Because of its relative rapid growth, some other country like CAR, Gabon, Ivory Cost and Madagascar imported this species from Cameroon around respectively 1956, 1959, 1959 and 1963. (Moreau, 1982).

Importance and interest of Heterotis niloticus

The mean value of proximate composition of *Heterotis niloticus* in Nigeria are: moisture content (62.95%), ash (2.40%), protein (20.60), fat (12.20%), carbohydrate (1.85%) (Olanrewaju *et al*., 2016). In Cameroon, Monentcham, *et al*. (2010) found that the body composition of *Heterotis niloticus* rearing in ponds have the following respective composition: moisture content (80.50%), ash (4%), proteins (13.60), total lipid (1.10%), and carbohydrates (1.85%).

Heterotis niloticus farming

- **Growth and feeding**

In Africa, the Specific Growth Rate (SGR) of *Heterotis niloticus* varies between 3.22g/day to 4g/day. For artificial feeding, in Cameroon, the fingerlings of *H. niloticus* grows rapidly with a diet who have 36% of protein and 6% of lipids (Monentcham, 2010).

- **Reproduction**

The Gonad Somatic Index (GSI) of *Heterotis niloticus* varies between 0.52% to2.19% and 0.0035% to 0.35% respectively for females and males. The Gonad Somatic Index (GSI) of the females *Heterotis niloticus* was significantly higher in males at 5 % level according to the countries. The peak of GSI arises during the raining season and it declines progressively throughout the dry season. These indicated that the reproduction period of bonytongues was restricted to the wet and flood period margins. Many authors agree to recognize a definite correlation between the reproduction of *Heterotis niloticus* and the season of the rainfall causing heavy flooding and flooding of surrounding grassy areas which constitute preferred spawning grounds (Kiloso, 2016; Kouakou *et al*., 2016; Adite et al, 2017).

Genetics of Heterotis niloticus in Africa

- **Morphobiometric measurements of *Heterotis niloticus* in Africa**

In Cameroon, no study is done about diversity of this species, but the recent study by Djouatsa (2020) shows that in bimodal zone the diversity of *Heterotis niloticus* is very low but we have two types of colour patterns of body, grey and brown; the dominant colour of eye is golden. These results are similar to those got by Moreau (1982) in Africa. The standard lengths (cm) varies accros the countries: Benin (63.25±1.90), Cameroon (52.55±7.11), DRC (16.24±2.25), Ivory Coast (42.15±4.72) and Nigeria (25.45±6.29) (Adite *et al.*, 2017; Kouako *et al.*,2016 ; Kiloso, 2016, Mohammed *et al.*, 2019; Djouatsa, 2020).

Body weight of Heterotis niloticus in Africa

In West Africa, the body weight of *Heterotis niloticus* varies from 252.7 g to 2812.72g, whereas in Central Africa the body weight varies from 621g to 4505g. In Benin and DRC, males are heavier than females and there is a significant difference in weight between females and males of *Heterotis niloticus* (Adite *et al*., 2017; Kiloso, 2016, Djouatsa, 2020).

Conclusion

Aquaculture of *Heterotis niloticus* in Africa is much diversified because of varied rearing and fisheries patterns of this species. The said species is of nutritive importance for human and their diet in captivity is simple. The feed conversion is good for this species. The standard length and body weight vary with the environment conditions (climate, feeding…). The aquaculture of *Heterotis niloticus* has huge potentials in high fish demanding environment and therefore needs accurate data.

Perspectives for improving aquaculture of Heterotis niloticus in Sub-Saharian Africa

In this study, some core difficulties of *Heterotis niloticus* aquaculture in Africa are not clearly documented. Suggestions therefore focus on following aspects using appropriate tools and methods, including nuclear-derived techniques: cartography of *Heterotis niloticus* in Africa*;* morphobiometric characterisation of *Heterotis niloticus* in some countries (DRC, Ivory Cost, Cameroon); exploitation of *Heterotis niloticus* in Africa; genetic diversity of *Heterotis niloticus* in Africa; sex determination of *Heterotis niloticus* from controlling reproduction (artificial or semi-artificial reproduction), and actualisation of fish Statistics in Africa.

