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GENETIC DIVERSITY OF EXON 2 OF OVINE MAJOR HISTOCOMPATIBILITY COMPLEX OF (MHC) CLASS 11 *DRB1*GENE IN SOME NIGERIAN SHEEP BREEDS

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ABSTRACT

The genetic diversity of three Nigerian sheep of breeds was investigated based on the *DRBI* (exon2) an immune gene. Blood samples from 150 sheep (50 each from Balami, Uda, and Yankasa breeds) were collected from Maiduguri Abattoir. The samples were analyzed at the Biotechnology Centre of the University of Maiduguri. The analysis is based on PCR-RFLP; the genomic DNA of sheep was carried out from whole blood sample using DNA extraction kit to extraction DNA. PCR product was incubated and digested using the restriction enzyme Rsa1. The analysis of molecular variance (AMOVA) shows that only 11 percent of the total variations were observed in the sampled population and accounted for population differences, and 89 percent was accounted for within populations. The phylogenetic tree showed that Uda and Yankasa are closely related to each other than the Balami sheep.

Keywords: Sheep breed, *DRB1* Gene, PCR-RFLP, Major Histocompatibility Complex and Genetic diversity

INTRODUCTION

According to FAOSTAT (2022), Africa has a population of over 400 million sheep, representing approximately 33% of the world's total, while the estimated population of sheep in Nigeria stands at over 47 million. In savannah and semi-arid zones, the large Yankasa, Uda, and Balami sheep thrive well (Alaku, 2011). The importance of sheep production in Nigeria cannot be overemphasized. Disease is a major factor that contributes to low performance and genetic selection for disease resistance is the only way to improve performance of animals. The purpose of genetic improvement within-herd performance is a result of increasing the frequency of desirable genes and decreasing the undesirable ones in the herd. Genetic improvement from selection has no relationship with feeding, management or facilities used in a herd Daryl and Steve (2001). Selection for disease resistance is much more difficult than selection for production traits, which can be measured directly or indirectly in each animal. Consequently, in selection of animals for disease resistance, it may be unethical to challenge each animal with a pathogen to determine its disease resistance level.

Genetic selection for disease resistance is important for diseases that has no vaccines nor therapeutic. Selection may focus on diseases of interest as a result of variety of pathogens infecting the host in a similar way. In addition, Gibson and Bishop (2005) observed that genetic improvement is an efficient method of control for the target disease due to adequate genetic variations that existed for disease resistance between or within breeds. Hence, new techniques in molecular genetics are emerging which entails the isolation and identification of DNA markers that are linked to genes for disease resistance. Nigerian sheep have not yet been genetically characterized for disease resistance due to the dearth of information on molecular studies of candidate genes responsible for disease resistance. The new technologies involving DNA analysis are now the methods of choice for livestock characterization. This study aimed study aimed at assessing the genetic variability genetic analysis of the genetic variability of Exon 2 of the ovine MHC class II *DRBI* gene among Balami, Uda, and Yankasa indigenous breeds of sheep in Nigeria.

MATERIALS AND METHODS

Study Area

The study was carried out at the Maiduguri Abattoir and Biotechnology Centre, University of Maiduguri, Borno State. Located on latitude 11° 5' N and Longitude 30° 09 E and an altitude of 354m above sea level. Maiduguri falls within the Sahelian zones of West Africa and it is characterized by the fluctuation of climatic and seasonal variations. Furthermore, the area has a very short period (3–4 months) of rainfall which was recorded to be 645.9 mm/annum, with a long dry season of about 8–9 months (Adamu *et al*., 2008; Raji *et al*., 2010; and Aliyu, 2013).

Sample Collection

Blood samples were collected from a total number of one hundred and fifty (150) adult breed of sheep (50 each from Balami, Uda and Yankasa) via their Jugular vein using syringe and needle (5ml). The samples were preserved in EDTA bottle and put inside a cooler with icepack, stored at -4°C and conveyed to the Biotechnology Centre, University of Maiduguri for DNA extraction.

DNA Extraction

Genomic DNA was extract ed from blood using commercial DNA extraction kit which was provided from Promega (the Wizard genomic DNA extraction kit). Method is based on isopropanol-salt precipitation techniques (Green and Sambrook, 2012). Proteins were precipitated using protein precipitated solution and DNA was subsequently precipitated with isopropanol. 75% of ethanol was used for washing DNA, which was then dehydrated with DNA dehydration solution. DNA was stored at -20^{0}C for short-term and -80^{0}C for long-term storage. DNA concentration was determined using a spectrophotometrically NanoDrop spectrophotometer (Thermo Scientific, Wilmington, DE) in USA. The spectrophotometry analysis is based on the principle that double-stranded DNA absorbs ultraviolet light which was maximally at 260nm (nanometers). The ratio of the absorbance was 26nm and 280nm which was used to assess the purity of DNA. A ratio of ~1.8 is generally acceptable as pure.

