

Research Article

DOI: <http://dx.doi.org/10.22192/ijamr.2026.13.09.004>

Investigation of hemoglobin polymorphism and its association with phenotypic traits in indigenous Afar goat breed in selected districts of Afar Region, Ethiopia: A need for characterization

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Abstract

Keywords

far goat,
characterization,
hemoglobin,
polymorphism,
morphometric trait

Afar goat breed is the one the most important livestock asset of Afar community and contributed for cash income and source of food. The present study aimed to investigate the biochemical polymorphism of Hemoglobin and its genomic signature in Afar goat breed. For this study, a total of 200 blood samples were collected by jugular vein puncture from 123 female and 77 male goats. The sample was subjected to agarose gel electrophoresis (pH range 8.4-8.5) to study the polymorphic activities of haemoglobin. The results of the present study showed three types of genotypes (Hb^{AA} , Hb^{AB} and Hb^{BB}). Among the three genotypes the most frequent genotype of Hb^{AA} (0.535) was observed. The least frequent genotype Hb^{BB} appeared at gene frequency of 0.17.. The gene frequency of Hb^A allele was 0.68 and that of Hb^B allele was 0.32 in Afar goat breed. The study revealed that the population is not under Hardy-Weinberg equilibrium. This implies there is a force that affect gene and hence genotype frequency in a population i.e. small size of population, nonrandom mating and selection for certain genotypes and mutations may lead to the violation of Hardy Weinberg equilibrium principles. In this study the highest frequency of Hb^A allele may contribute to the breed since it is suggested that Hb^A allele has effect on blood parameters such as hemoglobin and packed cell volume and positively influence the resistance of animals against diseases such helminthic infestation and environmental adaptation. So, further research should be conducted to characterize Afar goat breed at DNA level.

1. Introduction

In Ethiopia, more than 85% of the human population depends on agriculture for their livelihoods (Solomon, 2014) and usually keep livestock as pastoralists or in mixed crop livestock systems. Livestock are an important section of agriculture in Ethiopia and provides milk, meat, draught power, transport, manure, hides, skins (Funk *et al.*, 2012). Ethiopia is endowed with huge livestock biodiversity adapted to varied agro ecological conditions. Among the farm animal genetic resources, indigenous goats have unique adaptive mechanisms which enable them to fit in varied agro ecologies and contribute to the livelihood of small holder farmers through producing valuable milk and meat products (Yakubu and Aya, 2012)

Ethiopia is home of about 52.5 million goats in which nearly all are of local breeds (CSA 2020/2021). They are reared in a crop-livestock and agro-pastoral farming systems, and are widely distributed across different agro-ecological zones of Ethiopia (Dea *et al.*, 2019). Goats are the most important livestock species for many smallholder farmers and pastoral communities due to their low maintenance requirements, excellent prolificacy, short generation interval, and potential to adapt a wide range of agro-ecological zones. Moreover, in areas where there is a financial and physical limitation for resources to keep large ruminants, goat milk production is valued as alternative source of income in the livelihood of the rural community (Hiwot Desta *et al.*, 2020). Goats (*Capra hircus*) are an integral part of economic and social life especially in developing countries. Goats also can have a role in tradition, social status, social payments, rituals and ceremonies, bride price, insurance, status display, dispute compensation and as a mobile bank (Arineitwe and Ndyomugenyi, 2013). Afar goat breed locally known as ‘widar’ also called Adal or Danakil kept mainly under pastoral extensive management system. Afar goat plays an important role in the livelihood of Afar pastoralists as source of cash, meat and milk.

Recent developments in biotechnology provide the opportunity for new approaches in the characterization, conservation and sustainable utilization of animal genetic resources (AnGR) (Gama and Bressan, 2011). A number of blood protein systems have been found to exhibit heterogeneity in different species. One of the important blood proteins is hemoglobin, an “evergreen” red protein (Bettati *et al.*, 2009) that has attracted attention because of its biochemical, biophysical and physiological properties, having relevance to the selection phenomenon of animals. Moreover, protein polymorphisms remain tremendously useful, especially in developing countries like Ethiopia. This is because of their utility, ease, cost, and amount of genetic information accessed, or simplicity of data interpretation (Akinyemi and Salako, 2012).

