INVESTIGATION OF HEAMOGLOBIN POLYMORPHISM IN TRYPANOTOLERANT SHEKO CATTLE (AFRICAN *Bos taurus*), BENCH MAJI ZONE, SOUTHWESTERN ETHIOPIA

MSc THESIS

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HAWASSA UNIVERSITY

COLLEGE OF AGRICULTURE

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A THESIS SUBMITTED TO THE SCHOOL OF ANIMAL AND RANGE SCIENCES, COLLEGE OF AGRICULTURE, SCHOOL OF GRADUATE STUDIES, HAWASSA UNIVERSITY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN ANIMAL AND RANGE SCIENCES (SPECIALIZATION: ANIMAL BIOTECHNOLOGY)

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FEBRUARY, 2021

COLLEGE OF AGRICULTURE

SCHOOL OF ANIMAL AND RANGE SCIENCES

ADVISORS' APPROVEL SHEET

(Submission sheet -1)

This is to certify that the thesis entitled **"Investigation of Heamoglobin Polymorphism in trypanotolerant Sheko cattle (African *Bos taurus*), Bench Maji Zone, Southwestern Ethiopia" in partial fulfillment** for the degree of Master of Science with specialization in **Animal Biotechnology** to graduate program of School of Animal and Range Sciences, College of Agriculture, and is a record of original research carried out by **Gizchew Fentahun** ,under our Supervision, and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged. Therefore I/We recommend that it be accepted as fulfilling the thesis requirements.

Name of Major Advisor Signature Date

Simret Betsha (Phd) ________________ _____________

Name of Co-advisor Signature Date

Prof. Dessie Shiferaw ________________ ________________

SCHOOL OF GRADUATE STUDIES
HAWASSA UNIVERSITY
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(Submission Sheet-2)

We, the undersigned, members of the Board of examiners of the final open defense by **Gizachew Fentahun Desta** have read and evaluated his thesis entitled "**Investigation of Heamoglobin Polymorphism in trypanotolerant Sheko cattle (African *Bos Taurus*), Bench Maji Zone, Southwestern Ethiopia"** and examined the candidate. This is therefore to certify that the thesis has been accepted in partial fulfillment of the requirements for the degree Masters of Science in Animal and Range science with Specialization in Animal Biotechnology.

Name of Chairperson	Signature	Date
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Name of External Examiner	Signature	Date
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Final approval and acceptance of the thesis is contingent upon the submission of the final copy of the thesis to the School of Graduate Studies (SGS) through the School of Graduate Committee (SGC) of the candidate's School.

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FINAL THESIS APPROVEL FORM
(Submission sheet-3)

As members of the Examining Board of the Final MSc Open Defense, we certify that we have read and evaluated the thesis prepared by Gizachew Fentahun entitled **Investigation of Heamoglobin Polymorphism in trypanotolerant Sheko cattle (African *Bos taurus*), Bench Maji Zone, Southwestern Ethiopia** and recommend that it be accepted as fulfilling the thesis requirement for the degree of the Master of Science in Animal and Range (Specialization: Animal Biotechnology).

Name of Chairman	Signature	Date
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Name of Major Advisor	Signature	Date
Simret Betsha (Phd)	_________________	______________
Name of Co-advisor	Signature	Date
Prof. Dessie Shiferaw	________________	_____________
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I hereby certify that I have read this thesis prepared under my direction and recommend that it accepted as fulfilling the thesis requirement.

Name of Thesis Advisor	Signature	Date
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DEDICATION

I dedicate this thesis manuscript to my father Fentahun Desta, my mother Workenesh Waleligne and my wife Helina Lake for nursing me with affection and love and for their dedicated partnership in the success of my life. I dedicate this work to my major advisor Dr. Simret Betsha for her irreplaceable support during my study.

STATEMENT OF AUTHOR

I declare that this thesis is my bona fide work and all sources of materials used for this thesis have been duly acknowledged. I solemnly declare that this thesis is not submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

Name: Gizachew Fentahun Signature __________
Place: College of Agriculture, Hawassa University, Hawassa
Date of Submission _____________

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ABBREVIATION AND ACRONYMS

BMZ	Bench Maji Zone
BMZRDMD	Bench Maji Zone Rural Development Main Department
CSA	Central Statistical Agency
DAGRIS	Domestic Animal Genetic Resources information System
EDTA	Ethylene-diamine-tetra-acetic acid
FAO	Food and Agriculture Organization of the United Nations
Hb	Haemoglobin
HWE	Hardy-Weinberg equilibrium
M.a.s.l	Meter above sea level
ILRI	International Livestock Research Institute
PCV	Packed cell volume
SNNPR	Southern Nations, Nationalities, and Peoples' Region

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Investigation of Hemoglobin Polymorphism in trypanotolerant Sheko cattle (African *Bos Taurus*), Bench Maji Zone, Southwestern Ethiopia

Gizachew Fentahun

Major Advisor: Simret Betsha (Phd), Hawassa University
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ABSTRACT

Sheko is one of the Ethiopian indigenous cattle breed and geographical restricted to Bench Maji Zone and partly in the adjoining parts of Kaffa and Shaka Zones of south west Ethiopia. The breed possess typical characteristics related to disease resistance such as trypanosomosis and well adapted to live in warm and humid environment, produce and reproduce in tsetse infested areas than any other indigenous cattle. The present study aimed to investigate the biochemical polymorphism of Hemoglobin in trypanotolerant sheko cattle. For this , a total of 200 blood samples were collected by jugular vein puncture from 165 Sheko and 35 Zebu cattle. 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^{AB} (0.60) observed in sheko while the most frequent genotype observed zebu breed was Hb^{AA} (0.52). The least frequent genotype Hb^{BB} appeared at gene frequency of 0.02 in sheko and 0.2 in zebu cattle respectively. The gene frequency of Hb^{A} allele was 0.68 and that of Hb^{B} allele was 0.32 in sheko cattle. On the other hand, Hb^{A} and Hb^{B} allele's frequencies in zebu cattle were 0.66 and 0.34 respectively. The Hemoglobin polymorphism was found to be statically significant in both breeds ($P<0.005$). The Chi-square ($\chi2$) test revealed that the population is not under Hardy-Weinberg equilibrium. This suggests there is a force that small population size might affect gene and hence genotype frequency in a population and lead to the violation of Hardy Weinberg equilibrium. To obtain a clear over view about gene and genomic structure of sheko; whole genome sequence should be conducted.

Keywords: Polymorphism, Sheko breed, Hemoglobin, Genotype, Bench Maji Zone

1. INTRODUCTION

Globally, there are about 40 species of domestic livestock (FAO, 2018), which have been domesticated by humans over the last 12,000 years. In the process of domestication, however, separate and genetically unique breeds and strains have developed within each species as a result of human development and occupation of new areas over the planet. According to FAO (2018), there are about 4, 000 to 5,500 breeds and strains of domestic animals in the world. Ethiopia is endowed with diverse cattle genetic resources adapted to various local environmental conditions and acquired unique features with a variety of genetically diverse population from *Bos taurus* to *Bos indicus* species with unique adaptation characteristics (Berhane, 2016). These cattle breeds are found all across the country from the rift valley highlands as well as below sea level in the Afar depression.

Sheko breed is one of the Ethiopian indigenous cattle breeds which represent the last remnants of Africa's original *Bos taurus* (humpless shorthorn) cattle that were probably the first to be domesticated in eastern Africa (Rege, 1999). They were originally classified as taurine, while recent genetic analyses indicates they are more of a sanga type with African taurine and Asian zebu genetic ancestry (Mbole *et al*., 2014) . They are smaller in body size, with narrower belly and hindquarters, and shorter or no horns (Takele *et al*., 2009). The presence of a small cervicothoracic hump in them alludes to their zebu ancestry (Mekuriaw and Kebede, 2015). 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. The breed is better for its milk yield, draft power, hardiness and adaptation to challenged environment than any other indigenous cattle populations found in Bench Maji Zone (Mekuriaw *et al*., 2015).

The breed is known to have evolved in the tsetse belt of south western part of Ethiopia under natural selection and patronage of the Sheko people and other ethnic groups that inhabited in its breeding tract (Takele, 2011).