Polymerase Chain Reaction for *DRB1* Gene

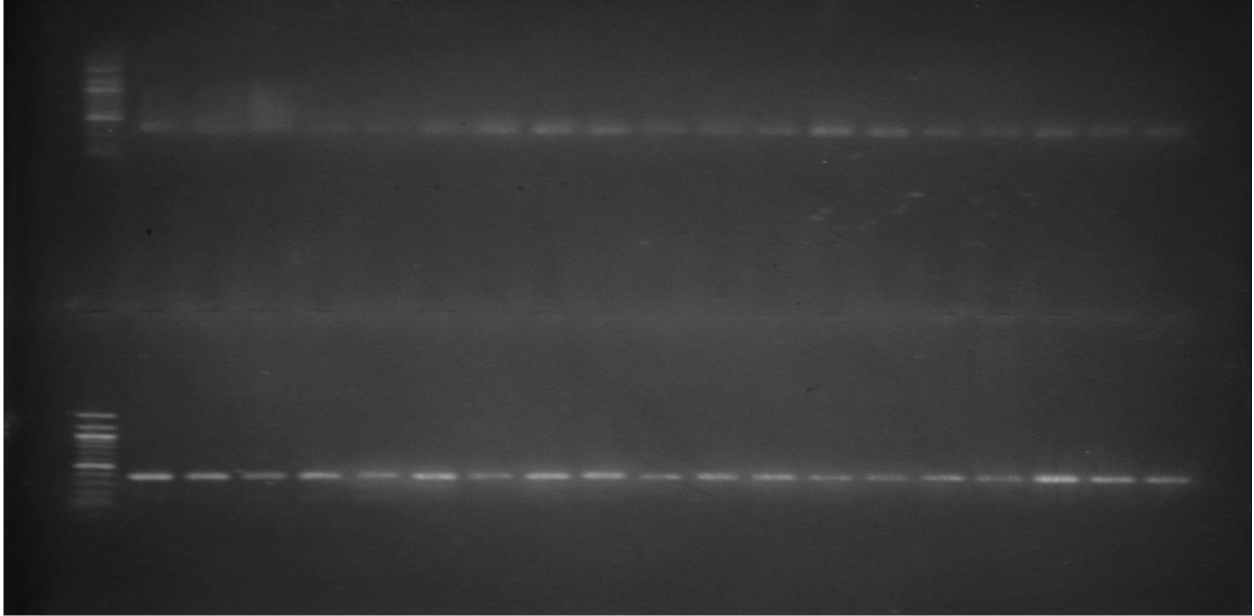
The primer sequence used in amplifying exon 2 of *DRB1* gene and the size of the PCR product (369 *bp*) are shown in Table 1.

Table 1: Primers used in the amplification sheep genomic DNA for *DRB1* gene analysed

DRB1 exon 2 primers	Product size	Annealing temperature (^{o}C)
Fw: 5’-ATTAGCCTCTCCCCAGGAGTC-‘3	*BP*	60
Rev: 5’-CACACACACTGCTCCACA-‘3	369	60