1.1. Statement of the Problem

Regardless of its paramount contribution particularly for the pastoral community, goat breed characterization has received very little attention. Although some studies were conducted on goat breed characterization using phenotypic variations in Ethiopia (Halima *et al.* 2012; Hulunim *et al.* 2015; Dereje *et al.* 2019), the Afar region with high goat production potential were not adequately represented in the study in general and molecular characterization in particular. Even though there were some attempts have been made at the morphological characterization of goats in Afar region (Theodros *et al.*, 2014) and reproductive performance and trait preferences (Amare *et al.*, 2021), there is a dearth of information on molecular characterization particularly using hemoglobin polymorphism in Afar region in general and study districts in particular. Therefore the present study aimed to characterize indigenous Afar goat breed using hemoglobin variants and also assesses its association with phenotypic traits in Afar region in general and study districts in particular.

1.2. Justification of the Study

According to FAO (2007) changes in population type and structure need to be documented regularly for all breeds at intervals of about five years for cattle, buffalo, sheep and **goats**. Characterization is the primary step to be undertaken for the sustainable utilization of the available animal genetic resources through conservation. Data obtained from this study can also provide valuable information on the suitability of animals for genetic improvement programs through selection. Identifying and characterizing indigenous Afar goat populations which are kept under extensive management condition is very important to integrate animals into various production systems and to make effective use of their potential. Moreover it is high tie to undertake characterization at genetic level; since decrease of genetic variation among farm animal and loss of genetic variation within and between breeds is detrimental not only from the perspective of culture and conservation but also of utility since lost genes may be of future economic importance and that genetic diversity when lost cannot be replaced (Ige *et al.*, 2013)

1.3. Objectives

1.3.1. General objective

- ❖ Investigation of the genetic structure from the level of the determinant locus of hemoglobin variants in Afar goats breed and its association with phenotypic traits

1.3.2. Specific objectives

- ✓ To quantify genetic diversity within Afar goat breed using hemoglobin using polymorphism
- ✓ Investigation of hemoglobin polymorphism in Afar goat breed
- ✓ To assesses the association between hemoglobin polymorphism and phenotypic traits

2. Materials and Methods

2.1 Description of the Study Areas

The study will be conducted from December, 2022 to May, 2024 in zone one particular selected districts namely; (Chifra, Dubti, Asayita and Afambo) Afar Region, Ethiopia. The Afar Pastoral Region is located in northeast of Ethiopia between 39° 34' to 42° 28'E longitude and 8° 49' to 14° 30' N latitude. The region shares common international boundaries with Eritrea in the northeast and Djibouti in the east and it is characterized by an arid and semi-arid climate with low and erratic rainfall. Rainfall is bi-modal throughout the region, with a mean annual rainfall below 500 mm in the semi-arid western escarpments and decreasing to 150 mm in the arid zones to the east. The altitude of the Region ranges from 120 m below sea level in Danakil depression to 1500 m above sea level. Temperatures vary from 20°C in higher elevations to 48°C in lower elevations. The region has a great potential of livestock resources comprising of 2.3 million cattle, 4.3 million goats, 2.5 million sheep, 0.8 million camels and 0.19 million equines that support the region and contributes to the national economy (CSA, 2010).

2.2 Study population

The study populations will be indigenous Afar goat breed which is kept by the Afar-ethnic group in the study districts during the study period.

2.3 Study Design

In the current study, a cross sectional study design will be employed for investigation of hemoglobin and association with morphometric traits in the study areas during the study period.

2.4 Sample size determination

Taking into Consideration the type data is genetic, laborious laboratory work, logistic related issues and time taking into consideration; a total of 200 representative blood samples and

with their record morphometric data will be collected from indigenous afar goat breed.

2.5 Sampling Technique and Sample Collection Approach

Multi-stage purposive sampling techniques will be applied to select the study districts, passant associations, and individual animals. In the first stage, three districts namely Chifra, Asayita, Dubti and Afambo were selected purposively based on their goat population and accessibility of transport and convenient of the area. In the second stage, three passant associations from each district will be selected randomly. In third stage, adult goats will be selected purposively to increase accuracy for quantitative traits since morphometric trait fully grown at adult stage of an animal. The final stage, simple random sampling will be used to select individual goat from adult one.