Sheko cattle is one of the trypanotolerant cattle strain in Africa which survives, produce and reproduce in a trypanosomiasis infested area of the country especially in wet areas of South and South-west Ethiopia (Takele *et al*., 2012). The breed has been recognized as having great potential related to disease resistance and adaptation to extreme environment that could prove the genetic backbone for future survival and the breed is fundamental to food security for the present and future human generations (ILRI, 2007). Even though, there is a potential of diversified genetic resources with unique features, cattle breeds are at risk to genetic dilution. There are different factors contributing, chief among them are uncontrolled breeding and indiscriminate cross breeding with exotic breeds (Berhane, 2016). According to (Takele, 2011) Only 3 % of households had a pure Sheko herd. Reported numbers of the Sheko population size vary greatly between different sources. The higher numbers of approximately 31,000 Sheko animals, were estimated based on the presumption that cattle in the Sheko area were predominantly Sheko (Rege, 1999). Later on, lower estimates of 4040 (Dadi *et al.*, 2008 and 2400 heads (Takele *et al*., 2012) were reported. Implicated that the breed is at risk to genetic erosion so 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).

Genetic diversity can be detected at three levels; morphological, biochemical and at DNA level. Although DNA-based technologies are now the method of choice for genetic characterization of livestock, 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). The role or potential of these alternatives approach in animal genetic diversity study should not be underplayed since genetic research in Africa is less fully developed than in Europe (Gifford-Gonzalez and Hanotte, 2011). Blood groups and blood proteins have been widely used to characterize animal population, especially haemoglobin (Ajayi *et al.*, 2013). Except, there are few information with respect to hemoglobin polymorphism in Ogaden cattle found Southeastern Ethiopia of Somali region (Sanjoy and Yesihak, 2014) to our knowledge, there is no information available on the hemoglobin polymorphism in trypanotolerant Sheko cattle. Therefore the present study is aimed with the following objectives;

General objective

- To investigate the hemoglobin polymorphism in trypanotolerant Sheko cattle

Specific objective

- To characterize sheko cattle at hemoglobin loci
- To investigate hemoglobin variation and its association with morphometric traits

2. LITERATURE REVIEW

2.1. Livestock Genetic Resources in Ethiopia

Ethiopia can be considered as a center of livestock diversity: it is a route of livestock migration from Asia into Africa and has large livestock population (FAO, 2018) and diverse traditional livestock breeds spread across diverse ecology, communities and production systems (Solomon, 2008). Central Statistical Authority of Ethiopia (CSA, 2019) showed that Ethiopia possess 65.35 million heads of cattle, 39.89 million heads of sheep, 50.50 million. Million heads of goat, 7.70 million heads of camel, 48.96 million poultry, 2.11 million horses, 8.98 million donkeys, and 0.38 million mules .There are also a variety of breeds/strains within each livestock species contributing to the total genetic pool. Different authors showed that there are around 24 cattle breeds in Ethiopia (DAGRIS, 2004). It ranks first in number of cattle and 17.10% of African cattle population found in Ethiopia.

The livestock subsector has an enormous contribution to Ethiopia's national economy and livelihoods of many Ethiopians, and still promising to rally round the economic development of the country. The subsector contributes about 16.5% of the national Gross Domestic Product (GDP) and 35.6% of the agricultural GDP (Metaferia *et al*., 2011). Despite high livestock population and existing favorable environmental conditions, the current livestock output of the country is little. This is associated with a number of complex and inter-related factors such as inadequate feed and nutrition and widespread diseases, poor genetic potential of local breeds (Negassa *et al*. 2011). Ethiopian indigenous cattle breeds have unique morphological features which distinguishes them from other cattle. These include horn shape and size (e.g. Afar and

Raya breed) and body size (large and small east African Zebu) (Mulualem *et al*., 2015). In addition to physical features, non-visible traits such as disease resistance, climatic stress resistance and productivity traits also differ among breeds. These characteristics are largely the result of natural and human selection. Some breeds are already known for their unique adaptive attributes (e.g. Sheko); one of the well-known outstanding features of trypanosomosis resistance cattle (Mulualem *et al*., 2015).

Table 1: Ethiopian indigenous cattle breeds with their characteristics

Group Name	Breed Name	Characteristics
Large East African Zebu	Begait	Active disposition and milk yield
	Ethiopian Boran	Walking ability, highly adapted to harsh conditions, herd instinct, mothering ability and longevity
Small East African Zebu	Arisi cattle	Poor milkers, extremely active and often very aggressive
	Jem-jem	Well adapted to wet and cold Climate
	Ogaden	Good dairy and beef characteristics
	Adwa cattle	high temperatures and are known to be more resistant to tick infestation
	Harar cattle	are known to be more resistant to tick infestation
	Jijiga cattle	adapted to dry environmental conditions
	Goffa and Guraghe	high temperatures and are known to be more resistant to tick infestation
	Ambo cattle	high temperatures and are known to be more resistant to tick infestation
East African Sanga	Raya Azebo	Good draught power
	Anugak	Tolerance to Trypanosomosis
Zenga	Fogera cattle	be able to survive on very poor pasture, scarce water and have good walking abilities

	Afar cattle	be able to survive on very poor pasture, scarce water and have good walking abilities
	Arado cattle	be able to survive on very poor pasture, scarce water and have good walking abilities
Humpless Shorthorn	Sheko cattle	Tolerance to Trypanosomosis

Source: Berhane *et al*., 2016

Currently, the indigenous livestock genetic resources in Ethiopia are becoming seriously endangered owing to the high rate of genetic erosion resulting from indiscriminate introduction of exotic genetic resources, before proper characterization, utilization and conservation. Besides, genetic dilution due to foreign or exotic use, changes in production systems, markets preferences and environmental hazards, natural catastrophes, unstable policies from public and private sectors and the availability of limited funds for conservation are deteriorate the diversity of livestock genetic resource in the country (Negassa *et al*., 2011).

2.2. Importance of Genetic Variation

Biochemical diversity or polymorphism is the occurrence of varieties attributed to biochemical differences which are under genetic control. It has created a leeway for the genetic improvement of farm animals. This is because it can be used as a useful tool for the characterization of livestock breeds and population. This way, the degree of similarity or differences within and between breeds can be ascertained and this differences or similarity are important raw materials for genetic improvement of animals (Egena and Alao, 2014).

Investigation of genetic variation is very important for future monitoring of gene flow in populations, conservation of species, determination of the level of inbreeding and crossbreeding within and between breeds. The loss of genetic variation within and between breeds is detrimental not only from the perspectives of culture, conservation and investigation but also for utility since lost genes may be of future economic interest, food security and meet unpredictable future environment (Kunene *et al*., 2009).

Before the advent of genetic studies, the classification of breeds was based on historical and anthropological evidence and morphological characteristics that were and are still not satisfactory or significant for the purpose and objective of conservation, parentage and future monitoring of breeds. Most of the indigenous livestock populations in developing countries have not yet been characterized and evaluated at phenotypic and genetic levels (Mwacharo *et al*., 2006).

2.3. Haemoglobin

Haemoglobin is iron-containing oxygen-transport metalloprotein in the red blood cells of almost all vertebrates and some invertebrates (Chineke *et al*., 2007). The molecule is tetramer of four sub units, each of which has two parts: a polypeptic chain, *globin* and a prosthetic group, *haeme*, which is an iron containing pigment that combines with oxygen and gives the molecule its oxygen-transporting ability (Chineke *et al*., 2007). The *haeme* portion is alike in all forms of haemoglobin, genetic variation is restricted to the structure of the *globin* portion only (Peters *et al*., 2004). Among other relevance, haemoglobins have revealed more about the molecular basis of human, animal and medical genetics than any other system (Chineke *et al*., 2007). They illustrate mechanisms of forming new genes other than point mutation, cast light on the process of evolution of both the molecular and population levels as well as provide a model of gene action during development (Peters *et al*., 2004).

Normal and different variants of haemoglobins are identified by standard laboratory techniques. Cellulose acetate electrophoresis technique is popular for detection of serum protein and its quantitation because of its simplicity and effectiveness in detecting most variants of haemoglobin at alkaline pH (Chineke *et al*., 2007).