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GENETIC IMPROVEMENT PROGRAMS IN NEPAL– CURRENT STATUS AND WAY FORWARD

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Abstract

Genetic improvement of farm animals is a prime concern over the years for researchers. Several pedigree selection programs using performance recording schemes have been implemented to select outstanding individual animal in order to improve the production and productivity. Similarly, together various assisted reproductive technologies like Artificial insemination, sexed semen, Embryo Transfer have been employed to reduce the generation intervals in farm animals. The main objective is that animal must be genetically merit and productive in its life term in order to yield maximum returns to farmers. The progress achieved during the recent few years through the combination of pedigree selection program and assisted reproductive technologies especially Artificial Insemination has been phenomenal for certain time. However, as in other developing countries, the pedigree selection program has always been a challenge in the village herds of smallholder farmers in Nepal due to lack of animal identification and not having a system in place for recording and analyzing performance data to make appropriate breeding decision are the main constraints that limited the enhancement of animal productivity by breeding. Only advancement in modern biotechnologies such as assisted reproductive technologies, genomics and nuclear techniques will play an important role in the future prospective and vision that increase productivity of farm animals.

Keywords: assisted reproductive technologies, genomics and nuclear techniques

Introduction

Nepal is predominantly an agrarian country where livestock is an integral and important component in mixed farming system. Livestock plays crucial role in providing food (meat, milk, eggs) for human consumption, manure for maintaining soil fertility, major source of power for agricultural operation and wool and hides for cottage industries. Small ruminants (sheep and goats), yak and equines are also important means of transport in the rural road inaccessible areas. Besides, the animals also have socio cultural significance. Nepalese farmers regard livestock particularly small ruminants and non-ruminants as living bank to quench the cash need at the time of crisis. Around 27% of the Agricultural Gross Domestic Products and around 12% of National Gross Domestic Products are contributed by Livestock in the country. Around 3/4th households are keeping cattle, nearly half of the households are keeping buffaloes and more than 50% are keeping goats. The population of cattle, buffaloes, sheep, goats, pig and poultry are 7.2 million, 5.2 million, 0.8 million, 9.8 million, 1.16 million and 48 million respectively (SINA, 2012/13). Dairy is the most important livestock activity, accounting for 62.7% of the total livestock sector value added, followed by meat (32.4%) and eggs (5.0%). Over half of the cattle, buffalo, goats, and sheep are being maintained in the hills, and about one third in the Terai. The native breeds of these livestock species are still contributes significantly to the Nepalese economy, the exotic breeds and their crosses with native are gaining popularity due to population growth, income increases and urbanization which demand increase in the production of food from animal origin. Since a few decades, crossbreeding between indigenous and exotic breed is considered to be the approach for obtaining

exponential increase in productivity while retaining their adaptability to harsh environment and tolerance to tropical diseases. Crossbreeding technology does not always give satisfactory result. The present paper collects different genetic improvement program of farm animals including assisted reproductive technologies that were employed in Nepalese context and draw some recommendation as way forwards.

Genetic improvement through crossbreeding

Cross breeding is the process of breeding with the intention to create offspring that share the traits of both parent lineages or to produce an animal with hybrid vigor. Cross breeding allows the improvement of standard production traits such as egg production, milk production, growth rate and production of total animal protein. Unpredictable result might be obtained if the genetic merit of the animal and the production system is not considered. In Nepal, crossbreeding was introduced since 1960 in a small capacity in cattle (NABGRC, Annual Report, 2019).
Various research findings has concluded that crossbred cattle with 62.5% blood level of Jersey has shown increase productivity in terms of milk with retaining their adaptability to existing farming system. In contrary, Murrah buffalo has exhibited well adaptability in the Nepalese hill and terai environment. Chicken and goats are the species which shows high adaptation potential in different agro-ecological zone, many breeds were introduced and most of them exhibited hybrid vigor. Maintenance of nucleus herd has always been challenge for the government farm as they do not have mandate of research activities thus deteriorating the breeding stock without robust breeding program.