2.6 Phenotypic data collection

For sampling goat population, castrated goats, pregnant doe, kids, buck kids and doe kids were not include in the sample goat population to increase accuracy for quantitative traits and to represent the adult goat population (12 month to 4 years) according age classification of goat by Girma and Alemu, 2008 (annex 2) but in this study an average age of 3-4 years old goats will be selected to minimize confounding factors due to age variation in phenotypic data. Subsequently, sampled goats will be taken by using simple random sampling method. Record data for each animal according to the format adopted from the standard breed description list developed by FAO, 2012 (annex 3). Body measurements (cm) namely, heart girth (HG), body length (BL) and horn length (HL) and body weight (BW) measurement (Kg) will be measured. The coat color of each animal was also recorded for qualitative data. The anatomical reference points and procedure are as adopted by Yakubu *et al.*, 2011 (Annex 1)





2.7 Blood Sample Collection and transportation

Five ml of Blood sample will be collected from the jugular vein of the sampled goats. Blood samples will be collected following the international guidelines for ethical treatment. Blood samples will be drawn separately into vactioners containing Ethylene-diamine-tetra-

acetic acid (EDTA) as anticoagulant, using vactioner needles. The samples will be properly labeled in line with their phenotypic trait of an animal. The sample will be kept at 4°C for transportation in icebox (OIE, 2008) National Veterinary Institute (NVI) for laboratory analysis. Once the samples arrived at the laboratory, they will be placed at -20°C until processed.



2.8 Hemoglobin Genotyping

In this study the laboratory procedure followed same technique used by Chuku and Uwakwe, 2012 and Sanjoy and Yesihak, 2014 i.e. Red blood cells will be separated from whole uncoagulated blood by addition of equal volume of normal saline (9.0g of NaCl in 1 litre deionized water) and allow standing for 30 minutes. The washing solution will be removed by decanting. The separated RBC is finally lysis by addition of two volumes of deionized water, exposing the haemoglobin. Haemoglobin genotypes will be determined by agarose gel (1%) (Weigh 1 g agarose and dissolve it in 100 mL of 0.5x TBE

Buffer) electrophoresis. Carefully transfer the gel to the electrophoresis tank and filled with 0.5x TBE buffer of pH 8.4 - 8.5. A sample size of ten microliters will be loaded in the wells and applies constant voltage of 200 volts and allow running for about 2 hrs. After completion of the electrophoretic run the haemoglobin pattern can be read directly on the gel without staining and motility will be detected based on the molecular weight of the hemoglobin molecules one fast homozygous band (Hb^{AA}), one slow homozygous band (Hb^{BB}) and two heterozygous bands with intermediate migration level for the (Hb^{AB}) (Chuku and Uwakwe, 2012).



2.9 Statistical Analysis

Data on quantitative and qualitative variables will be analyzed using statistical analysis system of SPSS version 21. Descriptive statistics, chi-square test will be carried out to detect statistical differences among sampled Afar goat breed. Data obtained from Hb alleles and genotypes will be analyzed using descriptive statistical tool i.e SPSS version 21 to estimate the gene and genotype

frequencies at the haemoglobin locus. Genotype frequencies will be subjected to Chi-square analysis to test for goodness of observed and expected frequencies under Hardy-Weinberg equilibrium (HWE) (Ukwu *et al.*, 2018 and Abdulraheem *et al.*, 2018). The t-test will be employed to determine the effects of haemoglobin variants on the phenotypic traits of Afar goat breed.

Genotype frequencies will be calculated as follows

$$\text{Genotype frequency of Hb}^{AA} (P) = \frac{\text{No of individuals with Hb}^{AA}}{\text{Total no of individuals sampled}} \times 100$$

$$\text{Genotype frequency of Hb}^{AB} (H) = \frac{\text{No of individuals with Hb}^{AB}}{\text{Total no of individuals sampled}} \times 100$$

$$\text{Genotype frequency of Hb}^{BB} (Q) = \frac{\text{No of individuals with Hb}^{BB}}{\text{Total no of individuals sampled}} \times 100$$

Gene frequencies will be calculated according to Hardy-Weinberg equation as follows:

$$p = P + \frac{1}{2} H$$

$$q = Q + \frac{1}{2} H$$

Where,
 P= Genotypic frequency of allele AA
 H= Genotypic frequency of allele AB
 Q= Genotypic frequency of allele BB
 p= frequency of allele A
 q= frequency of allele B
 N= Total number of individuals sampled
 N_{AA}= Observed genotype number for AA
 N_{AB} = Observed genotype number for AB
 N_{BB}= Observed genotype number for BB

Three genetic diversity parameters namely: Heterozygosity (H), effective number of allele (ne) and percentage polymorphic (%P) will be used to assess genetic variability in the study

$$\text{Heterozygosity (H)} = 1 - \sum_{i=1}^n x_i^2 \text{ and } \text{the effective number of allele (ne) per locus was estimated as: } 1/1-H$$

Where,
 X = the gene frequency of the *i*th allele in a locus
i= the number of tested loci

Polymorphicity (%): a locus is polymorphic if the segregating genes are more than one and frequency of the rarest gene is at least 0.01% (Nyamsamba *et al.*,2003).

