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Genetic Differentiation and Metabolic Adaptation of Cattle Populations along the Slopes of Simien Mountains of North Western Ethiopia

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Dedicated To

My Father: Wuletaw Gebeyehu

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Blen
Bemnet

Abstract

Sustainable utilization of animal genetic resources demands comprehensive characterization of the populations involved. In most of the cases, however, characterization has been limited to production potentials, systems and rarely to genetic distances and adaptation parameters. Here an effort is made to investigate cattle breeds of North Western Ethiopia in terms of genetic distances and adaptive attributes as a contribution for designing breeding strategies to the rural farming communities. First we investigated characteristics of altitude adaptation of indigenous and crossbred cattle populations found in the altitude range of 1700 - 3500 m. In this study a total of 218 animals were tested for their pulmonary artery pressure (**PAP**), percent arterial oxygen saturation (**% SaO_2**), and 672 animals for haematological parameters. Results show that no sign of pulmonary hypertension was observed among all the cattle genotypes. Low % SaO_2 and absence of hematopoiesia (Red blood cells and Haemoglobin) were also evidenced as typical features of the high altitude cattle. Further analysis of histological data of muscular pulmonary arteries revealed that these cattle do have thick medial walls but wider lumen diameter. We concluded that, though further verification with large sample size is suggested, Simien cattle have a special mode of adaptation to high altitude hypoxia probably due to the anatomical feature of distal pulmonary arteries (wider lumen). Analysis of genetic variability of these populations using microsatellite markers confirmed little but significant genetic differentiation and inbreeding with out evidence for recent bottleneck. Although further molecular analysis evidenced two major clusters, the populations exhibited high within breed genetic diversity, which is important for future breed development. Thus, it is concluded that adopting effective breeding and management practices while maintaining their adaptive attributes is the most rational and sustainable way to facilitate conservation and utilization of these adapted and genetically diverse indigenous cattle resources.

Key words: adaptation, cattle, genetic diversity, histology, oxygen saturation, Ethiopia

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CHAPTER 1

General Introduction

1. General Introduction

Domestication of livestock species and a long history of migrations, selection and adaptation have created an enormous variety of breeds. The utilization of these farm animal genetic resources to achieve and maintain sustainable production systems, which are capable of responding to human needs, is necessary to maintain national and global food security (FAO, 2007; Groeneveld et al., 2010). Livestock genetic diversity has provided the material for the very successful breeding improvement programs of the developed world in the 19th and 20th centuries. In economic terms, diversity can provide insurance against changes in production circumstances, new diseases, or changes in market demands. Further more, global climate change requires diversity of livestock breeds (Seo and Mendelsohn, 2007; Hoffmann, 2010) that withstand greater extremes in temperature and rainfall.

FAO's latest global assessment of breed diversity identifies 7040 local and 1051 transboundary breeds (FAO, 2009). The risk status of 36% of these breeds is unknown, about 9% of reported breeds are extinct and 20% are currently classified as being at risk. Species level assessment showed that 31% of cattle breeds are currently at risk and already extinct (FAO, 2009). The impact of these losses on the global or the local diversity remains undocumented and loss of within-breed diversity remains unquantified (FAO, 2007; 2009). While it is already too late for many breeds in Europe, the situation is also particularly worrying in the developing world where rapid changes in production systems are leading to the replacement of breeds or at best crossbreeding. There is therefore an urgent need to document the diversity of our livestock genetic resources and design strategies for their sustainable conservation.

1.1 Characterization of Farm Animal Genetic Resources

Characterisation of animal genetic resources encompasses a broad range of exploratory research outcomes on description of the origin, development, population size, structure, distribution, typical features and phenotypic performance of these resources in defined management and climatic environments (FAO, 1986; Cunningham, 1992). Currently two complimentary characterization methodologies, phenotypic and genotypic, are accepted and being widely used for documenting domestic animals genetic resources. Phenotypic characterisation is undertaken to understand the extent, distribution, basic characteristics, comparative performance, utility, value and current status of the animal genetic resources (FAO, 1998; Rege and Gibson, 2003). Genotypic characterization on the other hand has recently been used as a method of describing and classifying livestock breeds using measures of genetic distances between populations (e.g. Nei et al., 1983; Reynold et al., 1983).

