Genetic Diversity in three Nigerian Indigenous Goats (Capra Hircus) using Thyroid Hormone Responsive Spot 14 Alpha Gene (THRSPα).

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Abstract: This study was carried out to determine the genetic diversity that exists between three indigenous goat breeds in Nigeria. Thyroid Hormone Responsive Spot 14 Alpha Gene (THRSPα) was used to determine the diversity that existed. A total of 83 goats were sampled. Four (4) ml blood sample were collected from each goat into a 5ml Ethylene Diamine Tetra Acetic (EDTA) bottles and were stored at -4°C for DNA extraction. The genetic distance between Sahel vs. Red Sokoto was 0.0131; Sahel vs. WAD Goat is 0.0146 while that of Red Sokoto vs. WAD Goat is 0.0561. The genetic identity between Sahel vs. Red Sokoto was 0.987, Sahel vs. WAD is 0.9855 while Red Sokoto vs. WAD Goat is 0.9454. The Hardy-Weinberg's Equilibrium, the number of genotype observed and expected for genotype AA was 9 and 11.4083, 9 and 8.4706 and 2 and 4.7647 for Sahel, Red Sokoto and WAD Goat respectively. The genotype AB had an observed and expected genotype of 19 and 14.1833, 6 and 7.0588 and 14 and 8.4706 for Sahel, Red Sokoto and WAD Goat each. Whole for genotype BB, the values were 2 and 4.4083, 2 and 1.4706 and 1and 3.7646 for Sahel, Red Sokoto and. WAD Goat respectively. The Exact Probability across breed was 0.1219 for Sahel, 0.5729 for Red Sokoto and 0.0173 for WAD Goat. In conclusion, the result shows a higher identity rather than diversity which is an indication that there could be a MIXED breeding among these breeds. This result however gives a clearer understanding of the genetic constituent of these breeds that will help in formulating better policies for conservation and improvement of these breeds.

Keywords: Nigerian Indigenous goats, Thyroid Hormone Responsive Spot 14 Alpha Gene, Genetic identity

INTRODUCTION

Goats constitute the largest group of small ruminant livestock in Nigeria totalling about 53.8 million, goats in Nigeria constitutes 6.25% of the world's goat population (FAO,STAT 2011). They also play significant role in the sustenance of the livelihood of rural farmers in the tropics (Ojo, 2014). The Nigerian indigenous goat breeds possess adaptive features that enable them to survive in their different environments. Some of these adaptive features include small body size and short generation interval (Abdul-Aziz, 2010); ability to thrive in harsh climate conditions and presence of trypanotolerance in some breeds (Salako, 2004) and ability to survive on poor quality diets provided by scarce grazing on marginal lands (Adedeji *et al*., 2011). The breeds of goat in Nigeria in order of their importance are Red Sokoto (50%), West African Dwarf (45%) and Sahel (5%) (Osinowo *et al*., 1992). The Red Sokoto (Maradi) are found mainly in the Northern part of the country and well adapted to arid conditions,

the Borno Sahel white are predominantly in the North East region whereas the West African Dwarf spread along the coastal and Southern parts of the nation. The adaptability of these breeds in different zone, present variation in the gene pool and this variation is the basis for conservation of germplasm (Kummar *et al*., 2008).

Genetic diversity plays an important role in the survival and adaptability of a species (Frankham, 2005). When a population's habitat changes, the population need have to adapt to survive; the ability of the population to adapt to the changing environment will determine their ability to cope with an environmental challenge (Putin, 2002).