The model used for statistical analysis of association between hemoglobin polymorphism and morphometric trait as $Y_j = \alpha + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + e_j$

Where, Y_j = the response variable (hemoglobin polymorphism) , α = the intercept
 X1..., X3 =explanatory variables (body length, heart girth and body weight and β_1 ..., β_3 = regression Coefficients of the variables X1..., X3, e_j = random residual error.

3. Results

3.1 Phenotypic Data

Both phenotypic data and blood samples were taken from 200 Afar goat breeds (123 from male and 77 female) to conduct this research. A phenotypic data taken from Afar goat exhibiting specific descriptor states of quantitative and qualitative characters as showed (Table1). Majority of Afar goat breed were white type

(34.5%) while minority were found brown and white (7.5%) (Table1). Most of Afar goat breed have possessed horn size of range <20cm (46%) but some goats were found a horn size 30 to 40 cm (20.5%). of zebu cattle have horn with large size (Table1). Regarding to body length, heart girth and body weight; majority of Afar goat breed were found with a range of 50-60cm (47%), 60-70cm (48.5%) and 20-30 kg respectively (Table1).

Table 1: Frequency distribution qualitative and morphometric traits of Afar goat breed

Characters	Attributes	No. animal observed	Percentage
Coat color	Black	17	8.5%
	Black and white	18	9%
	Brown	36	18%
	Brown and white	15	7.5%
	Red	24	12%
	Spotted	21	10.5%
	White	69	34.5%
Horn size	>30 cm	41	20.5%
	20-30 cm	67	33.5%
	<20 cm	92	46%
Body length	>60 cm	75	37.5%
	50-60cm	94	47%
	<50cm	31	15.5%
Chest girth	>70cm	51	25.5%
	60-70 cm	93	48.5%
	<60cm	56	28%
Body weight	>30kg	55	27.5%
	20-30kg	97	48.5%
	<20kg	48	24%
Total		200	100%

3.2. Genotypic Data

Hemoglobin loci were found to be polymorphic in afar goat breeds and three types of genotypes, a slow moving (Hb^{AA}) band, fast moving (Hb^{BB}) band and a combination of slow + fast moving bands (Hb^{AB}) were detected (figure2).

Figure 1: The observed haemoglobin polymorphism before gel electrophoresis

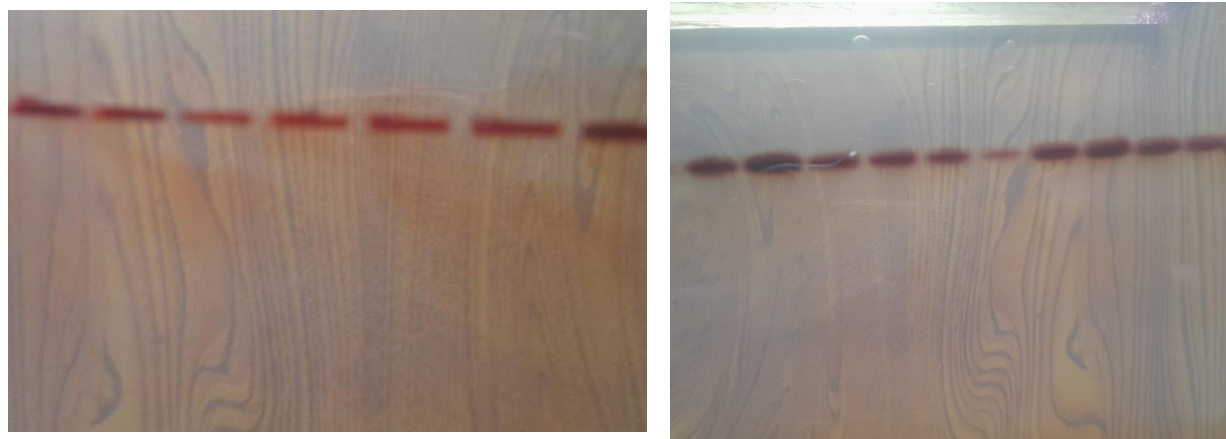
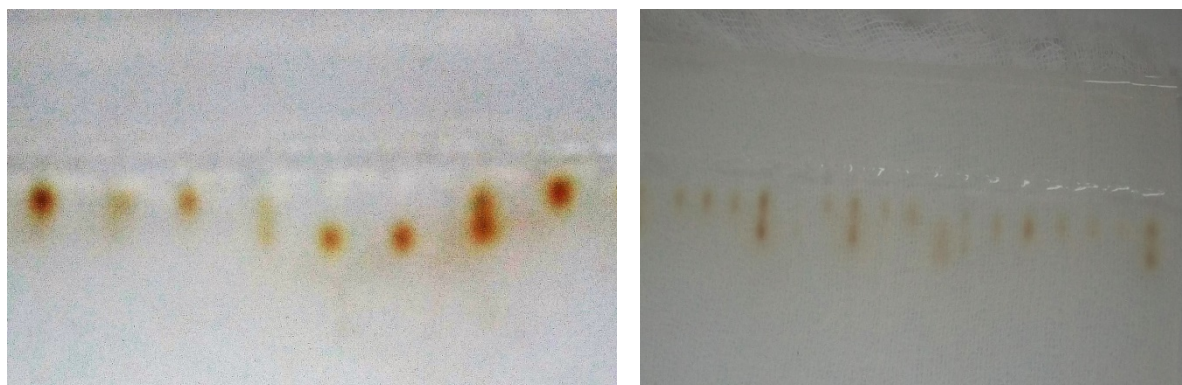