2.4. Heamoglopin Polymorphism

Haemoglobin has been one of the most studied polymorphisms in vertebrate species since the infancy of both the population and evolutionary genetics (Pieragostini *et al*., 2010). This blood protein has been reported to exhibit polymorphism at its globin portion, but is known to be the same in its "haem portion" in all vertebrates (Chineke *et al*., 2007). The use of haemoglobin polymorphism in genetic analysis of farm livestock species has been tried out. For instance, an investigation of hemoglobin polymorphism at the hemoglobin locus had been investigated in Nigerian indigenous chickens and ducks (Ajayi *et al*., 2013) and in sheep (Akinyemi *et al*., 2010) and the existence of three hemoglobin genotypes HbAA, HbAB and HbBB in the Bunaji cattle in Zaria, Nigeria had been reported (Essien *et al*., 2011)

Haemoglobin polymorphism in cattle seems to be breed influenced as some breeds to show a clear polymorphism with two alleles (AA, BB) and their possible phenotypes (AA, AB and BB) and also the occurrence of the two co-dominant alleles Hb A and Hb B (Egena *et al*., 2014) , while others present only one allele type-AA *(*Pal and Mummed*,* 2014*)* . Ahmed *et al.*

(2010) reported four Hb variants in their study with cows and buffaloes with allelic frequencies of 0.51, 0.33, 0.014 and 0.01 for Hb ^{A}, Hb ^{B}, Hb ^{C} and Hb ^{D}, respectively.

In Southeastern Ethiopia of Somali region in ogaden cattle revealed three haemoglobin genotypes Hb^{AA}, Hb^{AB} and Hb^{BB}, with Hb^{A} as the most prevalent allele in the population (Sanjoy and Yesihak, 2014). In eastern Ethiopia sheep breeds Hemoglobin showed three phenotypes (Hb^{AA}, Hb^{AA} and Hb^{BB}) in three sheep breeds by (Helen *et al*., 2016).

2.5. Trypanosome and Trypanosomosis

Trypanosomes are unicellular flagellate protozoa. All trypanosomes are heteroxenous which means they require more than one host to complete a life cycle. There are numerous different trypanosomes and the effect of an infection varies greatly based on the trypanosome species. Trypanosomes cause a large number of diseases in different species of vertebrate hosts. Some trypanosomes can infect several vertebrate hosts whereas others are host-specific (Jennie, 2011).

Trypanosomosis is a blood parasite disease transmitted when tsetse flies feed primarily on cattle, domestic and wild animals (Jan *et al*., 2010). There are three species of the trypanosomosis: *T. congolense*, *T. vivax*, and *T. brucei.* Research have been done with respect to these three strains have found *T. vivax* and *T.* congolense to be the most prevalent strains in cattle and *T. congolense* to be the most deadly one (Cherenet *et al*., 2004). These blood parasites are called trypanosomes and multiply rapidly and cause lysis in red blood cells to occur, thus making the animal anemic, rapidly loses weight and dies. For the past half century, trypanocidal drugs have been used to control trypanosomes in cattle and maintain productivity. However, mounting evidence suggests trypanocidal drugs are only 60% effective in treatment of trypanosomosis and that strains have developed resistance to widely used trypanocidal drugs (Adams *et al.*, 2010).

2.6. Bovine Trypanotolerance

Trypanotolerance is "the relative capacity of an animal to control the development of the parasites and to limit their pathological effects. In other words, trypanotolerant animals can be defined as animals that do not get infected as often, and when infected, they survive, produce and reproduce better than susceptible animals (Courtin *et al.*, 2008). It is commonly believed that trypanotolerance in cattle is a result of natural selection where cattle and trypanosomes have co-existed for a long time (Mugambi, 2009). Differences between breeds and breed groups are explained by the way cattle arrived to the African continent. The first cattle to arrive were of *Bos taurus* type, whereas *Bos indicus* (zebu) cattle came later (Mugambi, 2009).

Taurine cattle types in Africa are often characterized by a small body size and a relatively low productivity compared to most zebus. However, they have over the years acquired hardiness to harsh climates and different disease resistances among which trypanotolerance is the most notable one. Trypanotolerance can therefore most easily be found in taurine cattle breeds, whereas indicus breeds more often are trypano-susceptible (Murray *et al.*, 2004). The ability to control anemia is an important characteristic of trypanotolerant cattle and it is highly linked to the overall production and reproduction. After the initial breakdown of red blood cells, leading to a lowered packed cell volume (PCV), trypanotolerant cattle recover, whereas susceptible animals continue to have a lower PCV (Murray *et al.*, 2004).

The lack of vaccine and the limitations of the present methods of control, namely chemotherapy and tsetse control have stimulated the desire to develop additional approaches that might allow more efficient land utilization in the vast areas of Africa dominated by the tsetse fly. Thus, there is now considerable interest in the potential use of trypanotolerant livestock. Particular attention has been focused on the N'Dama breed of cattle because it has a relatively fixed phenotype and can be improved in productivity (Roberts and Gray, 1973).

2.7. Mechanisms of Trypanotolerance.

There is evidence indicating that host genetic factors play a significant role in determining an individual’s susceptibility/resistance status to trypanosoma infection (Hanotte *et al*., 2003; Courtin et al., 2008). The basic mechanism of trypanotolerance is still to be precisely defined, there is at least circumstantial evidence that the mechanism is related to a host response factor and it is a heritable trait that trypanotolerant animals were reported to switch from innate immune response to adaptive immune response with the induction of active macrophages (M2) following trypanosome infection (Bosschaerts *et al*., 2011).

The trypanotolerant nature of the bovine (e.g N'Dama) and the capacity of certain strains of mice to survive a trypanosome infection longer than others (Morrison *et al*, 1978) would appear to be related to their ability to limit the level of peaks of parasitaemia and subsequently to control, reduce or even eliminate the parasite. Evidence that a more effective immune response might be responsible for the differences in susceptibility between N'Dama and zebu cattle comes from the work of Desowitz (1959). He found that N'Dama with previous experience of trypanosomiasis were able to eliminate trypanosomes more rapidly than their zebu counterparts following a renewed challenge.

There is a considerable body of evidence to show that trypanotolerance has a genetic basis. Thus, studies on cattle that have had no previous experience of trypanosomiasis have clearly established that N'Dama is significantly more resistant than zebu (Roberts and Gray, 1973). It is likely that trypanotolerance has evolved in tsetse fly infested areas by natural selection of the more resistant animals within a breed. In this respect a range of susceptibility is found both within groups of N'Dama and zebu cattle. Under such circumstances it is likely that the factors governing the susceptibility have a complex genetic basis. In this regard, N'Dama cattle showed almost 100 percent gene frequency for Haemoglobin (Hb) A, while zebu is a mixture of A and B; it has been proposed that animals could be selected on the bases of Hb type (Roberts and Gray, 1973).

2.8. Sheko Cattle Breed and Trypanotolerance

Sheko cattle is found only in the remote corner of southwestern Ethiopia specifically at the humid Sheko and Bench districts owned by small holder farmers who breed them for millennia of years for their natural resistance to disease, particularly tsetse transmitted trypanosomosis (ILRI, 2007). The breed is well adapted to live in warm and humid environment, produce and reproduce in tsetse infested areas and the breed manifests strong favorable trypanotolerant (Takele *et al.*, 2009).

Sheko cattle have better feed conversion efficiency, longevity, and fertility good mothering ability compared to other cattle breeds in adjacent areas (Takele *et al*., 2009). They also have good potential as dairy cattle for Africa, having large teats and the ability to yield on average 2.79 liters of milk daily and 850.6 liters per lactation period, which is 307.69 days, depending on the on-farm management practice (Mekuriaw *et al*., 2015). On the other hand, their occasional aggressive temperament and voracious feeding habits, particularly during the dry season, were mentioned as undesirable traits which trigger its keepers to intentionally reinstate with smaller breeds of lower feed intake (Takele *et al*., 2009). Sheko is now considered endangered by gradual interbreeding with local zebu (DAGRIS, 2005).

3. MATERIAL AND METHODS

3.1. Study Area Description

This study was conducted in the known current geographical distribution of Sheko breed of cattle in Bench Maji Zone (BMZ) and Keffa zone, southwestern Ethiopia. The study area also included Yem special district. Yem district was included to sample cattle which are geographically located far from Bench Maji zone (which is the natural breeding tract of Sheko).

3.1.1. Bench Maji Zone

Bench Maji is one of the Zones of the Ethiopian Southern Nations, Nationalities, and Peoples' Region (SNNPR). It is bordered on the south by the Ilemi on the west by South Sudan, on the northwest by the Gambela Region, on the north by Sheka, on the northeast by Keffa, and on the east by Debub Omo. The administrative center of Bench Maji is Mizan Teferi; other towns include Maji. The zone has 10 districts with a total of 236 Kebelles (Takele *et a*l., 2009). The altitude of Bench Maji ranges from 850-3000 m. above sea level and its geography coordinates between $5^{o}33'$ and $7^{o}21'$ N Latitude and $34^{o}88'$ and $36^{o}14'$ E Longitude. The temperature of the agro-ecological zones ranges from 15.1-40°C with mean annual rainfall ranges from 400 to 2000 mm (Hailu, 2018).