Thyroid Hormone Responsive Spot 14 Alpha gene (THRSPα) has been regarded as a candidate gene for lipogenesis in domestic animals (Chen *et al*., 2012). Polymorphism in the THRSPα gene has been associated with remarkable growth traits at the P2 locus in Boer goats (Xiaopeng *et al*., 2012). The work of Chen *et al*. (2012) also suggest that adaptability of goats to ecological environment depend on the change in THRSPα gene with fat metabolism regulation which results in reduced fat deposits in muscle and carcass of goats. The fat traits of chicken were related to the polymorphism of THRSPα gene. Though indigenous goat breeds in Nigeria has been classified into three distinct breeds based on the known phenotypic features, there is a problem of knowing the actual genetic make-up of these breeds of goat. Due to the explosion in human population globally, there is emphasis in improving food supply by manipulating both the gene of plants and animals to yield better result. To this end, there is need to know the genetic make-up of these breed of animal (goat) so as to effectively organise improvement and conservation strategies for these goats. Most indigenous breeds of livestock in Nigeria have not been characterized at the molecular level. This research is therefore designed to evaluate the genetic diversity and relationships in three indigenous goat breeds in Nigeria using Thyroid Hormone Responsive Spot 14 Alpha Gene (THRSPα).

Materials and Methods

Experimental location

Experimental location (study area) the research was carried out in the Department of Animal

science laboratory Faculty of Agriculture, University of Port Harcourt.

Study Population

A total of eighty three (83) goats: 33 Sahel (Long leg), 30 Red Sokoto and 20 West African Dwarf goats were used for this study. These goat breeds were sampled from the Trans-Amadi Abattoir in Port Harcourt and Bomu village in Gokana Local Government Area both in Rivers State. Four (4) mls of blood sample was collected into Ethylene Diamine Terra Acetic acid (EDTA) bottle from the jugular vein of each goat stored on ice before they are transported to the laboratory for DNA isolation using ZymoBeadTM Genetic DNA KIT (Irvine, CA.USA) following the instructions of the manufacture.

DNA Extraction protocol using a ZymoBeadTM Genomic DNA KIT

50µl whole blood was collected into a 1.5ml ependoff tube. The ZymoBead™ slurry was fully re suspended by Vortexing. After wards, 200µl of Genomic Lysis Buffer was added to the 50µl of blood then 10µl ZymoBead™ was added and mixed by inversion, and then incubated at room temperature for 5 minutes. Centrifuge the tube at 1,500xg for one minute. Then the supernatant was removed carefully without disturbing the bead pellet.

200µl of Genomic Lysis Buffer was added to the ZymoBeads™ then re suspend the pullet by pipeting up and down, centrifuge at 1,500xg for one minute. Afterwards the supernatural was discarded. 200µl of DNA pre-wash Buffer was added to the ZymoBeads™ then the pellet was re suspended and the transferred into a new 1.5ml ependoff tube and then centrifuges again at 1,500xg for one minute. 500µl of g-DNA wash Buffer was added to the ZymoBeads™ re suspend the pellet and then centrifuge at 1,500xg for one minute. The supernatant was later discarded then centrifuge briefly (for 30 sec) and then remove any residual wash buffer.

45µl of Elution Buffer was added and then re suspend pellet by pipetting up and down, and then centrifuge at 10,000xg for one minute. The supernatant was collected. This is because the supernatant contain, purified DNA that can be used immediately or stored at -200c for later use.

Polymerase Chain Reaction (PCR)

The PCR reaction followed that described by Hirwa *et al.*, 2009. The DNA was amplified via PCR

in a PTC-100 Thermal Cycler (Biorad, Hercules, CA) using forward and reverse primer (deletion R:5'-CGG TCA GAA - GCC TCC GTC ACC GAT CAG-3').The 20µl amplification reaction contained 50ng template DNA, 1.0µlM of each primer. 16µl Nuclease free water in a BIONEER AccuPower® TLA PCR Premix. PCR was performed of 33 cycles of 30 sec at 94°C and 1 min at 72°C after denaturation at 94°C for 2min, final extension was carried out for 10 min. The forward and reverse primers produced a 127 or 136bp. The 136bp is Representative of THRSPα. AA genotype and 127bp is representative of THRSPα BB genotype, which is indicated by 9bp deletion.

Gel Electrophoresis and Scoring of gels.