Figure 3: The observed haemoglobin polymorphism after gel electrophoresis



The present study revealed that among the three genotypes the most frequent genotype Hb^{AA} (0.535) and least frequent Hb^{BB} (0.17) were observed (Table 2). The overall heterozygosity of

Afar goat breed was 0.6 with an effective number of allele of 2.5; which is an indication of moderate genetic diversity at the hemoglobin locus in Afar goat breed.

Table 2: Allele and genotype frequency distributions of blood proteins across the breed

Genotype	Allele frequency	Genotype	Genotype Observed	Genotype Frequency	Heterozygosity (HE)	Effective no of allele (NE)
HB	A=0.68 B =0.32	Hb^{AA}	107	0.535	0.6	2.5
		Hb^{AB}	59	0.295		
		Hb^{BB}	34	0.170		
Total	1.00		200	1.00		

The results of this study indicated that three types of hemoglobin genotypes were observed across the sex of Afar goat breed (Table 3). Even though, there was apparent difference of genotype and

allele frequency across the sex of animal, the hemoglobin polymorphism is not found statically significant at ($P > 0.05$).

Table 3: Allele and genotype frequency distributions of heamoglobin across the of animals

Sex	Allele frequency	Genotype	Genotype Observed	Genotype frequency	$X^2df=2$
Female	A=0.40	Hb ^{AA}	59	0.295	0.225 ^{ns}
		Hb ^{AB}	39	0.195	
		Hb ^{BB}	25	0.125	
Male	A=0.31	Hb ^{AA}	48	0.240	
		Hb ^{AB}	20	0.100	
		Hb ^{BB}	9	0.045	
Total	1.00		200	1.00	

Heamoglobin genotypes were found polymorphic across the coat color of Afar goat breed with most frequent genotype of Hb^{AA} (0.155) and allele of Hb^A (0.21) in white coat color of Afar goat breed. But only two types' heamoglobin genotypes (Hb^{AA} and Hb^{BB}) were observed across the coat

color of brown and white (Table 4). Heamoglobin genotypes were found polymorphic across the coat color of Afar goat breed. But the difference is not statically significant ($P > 0.05$) irrespective of coat color of animal.

Table 4: Allele and genotype frequency distributions of heamoglobin across the coat color of Afar goat breed

Coat color	Allele frequency	Genotype	Genotype Observed	Genotype frequency	$X^2df=12$
Black	A=0.0525	Hb ^{AA}	8	0.04	0.56 ^{ns}
		Hb ^{AB}	5	0.025	
		Hb ^{BB}	4	0.02	
black and white	A=0.05	Hb ^{AA}	5	0.025	
		Hb ^{AB}	10	0.05	
		Hb ^{BB}	2	0.01	
Brown	A=0.138	Hb ^{AA}	21	0.105	
		Hb ^{AB}	13	0.065	
		Hb ^{BB}	2	0.01	
brown and white	A=0.065	Hb ^{AA}	13	0.065	
		Hb ^{AB}	0	0.00	
		Hb ^{BB}	2	0.01	
Spotted	A=0.077	Hb ^{AA}	13	0.065	
		Hb ^{AB}	5	0.025	
		Hb ^{BB}	3	0.015	
White	A=0.21	Hb ^{AA}	31	0.155	
		Hb ^{AB}	22	0.11	
		Hb ^{BB}	16	0.08	
Total	1.00		200	1.00	