3.1.2. Keffa Zone

Keffa is a Zone in the Ethiopian Southern Nations, Nationalities, and Peoples' Region (SNNPR). Keffa is bordered on the south by Debub Omo, on the southwest by Bench Maji, on the west by Sheka, on the north by the Oromia Region, and on the east by Konta. Gojeb River runs along part of the northern border of this zone. The administrative center of Keffa is Bonga. Keffa formed the main part of former Keficho Shekicho Zone. Current districts of Keffa Zone are: Bita, Bonga Town, Chena, Cheta, Decha, Gesha, Gewata, Ginbo, Adiyo, Sayilem, Telo, Shisho inde and Goba. Kafa Zone shows bimodal rainfall distribution pattern; the main rainy (high rain) season extends from June to the end of September and the short rainy period from February to April. The average annual rainfall is 1500 mm in the lowland areas and 2000 mm in the high-land areas of the zone. The annual minimum temperature of the area is 15°C and the maximum is 24 °C (NABU, 2017).

3.1.3. Yem Special District

Yem is a special district in the Southern Nations, Nationalities, and Peoples' Region (SNNPR) of Ethiopia. Because Yem is not part of any Zone in the SNNPR, it is considered a Special woreda, an administrative subdivision which is similar to an autonomous area. Yem is named for the Yem, people whose homeland lies in this special woreda (Gezahegn *et al*., 2015). It is bordered on the west and north by the Oromia Region, and separated from Gurage on the northeast and Hadiya on the east by the Omo River. The administrative center of Yem is Fofa. It is situated in the northwestern apex of SNNPR and is located between 7° 57′N to 8° 02′N latitude and 37° 40′E to 37° 61′E longitude (Zerfu *et al*., 2018).

Yem special district receives a mean annual rainfall of 900–2200 mm in a bimodal pattern, from mid-February to April, and June to September. The mean annual temperature is in the range of 12– 30°C (Gezahegn *et al*., 2015). The topography of Yem district is characterized by rolling mountains include Mount Bor Ama, Mount Azulu and Mount Toba, long deep gorges of Gibe River to the east, steep slopes and flat to undulating plateaus (Zerfu *et al*., 2018).

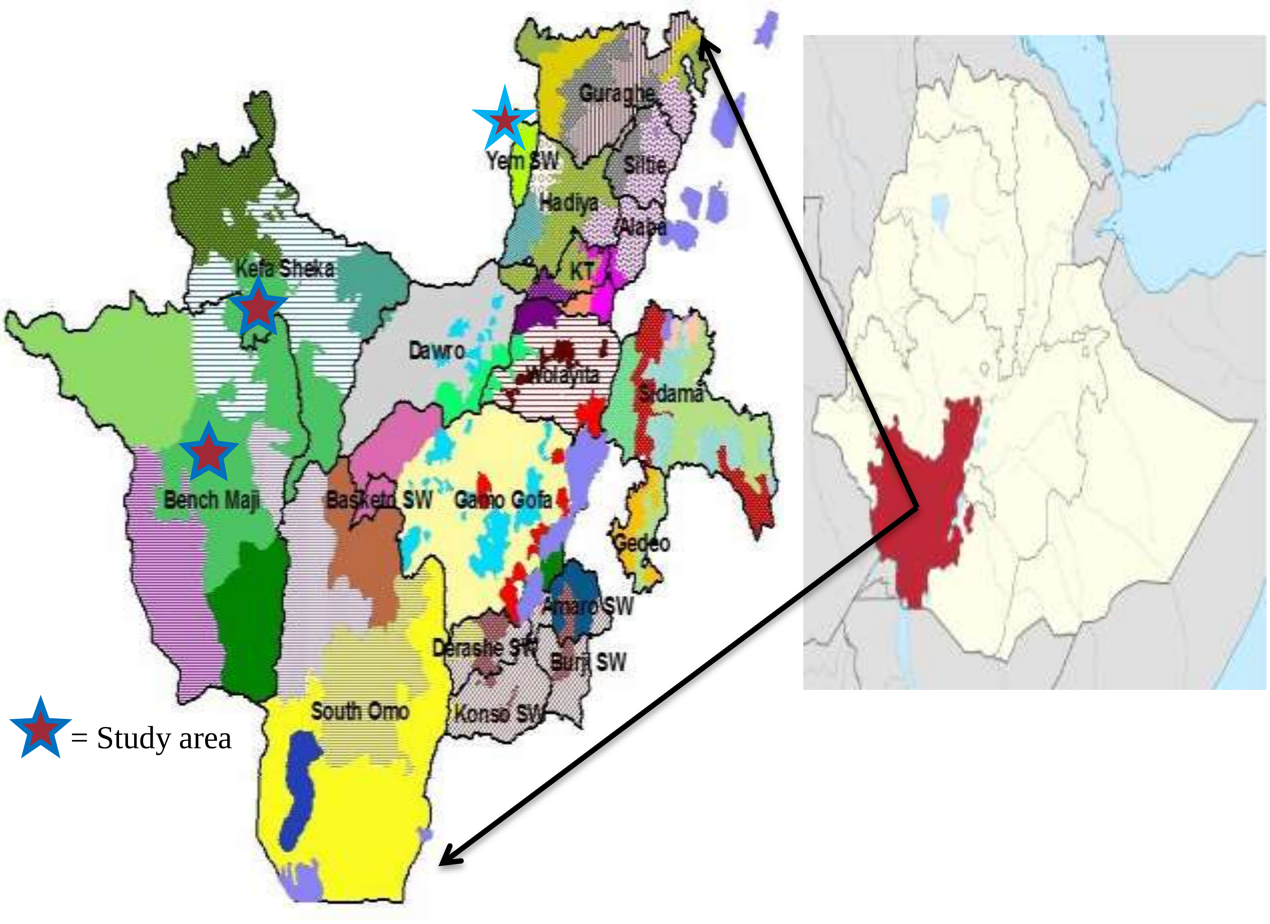

Figure 1: Geographical map of the study area (Bench Maji, Kefa Zone and Yem special District).

3.2. Study Methodology and Sampling Strategy

The study used both experiment and survey. For the experiment the study used blood samples collected from 200 cattle (165 and 35 from sheko and zebu respectively). For this out of the ten districts except Surma which were excluded from the study due to security reason, nine districts namely (Sheko, Mizan, Semein Bench, Debub Bench, Gura Ferda, Shei Bench,Maji, Yeki and Minit Goldiya) were purposively included from Bench Maji Zone. In addition, based on sheko cattle availability and accessibility Chena and Bita districts were selected from Keffa zone. Moreover, to make the sampling representative blood samples were also taken from sheko cattle owned by institutions such as Mizan Tepi University, Tepi-research center and an Association which claimed they have pure Sheko cattle. On the other hand Yem, district was also included, to collect blood samples from zebu cattle which are far from the breeding tract of Sheko cattle. Households which owned at least one Sheko cattle were selected purposely. Accordingly a total of 99 (72, 17 and 10) households were selected from Bench Maji , Keffa Zones and Yem special distric respectively. Thus, a total of 165 Sheko and 35 zebu animals were used. Sheko cattles were identified based on phenotype description documented by different researchers (Takele et *al.*, 2011 and Tatek and Abegaz, 2013), consultation with local administration and agricultural extension officers, key informants (cattle traders, village chiefs, elders) and researchers. The existing true–to–type Sheko cattle were identified, located and enumerated through extensive consultation with Sheko cattle owners.