Electrophoresis of Gel

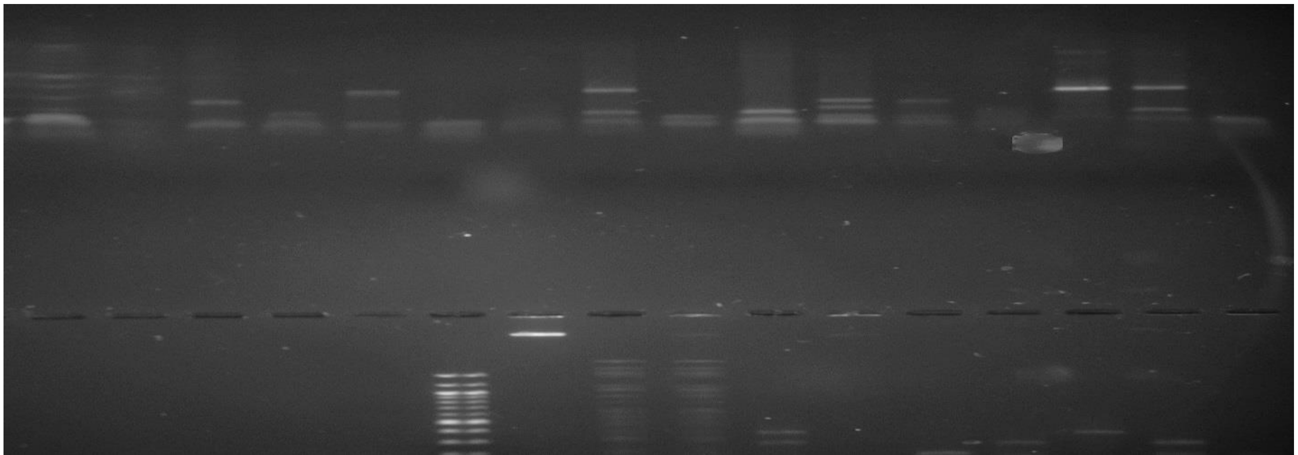
A 1.5% of agarose was prepared by weighing 1.5g of agarose powder and dissolved into 100 ml of 1x TAE buffer and then swirled to ensure thorough mixed. The electrophoresis tank was filled with 1x TAE buffer. Agarose was dissolved in a microwave for about 3–4 minutes and allowed to cool at the temperature of 60^{0}C. The ethidium bromide (10µl) was added and mixed to avoided bubbles. The gel cassette was assembled and the gel was poured into the gel trough. The comb was inserted into a running chamber and solidified for about 30 minutes. The gel comb was carefully removed from the solidified agarose. The set agarose gel was transferred into the gel tanks to ensure that the buffer flood was at least 2mm above the gel. The comb grooves were labeled according to the samples to identify the lines on the gel photography’s. Loading dye (2µl) and 5µl of PCR amplicons were transferred onto a separate sheet of paraffin material and was mixed with 10µl solution in pipette and the mixed solution was transferred to the gel well. The gel well was closed and the power cord was connected to the power source. The gel electrophoresis machine was switched on and allowed to run for 45 to 90 minutes at 120 volts. The progress of the gel was monitored and the resulting bands were visualized with a fitted camera (Canon Power Shot G12 UK) under ultraviolet (UV) trans-illuminator (CSL-MICRODUC system, Clemmer Scientific UK).



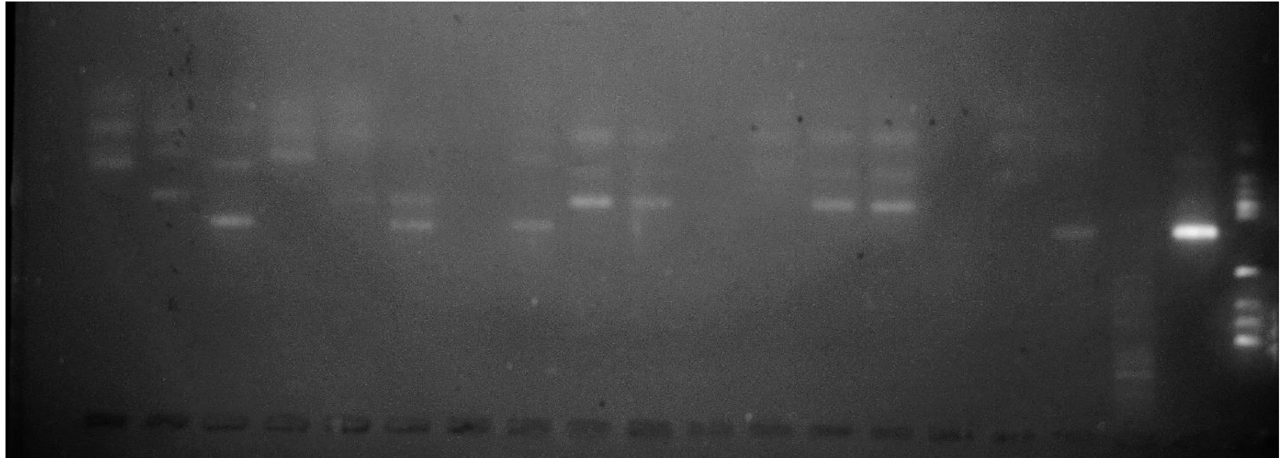
Gel Electrophoresis of PCR Amplicon

Evaluation of Restriction Fragment Length Polymorphism (RFLP)