Heamoglobin genotypes were found polymorphic across the body weight of Afar goat breed with most frequent genotype of Hb^{AA} (0.255) and allele of Hb^A (0.272) in animals having > 30kg (Table 5). But only two types' heamoglobin

genotypes (Hb^{AA} and Hb^{AB}) animal having a kg of >30. The difference also found hemoglobin polymorphism is statically significant ($P < 0.05$) (Table 5).

Table 5: Allele and genotype frequency distributions of heamoglobin across the body weight of animal

Body weight	Allele frequency	Genotype	Genotype Observed	Genotype frequency	X ² df=4
>30	A=0.225	Hb ^{AA}	35	0.175	0.003
		Hb ^{AB}	20	0.1	
	B=0.05	Hb ^{BB}	0	0.0	
20-30	A=0.2725	Hb ^{AA}	51	0.255	0.095
		Hb ^{AB}	19	0.095	
	B=0.1825	Hb ^{BB}	27	0.135	
<20	A=0.1725	Hb ^{AA}	23	0.115	0.115
		Hb ^{AB}	23	0.115	
	B=0.0585	Hb ^{BB}	2	0.001	
Total	1.00		200	1.00	

Heamoglobin genotypes were found polymorphic across the heart girth of Afar goat breed with most frequent genotype of Hb^{AA} (0.245) and allele of Hb^A (0.322) in animals having 60-70cm heart

girth (Table 6). Even though hemoglobin genotype were polymorphic across the heart girth of Afar goat breed; the difference was not found statically significant ($P > 0.05$) (Table 6).

Table 6: Allele and genotype frequency distributions of heamoglobin across the body heart girth of animal

Heart girth	Allele frequency	Genotype	Genotype Observed	Genotype frequency	X ² df=4
>70cm	A=0.1825	Hb ^{AA}	35	0.175	0.38 ^{ns}
		Hb ^{AB}	3	0.015	
	B=0.0725	Hb ^{BB}	13	0.065	
60-70cm	A=0.3225	Hb ^{AA}	49	0.245	0.155
		Hb ^{AB}	31	0.155	
	B=0.1425	Hb ^{BB}	13	0.065	
<60cm	A=0.19	Hb ^{AA}	23	0.115	0.15
		Hb ^{AB}	26	0.15	
	B=0.205	Hb ^{BB}	7	0.13	
Total	1.00		200	1.00	

Heamoglobin genotypes were found polymorphic across the body length of Afar goat breed with most frequent genotype of Hb^{AA} (0.285) and allele of Hb^A (0.365) in animals having 50-60cm

(Table 7). The difference also found hemoglobin polymorphism is statically significant ($P < 0.05$) (Table 7)

Table 7: Allele and genotype frequency distributions of heamoglobin across the body length girth of animal

Body length	Allele frequency	Genotype	Genotype Observed	Genotype frequency	X ² df=4
>60cm	A=0.2325	Hb ^{AA}	39	0.195	0.032
		Hb ^{AB}	15	0.075	
		Hb ^{BB}	21	0.105	
50-60cm	A=0.365	Hb ^{AA}	57	0.285	
		Hb ^{AB}	32	0.16	
		Hb ^{BB}	5	0.025	
<50cm	A=0.0875	Hb ^{AA}	11	0.055	
		Hb ^{AB}	13	0.065	
		Hb ^{BB}	7	0.035	
Total	1.00		200		

Heamoglobin genotypes were found polymorphic across the horn length of Afar goat breed with most frequent genotype of Hb^{AA} (0.185) and

allele of Hb^A (0.237) in animals having 20-30cm (Table 8). But the difference was not found statically significant (P >0.05) (Table 8).