Ten µl of the PCR product was loaded in a 1.5% agarose gel pre-stained with 0.5µl/ml ethidium bromide. Electrophoresis apparatus (Biorad, Hercules, CA, USA). The resulting amplified bands were visualized with UB light and photographed and were scored using GENEMate Quanti-Marker 100 bp DNA ladder (Bioexpress, UT USA).

Statistical Analysis

The allelic frequency and genotype frequencies were estimated by GENEPOP software package (Raymond and Rousset, 1995). Other genetic analysis is data were performed using PAST. SPSS version 16 and Tools for population Genetic Analyses (TFPGA) version 1.3 (Miller, 1997).

Results and Discussion

The results from analysis of data are shown in the tables below. In Table 1 two alleles were identified in all the populations of indigenous goat breeds examined. Observed number of alleles ranged from 10 – 24 in Red Sokoto, 23 – 37 in Sahel and 16 – 18 in WAD goats. These values were at variant with 4 – 11 reported for Barbari goats (Ramamoorthi *et al.*, 2009); 5 – 11 reported for Brazilian goats using DNA microsatellites (Araujo *et al.*, 2010) Number of heterozygosity was highest in Sahel goat (19.00) versus 6.00 for Red Sokoto. Average heterozygosity in the population ranged from 0.415 in Red Sokoto to 0.498 in WAD. These values represent the average proportion of individuals that are heterozygous for a particular trait in the population. Percent polymorphic loci of 100 were reported for all the goat breeds used in this study. These values were at variance with earlier works reported by other investigators; 69.23

- 93.33 for Iranian Mohair goats using Inter Sequence Repeat (ISSR) Marker (Mohamed et al., 2014); 40.9 – 70 for study of genetic relationship with six Iranian goats using Random Amplified Polymorphic DNA marker (Saeid *et al*., 2007).

Table 2 shows the test of Hardy-Weinberg's equilibrium for the two alleles identified in the population. At the AA locus for Sahel and WAD goats the observed number of genotype was lower than the expected number of genotype whereas the reverse is the case for Red Sokoto goat. The exact probability value ranged from 0.0173 for WAD to 0.5729 for Red Sokoto. These values corroborates the report of Mastrangelo *et al.* (2013) but at variance with the report of Baghizadeh *et al.* (2009) who reported that there was no deviation from the normal Hardy-Weinberg's equilibrium of one (1) in allelic variation of gene studied. Differences obtained may be attributed to difference in population structure of the breeds. Populations with less genetic variability are less adaptable to sudden environmental changes whereas populations showing a great deal of variation will be able to adapt to changing circumstances in the environment (Ojango et al., 2011).

Genetic distances between the three Nigerian indigenous breeds of goat are shown in Table 3 and Figure 1. The closest genetic distance was between Sahel and Red Sokoto whereas the farthest was between WAD and Red Sokoto. Calculation of genetic distances between the breeds indicated a relatively divergent position of the WAD goats, relative to the two other breeds. The reason for this may not be far from the fact that Sahel and Red Sokoto are predominantly found in the arid and semi arid regions of northern Nigeria with almost similar vegetation cover and weather conditions. There is also an indication that Sahel and Red Sokoto goats that are closely related may have a recent common ancestor.

Nei's Genetic identities followed the same trend as genetic distances in Sahel, Red Sokoto and WAD goats. The genetic identities ranged between 0.9454 – 0.9870 among the three breeds with closest identity between Sahel and Red Sokoto goats while the farthest (lowest)exist between Red Sokoto and WAD goats. Figure 1 substantial further the close relationship between Sahel and Red Sokoto as revealed in cluster one of the dendogram while the WAD goats occupied the second cluster.

Conclusion

The results from this study revealed that Thyroid Hormone Responsive Spot 14 Alpha Gene (THRSPα) can be used as a genetic marker for the Nigerian indigenous goat breeds. The result also revealed a higher identity rather than diversity within the three goat breeds studied. There is an indication that there could be mixed breeding among these breeds. This result however gives a clearer understanding of the genetic constituent of these breeds that will help in formulating better policies for conservation and improvement of Nigerian indigenous goat breeds.