Table 2: Sampling distribution across district, kebele, conservation and research center

Zone	District	Kebeles	No. animal selected	Sex		Breed		No. household Selected
				M	F	Sheko	Zebu	
BMZ Zone	Debub-Bench	AmanZemika Danchu	23	9	14	23		14
	Gura Ferda	Alenga	6	4	2	6		3
	Minit-Goldiya	kushanta bachuma-01 bachuma-02	15	8	7	15		11
	Semen-Bench	Genja Garika	14	6	8	14		9
	She- bench	Zeyagen Kesheta	18	13	5	18		12
	Sheko	Mehalsheko Shasheka	17	10	7	17		10
	Maji	Tum	15	6	9		15	7
	Yeki	Beko Mariyam sefer	14	4	10	14		6
Kafa zone	Chena	Koda Kulesh	14	7	7	8	6	11
	Bita	Odda Yina	13	7	6	13		6
Yem special district	Yem		14	8	6		14	10
Associations and research center								
Association	Sheko	Mehalsheko	8		8	8		
Mizan Tepi Research center	Mizan		15	2	13	15		
Mizan Tepi University	Mizan		14	5	9	14		
Total			200	89	111	165	35	99

3.3. Sample size determination

The sample size of the study was determined by the number of sheko cattle owners who expected to have pure sheko that priory told to participate in the study at specific time and place and the information obtain from where pure sheko cattle was found; that was institution and research center. Accordingly a sample size of 200 cattle was sampled based on phenotypic data record sheet and cattle owner information.

3.4. Survey Data Collection

Survey data was collected from the same households which the blood samples were collected through interview using check list, observation and measurement. Accordingly, some morphometric trait (horn size and hump size) categorized as large, medium, small, very small and absent and qualitative (Sex, age and coat color) data were recorded for each animal according to the format adopted from the standard breed description list developed by FAO, 2012 (in annex of table 2 and 3).

3.5. Blood Sample Collection

Ten ml of Blood sample was collected from the jugular vein of the sample animals. Blood samples were collected following the international guidelines for ethical treatment. Blood samples were drawn separately into vactioners containing Ethylene-diamine-tetra-acetic acid (EDTA) as anticoagulant, using vacationer needles. The samples were properly labeled according to breed and sex of animal and stored at -20^{o}c until processing.

3.6. 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 were separated from whole uncoagulated blood by addition of equal volume of normal saline (9.0g of NaCl in 1 liter deionized water) and allow standing for 30 minutes. The washing solution was removed by decanting. The separated RBC was finally lysis by addition of two volumes of deionized water, exposing the haemoglobin. Haemoglobin genotypes was 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 was 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 could be read directly on the gel without staining and motility was detected based on the molecular weight of the hemoglobin molecules one slow band (Hb^{AA}), one fast band (Hb^{BB}) and intermediate speed of two bands for the heterozygous (Hb^{AB}) (Chuku and Uwakwe, 2012).

3.7. Statistical Analysis

Survey data were analyzed using statistical analysis system of SPSS version 21. Descriptive statistics for frequency of observation and chi-square test to detect statistical differences among sampled cattle breeds were carried out. Data obtained from Hb alleles and genotypes were analyzed using descriptive statistical tool of SPSS version 21 to estimate the gene and genotype frequencies. Logistic regression analysis was carried out for statically significant variables to measure the degree of variation. Genotype frequencies were subjected to Chi-square analysis to test for goodness of observed and expected frequencies under Hardy-Weinberg equilibrium (HWE) (Chuku and Uwakwe, 2012). Genotype frequencies were calculated as follows

$$\text{Genotype frequency of Hb}^{AA}\ (P) = \frac{\text{No of individuals with Hb}^{AA}}{\text{Total no of individuals sampled}}\ \text{X100}$$

$$\text{Genotype frequency of Hb}^{AB}\ (Q) = \frac{\text{No of individuals with Hb}^{AB}}{\text{Total no of individuals sampled}}\ \text{X100}$$

$$\text{Genotype frequency of Hb}^{BB}\ (H) = \frac{\text{No of individuals with Hb}^{BB}}{\text{Total no of individuals sampled}}\ \text{X100}$$

Gene frequencies were calculated according to

Hardy-Weinberg equation as follows:

$p = P + ½ H$

$q = Q + ½ H$

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) were used to assess genetic variability in the sample used in this study

Heterozygosity (H) = 1- $\sum_{ί=1}^{n} x^2$ ί

Where:

X = the gene frequency of the ίth allele in a locus

ί= the number of tested loci

The effective number of allele (ne) per locus was estimated as:1/1-H

Where:

H = heterozygosity (Nyamsamba *et al*., 2003).

Polymorphicity (%): a locus is polymorphic if the segregating genes are more than one and frequency of the rarest gene is at least 0.01% (Sanjalj *et al*., 2000).

The model for the observation of genotype described as

yijk = β0 + BX1 + SX2 + EX3 + + eijk

Where:

Yijk = the observation of genotype

β0 = the intercept; B, S and E which are the independent variables sex, breed and environmental effect.

X1, X2 and X3 are the regression coefficients of the variables B, S and E

eijk = the residual error

4. RESULTS

4.1 Phenotype

A phenotypic data taken from sheko and zebu cattle exhibiting specific descriptor states of qualitative characters and morphometric traits as showed (Table3). The observed frequency distribution of morphometric and qualitative characters among each cattle are different. Majority of sheko breed were brown type (49.1%) and multi-colored (45.5%) while zebu were brown type (51.4%) (Table3).. Most of sheko cattle have no horn (66.1%) but (80.0%) of zebu cattle have horn with large size (Table3). Majority of sheko cattle (73.9%) were found with small hump size while less number (3.1%) of sheko cattle was found with large hump size (Table3). Almost half of zebu cattle (45.7%) were found with small hump size while (28.6%) were found with large hump size (Table3). The difference observed in the recorded phenotype of both breeds also found statically significance (p<0.05)

Table 3: Frequency distribution of coat color, horn and hump size across each breed

Characters	**Attributes**	**Breed**				
		No. Sheko	Percentage	No. Zebu	Percentage	p-value
Coat color	Black	8	4.8%	6	17.1%	0.001
	Brrown type	81	49.1%	18	51.4%	
	Dalecha	1	0.6%	3	8.6%	
	Multi-colored	75	45.5%	5	14.3%	
	White	0	0.0%	3	8.6%	
Total		165	100%	35	100%	
Horn size	Large	0	0.0%	28	80.0%	0.0001
	Medium	2	1.2%	6	17.1%	
	Small	44	26.6%	1	2.9%	

	Very small	10	6.1%	0	0.0%	
	Absent	109	66.1%	0	0.0%	
Total		165	100%	35	100%	
Hump size	Large	5	3.1%	10	28.6%	
	Medium	32	19.4%	7	20.0%	0.001
	Small	122	73.9%	16	45.7%	
	Very small	6	3.6%	2	5.7%	
Total		165	100%	35	100%	

4.2. Genotype

Hemoglobin loci were found to be polymorphic in both cattle 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 both sheko and zebu cattle (figure 2 and 3) respectively.

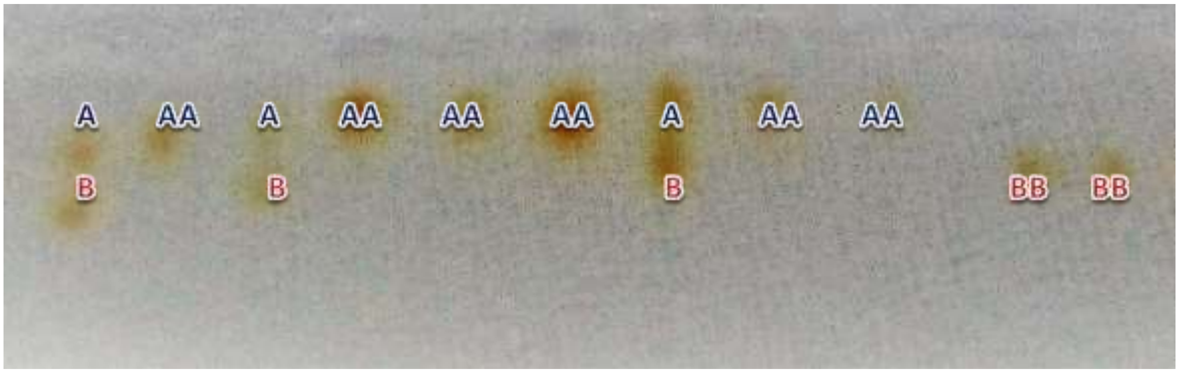

Figure 2: The observed hemoglobin polymorphism in Sheko cattle.