Table 8: Allele and genotype frequency distributions of heamoglobin across the body horn of animal

Horn size	Allele frequency	Genotype	Genotype Observed	Genotype frequency	X ² df=6
>30	A=0.1525	Hb ^{AA}	29	0.145	0.097 ^{ns}
		Hb ^{AB}	3	0.015	
		Hb ^{BB}	9	0.045	
20-30	A=0.2375	Hb ^{AA}	37	0.185	
		Hb ^{AB}	21	0.105	
		Hb ^{BB}	9	0.045	
<20	A=0.2925	Hb ^{AA}	38	0.19	
		Hb ^{AB}	41	0.205	
		Hb ^{BB}	13	0.065	
Total	1.00				

4. Discussion

The hemoglobin locus in Afar goat breed was polymorphic with three distinct genotypes – Hb^{AA}, Hb^{AB} and Hb^{BB} with most the frequent genotype Hb^{AA} controlled by two alleles with genotype frequency of 0.535, 0.295 and 0.170 and allele frequencies of 0.68 and 0.32 for Hb^A and Hb^B respectively with Hb^A being the most frequent. The hemoglobin genotypes observed in this study were in line with the observation of Sanjoy and Yesihak (2014) who reported three

haemoglobin variants with genotype frequency of 0.9, 0.4, 0.1 for Hb^{AA}, Hb^{AB} and Hb^{BB} and allelic frequency of 0.70 and 0.30 for Hb^A and Hb^B respectively with Hb^A being the most frequent. Ukwu *et al.* (2018) reported that genotype frequencies of 0.50, 0.23 and 0.27 for Hb^{AA}, Hb^{AB} and Hb^{BB} and allele frequencies of 0.61 and 0.39 for Hb^A and Hb^B respectively, with Hb^A allele being the most frequent in Nigeria and Al-Samarrae *et al.* (2010) reported that genotype frequencies of 0.47, 0.36 and 0.17 for Hb^{AA}, Hb^{AB} and Hb^{BB} and allele frequencies of 0.65 and 0.35 for Hb^A and Hb^B and respectively with Hb^A being

the most frequent in, north of Iraq. But this finding was in contrary to the finding of Ahmed *et al.* (2010): reported four Hb variants of allelic frequencies of 0.51, 0.33, 0.014 and 0.01 for Hb A, Hb B, Hb C and Hb D in Egypt respectively. This variation may come from sample size or may be the type and number of allele controlled the genotype of goat breed vary one from other.

Hemoglobin locus was found polymorphic across the sex Afar goat breed and statically non-significant at ($p > 0.05$) agree with the previous study of Essien *et al.* (2011) that hemoglobin polymorphism was not statically significant across sex animal in Shika, Zaria Nigeria and Abdussamad *et al.* (2004) who reported hemoglobin genotypes were not statically significant in sex of animal in Zaria. The results of chi-square showed that genotype frequencies at hemoglobin locus in Afar goat breed were not in Hardy Weinberg equilibrium and in agreement with (Ukwu *et al.*, 2018) who conducted hemoglobin polymorphism in Nigeria. The reason could be, forces that affect gene and hence genotype frequency in a population i.e small size of population, mating may be nonrandom, selection for certain genotypes, emigration or immigration and mutations since Hardy Weinberg equilibrium states that both gene frequencies and genotype frequencies will remain constant from generation to generation in an infinitely large interbreeding population in which mating is at random and no selection, migration or mutation.

The disequilibrium of genotype frequency at the hemoglobin locus in the Afar goat breed observed in this study is not in agreement with the earlier observation Essien *et al.* (2011) in Nigeria and Sanjoy and Yesihak (2014) in eastern part of Ethiopia, that their studies agree with Hardy Weinberg equilibrium. The preponderance of homozygous (Hb^{AB}) against heterozygotes of both types (Hb^{AB} and Hb^{BB}) in Afar goat breed was agree also with finding of Essien *et al.* (2011) who reported three genotypes (Hb^{AA} , Hb^{AB} and Hb^{BB}) with frequencies of 0.47, 0.40 and 0.13 respectively, and allele frequencies of 0.64 (Hb^A) and 0.36 (Hb^B) with Hb^A being the most frequent in Nigeria.

Hemoglobin type A has been speculated to be associated with increased hemoglobin concentration and packed cell volume values (Pieragostini *et al.*, 2006). The higher oxygen affinity of the allele A has been suggested to be due to its biophysical, biochemical and physiological peculiarities such saturation capacity with oxygen, dissociation curve of oxyhaemoglobin, erythrocyte load with hemoglobin, and metabolic profile of the erythrocyte (Raushenbach and Kamenek, 1978). These properties of the Hb A with its effects on some blood parameters such hemoglobin and packed cell volumes has been suggested to positively influence the resistance of animals carrying this type of hemoglobin against diseases such helminth infestation (Di Stasio, 1997). A study by Achukwi *et al.* (1997) in the Central and West African region, Namchi, and N'Dama taurine breeds resistant to devastating trypanosomiasis disease believe that their genotype solely Hb^A allele may be responsible for this resistance.