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Table 1: Allele, heterozygosity and percentage polymorphic loci for the entire population

	Allele	Observed number of alleles	Allele frequency	Number of heterozygosity	Heterozygosity frequency	Average heterozygosity	Average heterozygosity (unbiased)	Average heterozygosity (direct count)	% polymorphic loci
Sahel	A	23	0.3833	19.0000	0.6333	0.4728	0.4808	0.6333	100.0000
	B	37	0.6167	19.0000	0.6333	0.4728	0.4808	0.6333	100.0000
Red Sokoto	A	10	0.2941	6.0000	0.3529	0.4152	0.4278	0.3529	100.0000
	B	24	0.7059	6.0000	0.3529	0.4152	0.4278	0.3529	100.0000
West African Dwarf	A	16	0.4706	14.0000	0.8235	0.4983	0.5134	0.8235	100.0000
	B	18	0.5294	14.0000	0.8235	0.4983	0.5134	0.8235	100.0000

Table 2 Test for Hardy - Weinberg equilibrium

	Sahel			Red Sokoto			West African Dwarf		
	Genotype	**Observed Number of genotype**	**Expected number of genotype**	**Genotype**	**Observed number of genotype**	**Expected number of genotype**	**Genotype**	**Observed number of genotype**	**Expected number of genotype**
	AA	9	11.4083	AA	9	8.4706	AA	2	4.7647
	AB	19	14.1833	AB	6	7.0588	AB	14	8.4706
	BB	2	4.4083	BB	2	1.4706	BB	1	3.7647
Exact Probability			0.1219			0.5729			0.0173

Table 3. Nei's Genetic Distances and Identities

Populations compared	Distances	Identities	Unbiased distances	Unbiased Identities
Sahel vs. Red Sokoto	0.0131	0.9870	-.0055	1.0055
Sahel vs. WAD	0.0146	0.9855	-0.0083	1.0084
Red Sokoto vs. WAD.	0.0561	0.9454	0.0299	0.9705

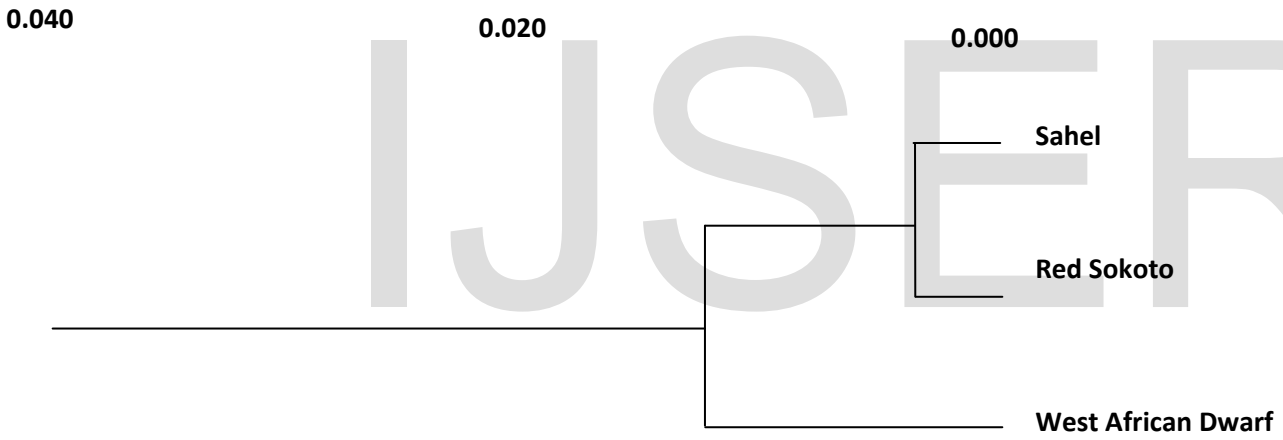

Figure 1: Dendogram showing the genetic diversity among Nigerian goat breeds