Assisted reproductive technologies

In recent years, livestock productivity has been increased by improved reproduction. Various techniques have been developed and refined to obtain a large number of offspring from genetically superior animals or obtain offspring from infertile animals. These techniques include: artificial insemination, cryopreservation of gametes or embryos, induction of multiple ovulations, embryo transfer, *in vitro* fertilization, sex determination of sperm or embryos, nuclear transfer, cloning, etc. These technologies have been introduced to overcome reproductive problems, to increase the offspring from selected females and to reduce the generation intervals in farm animals.
In Nepal, the progress achieved during the last few years in the assisted reproductive technologies field has been phenomenal in cattle. Artificial Insemination (AI) is the most effective method being used for the genetic improvement of animals. Reproductive capacity and efficiency has been improved tremendously since the introduction of artificial insemination. Embryo transfer technology was familiarized in 2000 using frozen embryo of New Zealand Jersey (NABGRC, Annual Report, 2000). Hitherto ET is done only by the government entities in order to produce pure bred high genetic merit animals. These successful reproductive technologies such as AI and Embryo transfer need be applied on a large scale.

Genetic selection including assisted reproductive technologies

Performance recording is a necessary pre-requisite to effective decision making on breeding policy. Dairy cattle improvement project is the combined program of pedigree performance recording system and assisted reproduction technology was a collaborative program between research (National Animal Breeding and Genetics Research Centre) and extension (National Livestock Breeding Organization) which was led by the research institute (NABGRC, Annual Report, 2009). This program was implemented in organized farms where animals are usually identified plastic tag with unique identity number for each individual animal. The aim of the project is to produce high milk yielder Dam/cow and Sire/bull of Nepalese Jersey and HF within the country. This project was undergone for first two years as TCP assistance of FAO and was considered as one of the most successful TCPs. After completion of TCP, Government of Nepal has given the high priority to this program and has given continuation as extension institute (National Livestock Breeding Organization) as leading organization. The similar

project on buffalo, on the other hand, was not as successful due to various reproductive problems in this species such as silent heat, low conception rate etc and buffalo meat is very popular in Nepal which might be the reason that when animal was not conceive for one season it is more economical to slaughter than wait for next season.

Earlier, pedigree recording and genetic selection in smallholder farmers have been deemed infeasible by researchers and development workers. Due to uncontrolled breeding practices, it is almost impossible to identify sire. Since community based goat breeding program is being successful worldwide (Gizaw et al 2011; Karnuah, Dunga, Rewe, 2018), Nepal has also introduced a pilot community based goat improvement program initiated from 2013 by the Ministry of Agriculture and Livestock Development (MoALD) with the financial support from the World Bank through the Agriculture and Food Security Project (AFSP) and International Fund for Agricultural Development (IFAD) through the Improved Seeds for Farmers Program. The main goals of these projects were to improve the non-descript goats by crossbreeding with Boer goat while at the same time, improving the identified indigenous goat breeds through breeding for genetic merit. This program had centralized the breeding scheme imposed by the government in order to improve the national goat population. The experience so far indicates that the Nepalese government and the private sector need to invest in strategic areas around community based goat improvement programs to make the program work for the poor and be sustainable in low-input systems (KUBK Report, 2019). Most of the local government has implemented the project on improvement of productivity of goat based on same strategy. Further, a community based goat breeding program was piloted by Nepal Agricultural Research Council in western hills and the project is funded by USAID under Livestock System Innovation Lab since 2020. In all above cases, pedigree selection was employed together assisted reproductive technology (Artificial insemination). This combination would help to use selected sire and dam to produce high genetic merit progeny. In this ongoing project, community-based performance and pedigree recording will be evaluated in terms of its reliability and accuracy to estimate genetic parameters, predict breeding values and estimate genetic trends across generations. To counter these challenges, it is crucial to generate genomic data of performance recorded animals, as this enables breeders and farmers to relate production traits with parentage and genetic admixture of animals, thereby allowing them to identify and select superior sires for breeding.

