Global Journal of Molecular Sciences 7 (1): 11-14, 2012
ISSN 1990-9241

DOI: 10.5829/idosi.gjms.2012.7.1.64168

Haemoglobin Polymorphism and its Distribution in Smallholder Goat Herds of Abuja Nigeria

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Abstract: Blood samples were collected in a heparinized test tubes from 94 smallholder goats in Abuja. The blood samples collected were analysed by electrophoresis to study the Haemoglobin polymorphism and its distribution in smallholder goat herds of Abuja, Nigeria. The electrophoretic test showed the presence of three co-dominant alleles HbA, HbB and HbC which produced 4 haemoglobin phenotypes; A, AB, B and AC. The allele HbC was detected in combination with the allele HbA, however at very low frequency (0.064). The preponderance of HbA allele (0.601) was almost twice it co-dominant allele HbB (0.335). The haemoglobin alleles control the phenotyping of two homozygous (HbAA, HbBB) and two heterozygous (HbAB and HbAC) genotypes. The most wide-spread individuals were heterozygotes allele HbAB (43.62) while the homozygotes allele HbBB had the lowest frequency (11.70%) within the population. In the whole aspect of zygosity, the haemoglobin heterozygotes (56.39%) were more frequent than the haemoglobin homozygotes (43.61%). The foetal haemoglobin type HbAC which migrates slightly faster than the adult type A and B appeared typically in the younger goats of between 1-12 months of age and disappeared thereafter with an insignificant trace beyond this age. The chi-square test showed no significant ($P< 0.05$) differences between observed and expected genotype frequencies, thus indicating that this flock was in Hardy- Weinberg equilibrium for the haemoglobin locus.

Key words: Goats · Haemoglobin Polymorphism · Smallholder Herd

INTRODUCTION

Goat breeds have been variously evaluated for genetic variation based on morphological, physiological, pathological physiology, productive and behavioural features [1, 2]. However, these characters underestimate the true levels of genetic variations. Therefore, the polymorphic variants of different proteins, enzymes, mineral elements or blood group factors represent accurate procedures for a better measurement of genetic variation in caprine species [3].

Blood and blood components are undoubtedly essential biological characteristics and warrant consideration for study of a breed. Studying the hematological picture is helpful for clinical diagnostics [4, 5], but it is also essential to reflect the particular evolution of a breed or a population; actually, the fact that some blood factors are related to the suitability of the breeds under particular environmental conditions has been repeatedly suggested [6-8].

Hemoglobin (Hb) is a large complex protein, consists of four polypeptide chains and one hem [9]. It is one of the most studied polymorphisms in vertebrate species since the infancy of both population and evolutionary genetics because of its accessibility and its obvious biological importance. However, owing to the close relationship between structure and function, this complex protein remains a fascinating subject from all points of view and especially in terms of its molecular, genetic and adaptive features. Accordingly, Hb has been recently defined as “an evergreen red protein” [10].

This paper therefore proposed to describe the genetic structure from the level of determinant locus of haemoglobin in goats belonging to small holder farmer in Abuja Nigeria.

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MATERIALS AND METHODS

The biological materials used for the investigation of the haemoglobin polymorphism composed of 94 goats belonging to local farmers at Dei-dei and Gwagwalada grazing reserved, Abuja-Nigeria. The bloods from the goats were sampled in heparinized test-tubes, by jugular vein puncture. The blood samples were centrifuged at 3000 rotations/minute for three minutes, the plasma supernatant was decanted and then the erythrocyte stored were washed three times with physiological serum solution; after each centrifugation, the washing solution was removed. After the third washing, erythrocyte store was mixed with 1 ml of haemolyset (red cell lysing agent) to lyse the blood cells and released the content of the erythrocyte including the haemoglobin.

The haemoglobin solutions resulted from the haemolysed red cells were ready for electrophoretic analysis to separate the globin fractions of the β-chains of haemoglobin.

At the end of an electrophoretic run, there were one or more bands on the acetate strip for each sample. Each band represents one type of haemoglobin. The haemoglobin was identified by comparing with the positions of the haemoglobins on the control sample. Human haemoglobin AA and AS were used as control for the first few samples, this helped in developing a control for the caprine samples. On developing the caprine control which was the caprine haemoglobin AA and BB, the procedures were repeated for all the samples. A haemoglobin gene, when subjected to electric current becomes negatively charged and migrates toward the anode. The genotype that migrated faster was labeled HbAA while the slow moving fraction was identified as Hb BB. The heterozygote (consisting of both slow and fast bands) was HbAB. However, there was another band HbC (in combination with HbA) found in some samples which was identified as foetal haemoglobin. This foetal haemoglobin type Hb C migrates slightly faster than the adult type A and B.

The frequencies of genes and genotypes from the Hb locus of goats were calculated. Chi square ($\chi 2$) test was used to estimate the conformation mode of the goat population to the Hardy-Weinberg genetic equilibrium law. Hardy-Weinberg's equilibrium used for testing the significance of genotypic ratios was based on the expansion of the binomial equation $(p + q)^2 = p2 + 2pq + q2$.