This agree with also the idea that breeds that were common to lower altitudes had a significant higher frequency of Hb^A and (Hrinca, 2008) who suggested that temperature is on the factor that determine the type i.e the extreme temperatures (hot), conditions favors the fixing of allele Hb^A and A moderate temperature favors the fixing of allele Hb^B since the natural breeding sheko breeding is in extreme environmental conditions (hot and humid) favors to have allele highest frequency Hb^A allele which is in line with the above idea of researchers. Hemoglobin locus was found polymorphic across the observed quantitative traits such as horn size, body length, and heart girth and body weight of Afar goat breed. Hemoglobin genotypes were found statically significant across the body weight and length of Afar goat breed. The observed Heterozygosity (0.6) in this studies same as Al-Samarrae *et al.* (2010) who reported Heterozygosity of 0.45 in Mousul, north of Iraq, And in line with the finding of Ukwu *et al.* (2018) who reported Heterozygosity of 0.47 in Makurdi, Nigeria, which was an indication of moderate level of genetic diversity at the

haemoglobin locus, and Sanjoy and Yesihak (2014) who reported Heterozygosity of 0.42 in eastern part of Ethiopia.

The heterozygosity value observed in this study is an index of moderate genetic diversity at the hemoglobin locus in Afar goat breed. The observed heterozygosity at the haemoglobin locus in the Afar goat breed is beneficial since genetic diversity within a population enables perpetuation of the species in the presence of changing climatic conditions (Egena and Alao, 2014). This shows clearly that haemoglobin locus in the Afar goat breed may not be controlled by only one allele. Genetic variability parameter indicates the allelic richness of a population and its value is a function of number of alleles; consequently in most cases, higher number of allele segregating at different loci will generate higher Effective number of allele (ne) and vice versa (Oguntunji and Ayorinde, 2015).

5. Conclusions and Recommendations

Afar goat breed is one of the most important livestock assets in afar community. More over goats are the most valuable for cash income and source food. So a study was conducted to assess the hemoglobin polymorphism and its genomic signature in trypanotolerance Afar goat breed. The results of this study suggest that the haemoglobin locus in the Afar goat breed is polymorphic in genotypes (Hb^{AA} , Hb^{AB} and Hb^{BB}) which are controlled by two codominant alleles Hb^A and Hb^B . The most frequent genotypes observed in this study was homozygote (Hb^{AA}) with allele Hb^A was the most frequent allele in the population of the Afar goat breed studied. There exists a moderate genetic diversity with heterozygosity value of 0.6 at the haemoglobin locus in the Afar goat breed. Data on genotype frequencies were subjected to chi-square analysis to test for goodness of fit differences between observed and expected genotype frequencies under Hardy- Weinberg equilibrium. The results showed that the deviation was observed and the test revealed that the population is not under

Hardy-Weinberg equilibrium, maybe there is forces that affect gene and hence genotype frequency in a population i.e. small size of population, nonrandom mating, natural or artificial selection genetic drift and mutation. In this study the higher Hb^{AA} was more favored in Afar goat breed. This properly allows an advantage of the breed that favored it survival, fitness and adaptation to environmental challenges and resistant to devastating disease.

Based on the above conclusion I have forwarded the following recommendations

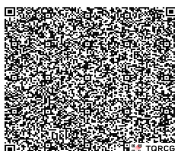
- ✪ A Research should be conducted to characterize Afar goat breed at DNA level.
- ✪ Further research should be carried out to determine the effect of the observed hemoglobin polymorphism and disease tolerance and environmental adaptation
- ✪ Further researches should be done on a larger sample size to determine association between hemoglobin polymorphism and qualitative and quantitative traits

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	Subject: Animal Biotechnology
Quick Response Code	
DOI: 10.22192/ijamr.2026.13.09.004	

How to cite this article:

Gizachew Fentahun, Haimanot Aragaw, Fanuael Bizayehu and Teshager Dubie. (2026). Investigation of hemoglobin polymorphism and its association with phenotypic traits in indigenous Afar goat breed in selected districts of Afar Region, Ethiopia: A need for characterization. Int. J. Adv. Multidiscip. Res. 13(9): 38-54.

DOI: <http://dx.doi.org/10.22192/ijamr.2026.13.09.004>