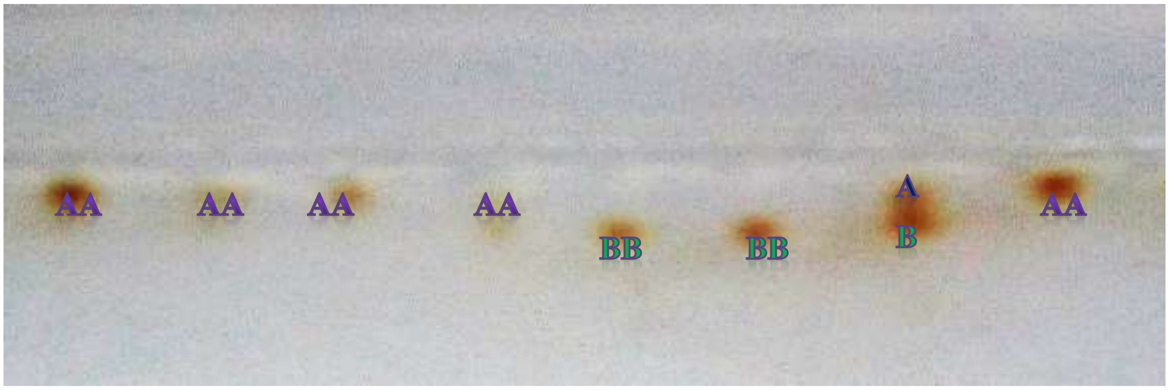

Figure 3: The observed haemoglobin polymorphism in zebu cattle.

The present study revealed that among the three genotypes the most frequent genotype Hb^{AB} (0.60) and Hb^{AA} (0.50) was observed in sheko and zebu breed respectively (Table 4). The gene frequency of Hb^{A} allele was 0.67 and that of Hb^{B} allele was 0.33 in sheko while in zebu cattle the gene frequency of Hb^{A} allele was 0.65 and that of Hb^{B} allele was 0.35 (Table 4). In this result majority of sheko cattle are heterogeneous (Hb^{AB}) in genotype while zebu cattle are homogenous (Hb^{AA}). This difference also found statically significant in both breeds at ($P<0.05$). In this study binary logistic regression analysis was conducted to determine the degree of association. In sheko cattle genotype Hb^{AB} is 17.31 times more likely to occur than genotype Hb^{BB} and genotype Hb^{AB} is 6.03 times more likely to occur than Hb^{BB} while in zebu cattle genotype Hb^{AA} is 10.51 times more likely to occur than genotype Hb^{BB} and genotype Hb^{AB} is 5.35 times more likely to occur than Hb^{BB}.

Table 4: Allele and genotype frequency distributions of heamoglobin across the breed

Breed	Allele frequency	Genotype	Genotype Observed	Genotype Expected	Genotype Frequency	p-value	OR(CI)
Sheko	A=0.67	Hb^{AA}	62	66.0	0.376	0.001	6.03(1.59-22.93)
		Hb^{AB}	99	89.9	0.6		17.31(4.32-69.56)
	B =0.33	Hb^{BB}	4	9.1	0.024		
Total	1.00		165	165.0	1.00		
Zebu	A=0.65	Hb^{AA}	18	14.0	0.50		10.51(0.66-165.10)
		Hb^{AB}	10	19.1	0.294		5.35(0.83-34.60)
	B =0.35	Hb^{BB}	7	1.9	0.206		
Total	1.00		35	35.0	1.00		

The results of this study indicated that three types of heamoglobin genotypes were observed across the sex of each breed of sampled cattle. The most frequent genotype of Hb^{AB} (0.352) and allele of Hb^{A} (0.39) were observed in female of sheko cattle. But the most frequent genotype of Hb^{AA} (0.286) and allele of Hb^{A} (0.36) were observed in male of zebu cattle (Table 5). Even though, there was apparent difference of genotype and allele frequency across the sex of each breed, the hemoglobin polymorphism is not statically significant ($P > 0.05$).

Table 5: Allele and genotype frequency distributions of heamoglobin across the of sex of each breeds

Breed Sheko	Allele frequency	Genotype	Genotype Observed	Genotype Expected	Genotype frequency	P- value
Sex						
Female	A=0.39	Hb^{AA}	36	36.1	0.218	0.943
		Hb^{AB}	58	57.6	0.352	
	B=0.19	Hb^{BB}	2	2.3	0.12	
Male	A=0.29	Hb^{AA}	26	25.9	0.158	
		Hb^{AB}	41	41.4	0.248	
	B=0.13	Hb^{BB}	2	1.7	0.12	
Total	1.00		165	165.0	1.00	
Zebu						
Female	A=0.30	Hb^{AA}	8	7.7	0.229	0.667
		Hb^{AB}	5	4.3	0.143	
	B=0.13	Hb^{BB}	2	3.0	0.57	
Male	A=0.36	Hb^{AA}	10	10.3	0.286	
		Hb^{AB}	5	5.7	0.143	
	B=0.21	Hb^{BB}	5	4.0	0.143	
Total	1.00		35	35.0	1.00	

Heamoglobin genotypes were found polymorphic in small hump sized of sampled sheko cattle with most frequent genotype of Hb^{AB} (0.43) and allele of Hb^{A} (0.50). But only two types' heamoglobin genotypes (Hb^{AA} and Hb^{AB}) were observed across the hump size of Large, medium and very small hump size of sheko cattle (Table 7). Heamoglobin genotypes were found polymorphic across the hump size of Large, medium and small with most frequent genotype of Hb^{AA} (0.25) and allele of Hb^{A} (0.30) in small hump sized zebu cattle. But only two type's heamoglobin genotypes (Hb^{AA} and Hb^{AB}) were observed across the hump size of very small (Table 7). But the difference is not statically significant ($P > 0.05$) irrespective of the hump sizes of both cattle breeds.

Table 6: Allele and genotype frequency distributions of heamoglobin across the hump size of each breed

Sheko	Allele frequency	Genotype	Genotype Observed	Genotype Expected	Genotype frequency	P-value
hump size						
Large	A=0.02	Hb^{AA}	2	1.9	0.12	0.533
		Hb^{AB}	3	3.0	0.18	
	B=0.01	Hb^{BB}	0	0.1	0.00	
Medium	A=0.12	Hb^{AA}	9	12.0	0.55	
		Hb^{AB}	23	19.2	0.139	
	B=0.06	Hb^{BB}	0	0.8	0.00	
Small	A=0.50	Hb^{AA}	47	45.8	0.285	
		Hb^{AB}	71	73.2	0.43	
	B=0.24	Hb^{BB}	4	3.0	0.24	
Very small	A=0.04	Hb^{AA}	4	2.3	0.24	
		Hb^{AB}	2	3.6	0.12	
	B=0.01	Hb^{BB}	0	0.1	0.00	
Total	1.00		165	165.0	1.00	
Zebu						
Large	A=0.22	Hb^{AA}	7	5.1	0.20	0.331
		Hb^{AB}	1	2.9	0.03	
	B=0.08	Hb^{BB}	2	2.0	0.06	
Medium	A=0.09	Hb^{AA}	1	3.6	0.03	

		Hb^{AB}	4	2.0	0.11
	B=0.12	Hb^{BB}	2	1.4	0.06
Small	A=0.30	Hb^{AA}	9	8.2	0.25
		Hb^{AB}	4	4.6	0.11
	B=0.15	Hb^{BB}	3	3.2	0.09
Very small	A=0.11	Hb^{AA}	1	1.0	0.03
		Hb^{AB}	1	0.6	0.03
	B=0.02	Hb^{BB}	0	0.4	0.00
	1.00		35	35.0	1.00

Heamoglobin genotypes were found polymorphic across the horn size of absent and small sized sheko cattle with most frequent genotype of Hb^{AB} (0.40) and allele of Hb^{A} (0.44) in absent horn size (Table 8). But only two types' heamoglobin genotypes (Hb^{AB} and Hb^{BB}) and (Hb^{AA} and Hb^{AB}) was observed across horn size of medium and very small respectively. The difference found hemoglobin polymorphism is statically significant ($P < 0.05$) across the horn of sheko cattle. Heamoglobin genotypes were also found polymorphic across the horn size of large and medium sized zebu cattle with most frequent genotype of Hb^{AA} (0.43) and allele of Hb^{A} (0.56) in large horn size (Table 8). But only one type heamoglobin genotype of Hb^{AA} was observed in small sized horn of zebu cattle. Even though there was difference in the horn size within zebu cattle it was not reached to significant level ($P > 0.05$).