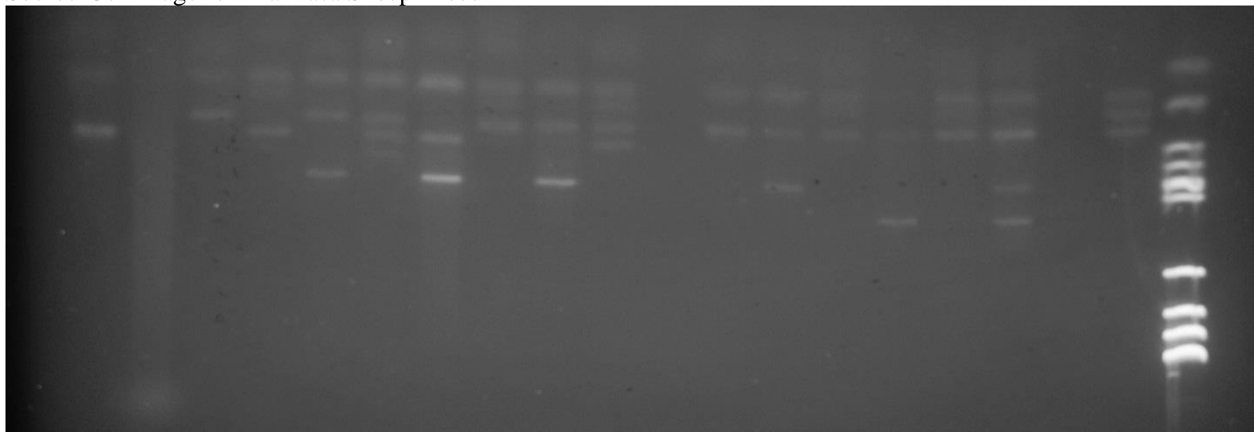
RFLP evaluation was performed by incubating the reaction volume of buffer at 2.5µl, amplicon at 5µl, Rsa1 at 1.2µl and water at 16.3µl, respectively for each sample and allowed it to incubate overnight at 37^0C inside a water bath. The RFLP was photographed with UV trans-illuminator to visualize the size of the fragment and the DNA was cut by the enzymes. PCR-RFLP genotyping was conducted at the online locus for the *DRB1* immune gene.



Scored Gel Image for Balami Sheep Breed



Scored Gel Image for Yankasa Sheep Breed



Scored Gel Image for Uda Sheep Breed

Data Analysis

Analysis of molecular variance (AMOVA) was carried out to determine the genetic variance due to differences among and within the population of each sample. Phylogenetic tree was constructed to compare the genetic relationships among the sheep breeds (Balami, Uda, and Yankasa, using Genpop (REF).The genetic distance (DA) was estimated according to the method of Nei (1987), and the unweighted pair group method with arithmetic means (UPGMA) was used for dendogram construction using the same programme.

RESULTS AND DISCUSSIONS

The genetic relationship among the populations

The analysis of molecular variance (AMOVA) presented in Table 2 revealed that only 11% was observed from the total variation of the population which was accounted for between population differences. The bulk of 89% variations were observed from individual or within population differences rather than between population differences. Similar trend was observed by Agaviazor *et al.* (2012), who observed that 0.23% of the variation was found among the populations compared to 99.7% variation within populations. According to Wang and Jang (2003), the result of pair-wise computation of AMOVA indicated that some breeds are different relative to a random collection of genotypes and reflected the differences in the spatial distribution of genetic variations. Similarly, Pariset *et al.* (2011) found that the primary mitochondrial variation (95.04%) occurred within breeds, whereas the variation among the regions (0.90%) and among breeds within regions (0.90%) and among breeds within regions (4.06%) was significantly lower. In a related study, Gizaw *et al.* (2011) stated that the important characteristics about genetic diversity in livestock populations is that, variation within a population is much higher than that between populations and this is well exemplified in the Ethiopian sheep population, which accounted for only 4.6% of the overall genetic diversity and 95.6% of within-population variation.

Table 2: Analysis of Molecular Variance (AMOVA)

Source	Df	SS	MS	Est. Var.	%
Among Population	2	6.472	3.236	0.113	11
Within Population	59	54.578	0.925	0.925	89
Total	61	61.047	1.038	1.038	100