Table 1: Allele and genotype frequency at haemoglobin locus

Genotype	Number of Genotype	Genotypic frequency	Alleles	Allelic frequency
AA	30	0.3191	A	0.601
AB	41	0.4362	B	0.335
BB	11	0.1170	C	0.064
AC	12	0.1277		

RESULTS AND DISCUSSION

The electrophoretic test showed the presence of 4 haemoglobin phenotypes; A, AB, B and AC (Table 1). These four haemoglobin phenotypes were produced by three co-dominant alleles HbA, HbB and Hb C. The allele HbC was detected (in association with the allele HbA) however at very low frequency (0.064), this observation conforms to earlier reports of Blunt [1110] and Pieragostini *et al*. [5]. The three haemoglobin alleles (A, B, C) had differential spreading within the breed; the allelic frequencies showed that the preponderance of HbA allele (0.601) was almost twice it co-dominant allele HbB (0.335). The haemoglobin alleles control the phenotype of two homozygous (HbAA, HbBB) and two heterozygous (HbAB and Hb AC) genotypes. The spreading of the haemoglobin genotypes in the goat population is relatively unbalanced. Thus, the most wide-spread individuals are heterozygotes for the allele HbAB (43.62%), a little less than half of the goat population being heterozygous for this allele. The homozygotes for the allele HbB had the lowest frequency (11.70%) within the population. In the whole aspect of zygosity, the haemoglobin heterozygote (56.39%) was more frequent than the haemoglobin homozygotes (43.61%). The neonatal or foetal allele HbC, that determines the synthesis of haemoglobin HbAC, had also been reported by Salako *et al.* [1] in Red Sokoto goats, Johnson *et al*. [12] in Omani goats and Deza *et al*. [13] in goats of Colon and Ischlin. Salako *et al*. [1] and Deza *et al*. [13] associated the observed HbC with incidence of anemia due to illness and environmental stress, while Johnson *et al*. [12] described it as a pre-adult form of haemoglobin.

In this study however, the neonatal or foetal haemoglobin type Hb AC which migrates slightly faster than the adult type A and B appeared typically in the younger goats of between 1-12 months of age and disappeared thereafter with an insignificant trace beyond this age (Table 2). The time of disappearance of this foetal haemoglobin in the blood need further studies. However, in an earlier study by Hubbert *et al*. [14] on "developmental polymorphism in Bovine haemoglobin" they observed 3 categories of haemoglobin; embryonic,

Table 2: The distribution of goats according to haemoglobin types in association with age

Age (months)	AA	AB	BB	AC
1-12	16	8	21	10
13- 18	11	2	11	1
19- 24	3	1	7	1
25-30	0	0	2	0

X^2 value =12.71; df = 9, Table value at 5% = 16.92; ns

Table 3: Observed and expected number of Haemoglobin Genotype in Red Sokoto goats

Sample size	Hb-phenotype	Observed	Expected	X^2 df=3
94	Hb AA	30	31.33	0.31004ns
	HbAB	41	41.78	
	HbBB	11	10.44	
	HbAC	12	10.44	
	Total	94	93.99	

foetal and adult haemoglobin, in which the time of the disappearance of the foetal haemoglobin from the blood varied from 21 weeks to at least 10 weeks of age postpartum. Also, Lorkin [15] reported that the amount of HbC present at birth is generally about 70-90% of the total haemoglobin and that this declines during the first 12 months after birth; less than 8% at 6 month and less than 2% at 12 months. Also, studies confirmed this observation; Brandis [16] reported that about 80% of the neonatal haemoglobin is foetal, this decrease rapidly so that by 6 month of age less than 5% of the haemoglobin is foetal and only very small amount (<1% of total Hb) are present in adult. Similarly, Zartal- Zidani *et al*. [17] reported that the developmental switch from foetal to adult haemoglobin occurs just before births and that the foetal haemoglobin constitutes less than 1% of the total Hb in normal animal by the end of first year. Therefore, the presence of foetal haemoglobin in the blood of young goats does not necessarily suggest incidence of anemia or illness especially in the kids of less than 6 months of age. It could however indicate abnormality if it persist in high percentage in the blood beyond the neonatal age. Hubbert *et al*. [14] reported that the persistence of foetal haemoglobin was observed in clinically abnormal cows. The appearance of a given haemoglobin type in the peripheral blood may be related to the predominant site of hematopoiesis; liver and bone marrow. It has been suggested that the period of activity of the respective sites governs the time sequence of appearance of the haemoglobin. It is evident that the normal pattern of Hb development is under genetic control, resulting in an expected time sequence for the switch mechanism to function.

To test the conformity of the haemoglobin locus of the flock to Hardy- Weinberg equilibrium, Chi-square analysis for the differences between observed and expected genotype frequencies was done and the results showed that the deviation was not significant (Table 3). The chi-square test yielded X^2= 0.31004, a non-significant value (P <0.05) for 3df. Thus we may consider this flock to be in Hardy- Weinberg equilibrium for the haemoglobin locus. This means that random mating occurred for the system under study (haemoglobin) and artificial selection has not much been practiced by the smallholder goats farmers in the study area.

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