Table 7: Allele and genotype frequency distributions of heamoglobin across the horn size of each breed

Sheko	Allele frequency	Genotype	Genotype Observed	Genotype Expected	Genotype frequency	P-value
horn size						
Absent	A=0.44	Hb^{AA}	41	41.0	0.25	0.001
		Hb^{AB}	66	65.4	0.40	
	B=0.20	Hb^{BB}	2	2.6	0.01`	
Medium	A=0.01	Hb^{AA}	0	0.8	0.00	
		Hb^{AB}	1	1.2	0.01	
	B=0.02	Hb^{BB}	1	0.0	0.01	
Small	A=0.19	Hb^{AA}	18	16.5	0.11	
		Hb^{AB}	25	26.4	0.15	
	B=0.08	Hb^{BB}	1	1.1	0.01	
Very	A=0.04	Hb^{AA}	3	3.8	0.02	
small		Hb^{AB}	7	6.0	0.04	
	B=0.02	Hb^{BB}	0	0.2	0.00	
Total	1.00		165	165.0	1.00	
Zebu						P-value
Large	A=0.56	Hb^{AA}	15	14.4	0.43	
		Hb^{AB}	9	8.0	0.26	0.073

	B=0.25	Hb^{BB}	4	5.6	0.11
Medium	A=0.07	Hb^{AA}	2	3.1	0.06
		Hb^{AB}	1	1.7	0.03
	B=0.09	Hb^{BB}	3	1.2	0.08
Small	A=0.03	Hb^{AA}	1	0.5	0.03
		Hb^{AB}	0	0.3	0.00
	B=0.00	Hb^{BB}	0	0.2	0.00
Total	1.00		35	35.0	1.00

Polymorphic heamoglobin genotypes were observed only in the district of Bita and Minit Goldiya of sheko cattle. But two types' of hemoglobin genotype Hb^{AA} and Hb^{AB} were observed in the district of Gura Ferda, Debub Bench, Shebench, Semen Bench, Chena, Sheko, Yeki and in conservation and research areas (Association, Research Center and MT University) with most frequent genotype of Hb^{AB} (0.10) and allele of Hb^{A} (0.09) in Debub Bench (Table 9) and least frequent allele of Hb^{B} (0.01) in Chena. Difference was observed across the district and the difference also was found statically significant ($P < 0.05$). But heamoglobin genotypes were polymorphic across the district where sampled zebu cattle were taken with most frequent genotype of Hb^{AA} (0.23) in Maji and Yem and most frequent allele of Hb^{A} (0.30) in Maji were observed in zebu cattle. Even though there was difference across the district of zebu cattle, hemoglobin polymorphism was not statically significant ($P > 0.05$).

Table 8: Allele and genotype frequency distributions of heamoglobin across the selected sites of each cattle breed

Sheko Selected sites	Allele frequency	Genotype	Genotype observed	Genotype expected	Genotype frequency	P-value
Kafa zone						
Bita	A=0.03	Hb^{AA}	2	4.9	0.01	0.001
		Hb^{AB}	8	7.8	0.05	
	B=0.05	Hb^{BB}	3	0.3	0.02	
Chena	A=0.04	Hb^{AA}	5	3.0	0.03	
		Hb^{AB}	3	4.8	0.02	
	B=0.01	Hb^{BB}	0	0.2	0.00	
BMZ Zone						
Debub Bench	A=0.09	Hb^{AA}	6	8.6	0.04	
		Hb^{AB}	17	13.8	0.10	
	B=0.05	Hb^{BB}	0	0.6	0.00	
Gura Ferda	A=0.03	Hb^{AA}	1	2.3	0.01	
		Hb^{AB}	5	3.6	0.03	
	B=0.02	Hb^{BB}	0	0.1	0.00	
Minit Goldiya	A=0.06	Hb^{AA}	7	5.6	0.04	
		Hb^{AB}	7	9.0	0.04	
	B=0.03	Hb^{BB}	1	0.4	0.01	
Semen Bench	A=0.06	Hb^{AA}	6	5.3	0.04	

		Hb^{AB}	8	8.4		0.05	
	B=0.02	Hb^{BB}	0	0.3		0.00	
Shebench	A=0.06	Hb^{AA}	5	6.8		0.03	
		Hb^{AB}	13	10.8		0.07	
	B=0.03	Hb^{BB}	0	0.4		0.00	
Sheko	A=0.08	Hb^{AA}	9	6.4		0.05	
		Hb^{AB}	8	10.2		0.05	
	B=0.03	Hb^{BB}	0	0.4		0.00	
Yeki	A=0.05	Hb^{AA}	4	5.6	.	0.02	
		Hb^{AB}	10	7.6		0.06	
	B=0.03	Hb^{BB}	0	0.8		0.00	
Association	A=0.04	Hb^{AA}	3	3.0		0.02	
		Hb^{AB}	5	4.8		0.03	
	B=0.02	Hb^{BB}	0	0.2		0.00	
Research Center	A=0.07	Hb^{AA}	8	5.6		0.05	
		Hb^{AB}	7	9.0		0.04	
	B=0.02	Hb^{BB}	0	0.4		0.00	
MT University	A=0.06	Hb^{AA}	6	5.3		0.04	
		Hb^{AB}	8	8.4		0.05	
	B=0.02	Hb^{BB}	0	0.3		0.00	
Total	1.00		165			1.00	
Zebu							p-value

Chena	A=0.09	Hb^{AA}	2	3.1	0.06	
		Hb^{AB}	2	1.7	0.06	0.059
	B=0.09	Hb^{BB}	2	1.2	0.06	
Maji	A=0.30	Hb^{AA}	8	7.7	0.23	
		Hb^{AB}	5	4.3	0.14	
	B=0.13	Hb^{BB}	2	3.0	0.06	
Yem	A=0.27	Hb^{AA}	8	7.2	0.23	
		Hb^{AB}	3	4.0	0.08	
	B=0.12	Hb^{BB}	3	2.8	0.08	
Total	1.00		35	35.0	1.00	

The estimated heterozygosities at the hemoglobin locus are shown in table 8 were 0.44 and 0.45 in sheko and zebu cattle respectively with mean of 0.45 in the entire population. The estimated Effective numbers of allele (ne) in this study were 1.79 and 1.82 in sheko and zebu respectively with **a** mean of 1.81(Table 10).

Table 9: Estimates of genetic variability within and across the breed of cattle

Breed	Locus	Heterozygosity (He)	Effective number of allele (ne)	Percentage polymorphism (% P)
Sheko	Hb	0.44	1.79	100
Zebu	Hb	0.45	1.82	100
Overall (mean)		0.45	1.81	100

5. DISCUSSIONS

5.1. Phenotype

In this study the most observed color in sheko breed were Brrown type (49.1%), Multi-colored (45.5%) and black (4.8%) are in line with the report of Alberro and Haile-Mariam, 1982 who observed mostly Brown coat color (82.5%). On the other hand this result disagree with Workneh , 2001 who reported coat color is predominantly red in plain (75%), patchy (15%) or spotted (9%) pattern and and Taye *et al*., 2007 who reported sheko cattle were dominated by glossy red hair coat. Majorty of sheko (80.0%) cattle have no horn which is in line with the report of (Alberro and Haile-Mariam, 1982 and Takele *et al*., 2007) who reported that many sheko cattle (87.35%) are polled and some (13.65%) with small jersey-like horns. Most of sheko have small hump (73.9%) which is in line with the report of (Workneh, 2001) who recorded that most of them have reduced type cervico-thoracic hump but in contrary to the report of (Alberro and Haile-Mariam, 1982) who recorded most of them are humpless.

5.2. Genotype

In this study heamoglobin was found polymorphic in sheko cattle with three distinct genotypes of Hb^{AA}, Hb^{AB} and Hb^{BB} controlled by two alleles with genotype frequency of 0.376, 0.60, and 0.024 for Hb^{AA}, Hb^{AB} and Hb^{BB} respectively and 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 in Ogaden cattle in eastern part of Ethiopia, 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 the Bunaji cattle, 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 native Sharabi cattle in Mousul, north of Iraq. But this finding was in contrary to the finding of Ahmed *et al*. (2010): reported four Hb variants in their study with cows and buffaloes with allelic frequencies of 0.51, 0.33, 0.014 and 0.01 for Hb A, Hb B, Hb C and Hb D in Egyptian cows respectively. This variation may came from sample size or may be the type and number of allele controlled the genotype of cattle breed vary one from other.

Heamoglobin locus was found polymorphic across the sex each breed and statically non-significant ($p>0.05$) agree with the previous study of Essien *et al*. (2011) that heamoglobin polymorphism was not statically significant across sex of Bunaji cattle and their Friesian crosses in Shika, Zaria Nigeria and Abdussamad *et al*. (2004) who reported heamoglobin genotypes were not statically significant in sex of Nigerian Zebu and their crosses in Zaria.