Genetic distance and identity

The genetic distances and identities of the three breeds of sheep are presented in Table 3. The genetic distances found in this study are within the range reported by Nei's (1987). The report of genetic distance obtained in this study revealed that the Yankasa and Uda populations were closer to each other than Balami. The genetic distance between Balami and Uda is higher. This indicates that they are genetically different from each other due to their adaptability to the environment. While Uda is found in Northern part of Nigeria and Cameroon, central Chad as well as Western part of Sudan as reported by Yunusa *et al*. (2013), Balami is confined to the semi-arid north. This may be attributed to high genetic diversity in a population to the adaptability of genotypes to environmental conditions. Similarly, Ordas and Primitivo (1986) estimated the genetic distance between Spanish dairy sheep breeds in the range of 0.0094 and 0.055 using data from 8 loci. Zonatti *et al*. (1990) observed from four blood groups and six protein-coding loci where they reported a distance of 0.012–0.060 in five Italian sheep breeds. A related study using data on five protein-coding loci by Mwacharo *et al*. (2002) on Kenyan sheep breeds gave a closer genetic distance of 0.044–0.283. Also, Boujenane *et al.* (2008) reported a genetic distance ranging from 0.006-0.026 among six Moroccan local sheep, namely BaniAhsen, Sardi, BeniGuil, Timahdite, D'man, and Boujaad.. Akinyemi and Salako (2012), also discovered that Balami was closer to Yankasa than Uda, based on microsatellite DNA and blood protein loci polymorphism, respectively. This shows differences in efficiency in the use of markers to determine the genetic identity of animals of different breeds. The disparity may be attributed to differences due to geographical locations.

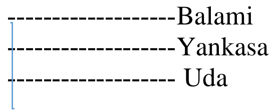
The highest identity (0.9887) was between Uda and Yankasa, compared with 0.9854 (between Uda and Balami) and 0.9883 (between Balami and Yankasa). This indicates that Yankasa and Uda are genetically identical or closer than Balami based on the DRB1 gene. Mohammed *et al*. (2018) made similar observations on the relationship between Yankasa and Uda based on the IGF-1 gene. The phylogenetic tree (Figure 1) indicates that all three breeds were originated from the same source. Balami diverged first followed by Yankasa and lastly Uda. However, Adebambo *et al*. (1999) reported that Nigerian sheep breeds were clearly separated from each other. Indigenous breeds tend to stand out as distinct groups. This is in relation with the well-known evolutionary history of Bovidae subfamily speciation (Floudas, 2007).

Similarly, the closeness between breeds of ruminants based on a particular gene may be as result of limitations in natural and artificial selection pressure that ruminants undergo during selection. Sun *et al.* (2015) reported that the close similarities of gene among breeds may be formed by a recent separation under similar selection pressure which indicated that breeds have suffered during evaluation. The genetic distance and tree construction methods gave insight into the genetic uniqueness of breeds under investigation (Dauda *et al.*, 2016). The Unweighted Pair Group Method with Arithmetic mean (UPGMA) phylogenetic tree (NJ) method clearly revealed that Yankasa and Uda were more closely related than Balami. The genetic identity between Yankasa and Uda was higher, with a range of 0.9887, while it was 0.9854 between Balami and Uda. The dendrogram generated based on genetic distances and identities revealed that Yankasa and Uda were more genetically close than Balami, while Balami and Uda were distinct apart.

Table 3: Genetic Distance (Below Diagonal) and Genetic Identity (Above Diagonal) of Balami, Yankasa and Uda Sheep Breeds

Pop	Balami	Yankasa	Uda
Balami	****	0.9883	0.9854
Yankasa	0.0117	****	0.9887
Uda	0.0147	0.0114	****

Dendrogram Based on Nei's (1987) Genetic distance:



Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

0 5 10 15 20 25

Y

Balami 23
Uda 62
Balami 20
Uda 60
Uda 58
Yankasa 28
Yankasa 32
Yankasa 36
Yankasa 38
Yankasa 27
Uda 45
Uda 52
Balami 21
Yankasa 33
Yankasa 37
Yankasa 25
Yankasa 29
Balami 22
Balami 24
Uda 48
Yankasa 26
Balami 17
Balami 1
Balami 4
Balami 8
Balami 5
Balami 11
Balami 13
Balami 10
Balami 14
Balami 12
Balami 7
Balami 9
Uda 46
Uda 50
Yankasa 39
Yankasa 42
Uda 49
Uda 51
Uda 47
Yankasa 40
Yankasa 43
Uda 44
Yankasa 34
Yankasa 35
Yankasa 30
Yankasa 31
Yankasa 41
Uda 54
Uda 55
Uda 56
Uda 61
Uda 53
Uda 57
Uda 59
Balami 6
Balami 2
Balami 3
Balami 15
Balami 16
Balami 18
Balami 19

Dendrogram of the Relationship among three breeds of sheep

CONCLUSION

The analysis of molecular variance (AMOVA) indicated that only 11% of the total variation observed among the population which accounted for population differences, while bulk (89%) of those variations came from individual differences rather than population differences. It can be concluded that sufficient genetic variation exists in studied Nigerian breeds of sheep and the performance of these populations are unlikely to decline due to inbreeding.

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