Nuclear and nuclear derived technologies

The intensification of livestock production systems is a prerequisite to meet the growing demand for animal products. Genetic selection, assisted reproductive technologies and optimized reproduction involving nuclear and nuclear-derived technologies support the production of animals that are more productive while retaining their ability to cope in harsh environments (FAO/IAEA, 2008). Improving native breeds through genetic selection in such a way that they retain their adaptability to local environments and their often innate tolerance to local diseases is crucial in addressing the challenge of supplying a persistently growing demand for food of animal origin. Various nuclear and nuclear-derived technologies exist to support such genetic selection procedures.

Radioimmunoassay (RIA) of hormones in milk, blood and other body fluids using iodine-125 is a mature and often used nuclear technique that can be easily performed in decentralized laboratories. It provides unique support in improving outcomes of artificial insemination services which ultimately increase the economic benefits to farmers (Khanal and Munankarmy, 2009). RIA however is still in the research stage.

Nuclear technique also help in breeding. Irradiation with cobalt-60 is used to construct radiation hybrid panels for the mapping of livestock genomes. High resolution radiation hybrid maps facilitate genome assembly by correctly ordering genes and genetic markers along chromosomes. Radiation Hybrid Mapping will ultimately utilized for whole genome mapping which assist the breeders to identify gene for the preferred traits.

Conclusion

Farm animal selection and reproduction are on the threshold of the application of new biotechnologies. Modern biotechnologies such as MOET, IVF and Cloning provides powerful tool for rapidly changing the animal populations, genetically. This advanced reproduction technologies will definitely play an important role in the future perspective and visions for efficient reproductive performance in livestock. Nuclear technique also help in breeding. Radiation Hybrid Mapping has created opening of a fascinating scientific arena.

Recommendation

A 70% increase in the consumption of animal-source protein as food is expected by 2050 due to population growth, income increases and urbanization. Consequently, the world will need to increase livestock production manifolds. Following way forwards can be recommended to catch up ever rocketing demand by brining positive changes in conventional breeding.

i. Since Nepal is using RIA in the research only, application of AI coupled with RIA will help in detecting the pregnancy in the early stage.
ii. Nuclear and nuclear derived techniques for genetic characterization of animals and identification of marker/s that drives favorable traits from parents to offspring.
iii. Genomic toots to be applied to improve livestock productivity by measuring breed composition of crossbred animals, verify purity of purebred animals and match data with appropriate genetics to select superior stocks for breeding.
iv. Farmers to improve productivity through better access to information about animals' performance.
v. Standardized animal identification and data recording procedures need to be in place for implementation of genetic improvement programs.

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NEW GENOMIC RESOURCES FOR A SUSTAINABLE UTILISATION OF OLD AND NEW WORLD CAMELS

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Abstract

Large and small camelids represent a key livestock resource in several countries. Due to their special assortment of biological and physiological traits adapted to extreme and harsh conditions, camelids better than other livestock species can thrive and produce high-quality food and fibre (wool) products. Recently, chromosome-assembled reference genomes of two Old World camel species, the dromedary (*Camelus dromedarius*) and the wild two-humped camel (*Camelus ferus*), as well as two domesticated New World camel species, the alpaca (*Vicuna pacos*) and llama (*Lama glama*), have become available. However, a high-resolution radiation hybrid (RH) chromosome map of the camel genome is still missing, as well as an affordable and easily applicable DNA microarray tool for large-scale phenotype-genotype association studies for traits of interest.
We designed a 180K Affymetrix-Axiom (Thermo Fisher Scientific) custom array for camelids, including 60K single nucleotide polymorphisms (SNPs) for dromedaries, 60K SNPs for two-humped camels (Bactrian camel and wild camel) and 60K SNPs for New World camels (llama and alpaca). This array is currently evaluated at the Animal Production and Health (APH) Laboratory of the Joint Food and Agriculture Organisation (FAO)/ International Atomic Energy Agency (IAEA) Division, Austria. This array will be used to (i) genotype the 5000 RAD dromedary RH panel previously constructed at the APH laboratory to create a high-resolution RH map of the dromedary genome, and (ii) to perform diversity and association studies in camelids of FAO/IAEA partner countries to understand the genetic basis of interesting production traits and local adaptation.
In addition, more camelid genomes sequenced at high coverage are on their way. With these new genomic resources we are a step closer to a sustainable utilisation of Old and New World camels in their challenging environments.