The results of chi-square showed that genotype frequencies at haemoglobin locus in the sheko cattle were not in Hardy Weinberg equilibrium and in agreement with (Ukwu *et al*., 2018) who conducted hemoglobin polymorphism in the Bunaji cattle, 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. In this study not only small size of population but also mating choice (selection) may lead to the violation of Hardy Weinberg equilibrium principles since farmers practices indiscriminate crossbreeding and replacement mainly with thoracic-humped zebu cattle due to Strong physique and aggressive temperament of Sheko cattle especially older individuals face difficulties in practicing tethered feeding feeding strategy since there is shrinkage of grazing land (Tatek and Abegaz, 2013) and also high feed requirements of Sheko cattle, which cannot match with ever increasing feed shortage because of expansion of farm land to feed the rapidly growing population (Takele *et al*.,2010).

The disequilibrium of genotype frequency at the haemoglobin locus in the sheko cattle observed in this study is not in agreement with the earlier observation Essien *et al*. (2011) in the Bunaji cattle and their Friesian crosses in Shika, Zaria Nigeria and Sanjoy and Yesihak (2014) Ogaden cattle in eastern part of Ethiopia, that their studies agree with Hardy Weinberg equilibrium.

The preponderance of heterozygotes (Hb^{AB}) against homozygotes of both types (Hb^{AA} and Hb^{BB}) in sheko cattle was agree also with finding of Essien *et al*. (2011) who reported three genotypes (Hb^{AA}, Hb^{AB} and Hb^{BB}) with frequencies of 0.40, 0.47 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 the Bunaji cattle in Shika, Zaria Nigeria. Heterozygote genotypes had superior advantage over the homozygotes in responding to tissue energy needs and output through tissue respiration, oxygen carbonic acid gradient in circulating blood and gaseous exchange between the animal and their environment (Gwaza *et al*., 2019) The higher Hb^{AB} was more favored in the sheko cattle than zebu cattle this properly allows an improved advantage of sheko cattle that favored it survival, fitness and adaptation to environmental challenges compared to the homozygotes (Hb^{AA} and the Hb^{BB}) genotypes (Aygun and Mert, 2007). Essien *et al*. (2011) probably suggested that the heterozygotes were favored by natural selection possibly for adaptation against the hot tropical weather and tolerance of tick and helminthic infections which is not achievable with exotic cattle breeds.

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 trypanosomiases disease believe that their genotype solely Hb^{A} allele may be responsible for this resistance.

In this study statically significant difference of genotype and alleles frequency were observed across the district, association and research institutions where Sheko cattle were sampled and this agree with the idea of (Evans and Turner, 1965) who has been documented that the frequencies of the two hemoglobin alleles vary from breed to breed within the same species and seem to be correlated with particular environments (Higher altitudes had a significant higher frequency of Hb^B, and the breeds that were common to lower altitudes had a significant higher frequency of Hb^A and (Hrinca ,2008) who suggested that temperatures 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.

Heamoglobin locus was found polymorphic across the observed quantitative traits such as hump size and horn size of sheko cattle. Heamoglobin genotypes were found statically significant across the horn size of sheko cattle. The most frequent genotype of Hb^{AB} was observed in sheko which have no horn. Heamoglobin genotypes were found polymorphic across the horn size of absent and small sized sheko cattle with most frequent genotype of Hb^{AB} (0.40) and allele of Hb^A (0.44) in absent horn size. In other hand the most frequent genotype of Hb^{AA} was observed in large horn size of zebu cattle.

Heamoglobin genotypes were also found polymorphic in small hump sized of sampled sheko cattle with most frequent genotype of Hb^{AB} and difference were observed across hump size sheko even though the difference is not reached at significant level. This indicates hemoglobin Haemoglobin types have been associated with some morphometric and performance traits in animals (Sam, 2012) reported that Hb^{AA} was superior to other genotypes in body conformation traits i.e animals with Hb^{AA} have longer horn length than Hb^{BB} and Hb^{AB} genotype. Study also indicated that the most valuable features, concerning shape and size of hair curls, as well as the quality and luster of hair fibers are associated with genotype Hb^{AB} and the weakest association of these features occurs with the phenotype Hb^{BB} (Hrincă and Vicovan, 2011). In this study Hb^{AB} genotype associated with small size of horn or polled and small hump size which are the typical phenotypic feature of sheko cattle.

The observed Heterozygosity (0.45) in this studies same as Al-Samarrae *et al*. (2010) who reported Heterozygosity of 0.45 in native Sharabi cattle in Mousul, north of Iraq, And in line with the finding of Ukwu *et al*.(2018) who **r**eported Heterozygosity of 0.47 in the Bunaji cattle in Makurdi, Nigeria, which was an indication of moderate level of genetic diversity at the haemoglobin locus, and Sanjoy and Yesihak (2014) who **r**eported Heterozygosity of 0.42 in Ogaden cattle in eastern part of Ethiopia. The heterozygosity value observed in this study is an index of moderate genetic diversity at the hemoglobin locus in sheko cattle. The observed heterozygosis at the haemoglobin locus in the sheko cattle is benefcial 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 sheko cattle 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).

6. SUMMARY AND CONCLUSION

Sheko cattle is one of the trypano-tolerant cattle strain in Africa which survives, produce and reproduce in a trypanosomiasis infested area of the country especially in wet areas of Ethiopia, South and South-west parts. Their Adaptation to extreme environments and tsetse challenge area breed could recognized as having great potential to prove food security for the present and future human generations. So a study was conducted to assess the hemoglobin polymorphism and its genomic signature in trypanotolerance sheko cattle. The results of this study suggest that the haemoglobin locus in the Sheko cattle in southwestern Ethiopia 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 heterozygote (Hb^{AB}) with allele Hb^{A} was the most frequent allele in the population of the sheko cattle studied. There exists a moderate genetic diversity with heterozygosity value of 0.45 at the haemoglobin locus in the sheko cattle. In this study the higher Hb^{AB} was more favored in the sheko cattle than zebu cattle. This properly allows an advantage of sheko cattle that favored it survival, fitness and adaptation to environmental challenges and resistant to devastating trypanosomiases disease. 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 significant ($P < 0.05$). The Chi- square (χ^2) test revealed that the population is not under Hardy-Weinberg equilibrium, due to small population size may affect gene and hence genotype frequency in a population.

7. RECOMMENDATIONS

- To obtain a clear over view about the gene and genomic structure of sheko breed A whole genome sequence should be conducted
- Further research should be carried out to determine the effect of the observed hemoglobin polymorphism and disease tolerance
- Further researches should be done determine association between hemoglobin polymorphism and all the remaining morphometric traits

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9. ANNEXES

Table 10: The standard breed descriptor list for Qualitative traits of cattle

No.	Type of Character	Description
1	Body hair coat color pattern	Plain, patchy, spotted
2	Body hair coat color	Black, dark red, light red, fawn, grey
7	Horn presence	Absent, present
9	Horn presence (at herd level; separately for males and females)	Percent of polled animals, percent of horned animals
10	Horn attachment (at herd level	Separately for males and females): percent of loose horns, percent of fixed horns
11	Horn shape	Straight, curved, lyre-shape, loose, stumps, polled
12	Horn orientation (at herd level, separately for male and females)	Tips pointing laterally, upward, downward, forward, backward (indicate also if animal is polled, or horns are loose or just stumps)
16	Hump size	Absent, small, medium, large

Source: FAO (2012)

Figure 4: Laboratory activities at Hawassa University in Animal biotechnology laboratory

BIOGRAPHY SKETCH

The author was born at Yechereka Kebele, Dembecha Woreda, West Gojam Zone, Amhara Regional State, on 21 September 1991. He attended his elementary and junior education at Anjenie Primary School from 1999-2007. He attended secondary and preparatory education at Dembecha Secondary and Preparatory School from 2008-2011. Upon passing the Ethiopian Higher Education Entrance Examination in 2011, He joined Samara University in 2012 and graduated with doctor of degree in Veterinary Medicine in 2017. After his graduation, He joined Samara University and served as Lecturer for one year from September 2017 until August 2018. He was married on 25 September 2018. Then He joined the Postgraduate Programs Directorate (PGPD) at Hawassa University in November 2018 to pursue his study leading to MSc Degree in Animal Biotechnology.