Protein polymorphisms, particularly during the 1970's, were the first molecular markers used in livestock for characterization of blood group and allozyme systems of livestock. However, with low level polymorphism of protein, development of Polymerase Chain Reaction (**PCR**), (Mullis et al., 1986) and sequencing technologies associated with automatic and/or semi-automatic large scale screening system, DNA-based polymorphisms became the markers of choice for molecular-based surveys of genetic variation (Sunnucks, 2000). Since then, microsatellites became the most popular markers in livestock genetic characterization due to their high abundance in the genome, extremely high degree of polymorphism and easy detection. They have been effectively exploited to understand bovine domestication and migration pattern (Loftus et al., 1994; Edwards et al., 2000) and to evaluate genetic diversity and relationships among cattle populations (MacHugh et al., 1998; Kim et al., 2002; Mukesh et al., 2004).

1.2 Cattle Genetic Resource of Ethiopia

Origin and domestication of African cattle is yet unclear. Currently two thoughts are available from the literature. The first hypothesis considers that present day cattle arose from three main phases of introduction from the centre of domestication, Asia, through the Nile Valley in Egypt, and via the Horn of Africa (Epstein, 1957). These cattle are originated from the two main types of cattle sub species, *Bos indicus* (Zebu) and *Bos taurus* (Taurine) (Loftus et al., 1994; Hanotte et al., 2000). It is believed that since their arrival in Africa, extensive crossbreeding had occurred between Zebu and Taurine cattle populations and resulted the present day cattle populations of the continent. The second hypothesis points towards a separate domestication event for African taurine cattle (Bradley et al., 1996; Hanotte et al., 2002) in the North Eastern part of the continent. Hanotte et al (2002) further revealed that African taurus was the earliest cattle originated within the African continent, but with Near East and European genetic influences. Fore example the Sheko cattle breed which is restricted to the humid Sheko and Bench districts in South Western Ethiopia is believed to represent the last remnants of Africa's original *Bos taurus* cattle (Dadi et al., 2008).The present day cattle of the content are therefore result of long time domestication events shaped with peoples migration, long time evolutionary adaptation and selection.

Currently Ethiopia has an estimated 50.88 million heads of cattle (CSA, 2010). It has the largest number of indigenous cattle breeds/strains in the continent, and a substantial diversity. The existence of the large livestock diversity in Ethiopia is due in large part to its geographical location, near the historical entry point of many livestock populations from Asia, its diverse topographic and climatic conditions, the huge livestock population size and the wide range in production systems. Currently some 32 breeds/strains of cattle, classified under five groups, are registered (DAGRIS, 2009). In general, proper definition

and classification of indigenous Ethiopian cattle breeds is deficient. Breeds are defined on the basis of subjective data and information obtained from local communities. Reliance on these criteria alone as the basis for classification, utilisation and conservation is in most cases quite misleading. Molecular genetic studies provide objective measures of diversity within and between breeds and evidence for unique genetic attributes. Genetic characterisation of these cattle breeds based on DNA studies is therefore necessary as it is more reliable, since it is based on precise genotypic information.

1.3 Environmental Adaptation

1.3.1 Definitions and Adaptation Strategies

Sustainable utilization of domestic animal genetic resources demands comprehensive characterization of populations. In most of the cases however, characterization has been limited to production systems, production potentials and genetic distances. It is rarely that researchers did characterization of the farm animal genetic resources in terms of adaptive attributes. Here an effort is made to investigate local cattle populations in terms of genetic distances as well as their adaptation characteristics. Extreme environments evoke physiological responses to be unperceivable, repeatable and adjusted to the constraint. Understanding the biochemical mechanisms that enable animals to survive and function under conditions of extreme environments can provide important insights into the nature of physiological adaptation. Young et al. (1989) defined adaptation as a modification in the animal's behaviour or metabolic responses resulting from an experience that improves the ability of animal to cope with subsequent challenges. Prayaga and Henshall (2005) also defined it as the ability to survive and reproduce within a defined environment. Barker (2009) defined adaptedness as the state of being adapted, the ability of breeds to produce and reproduce in a given set of environments, or the choice of particular breeds for specific environments. Adaptability is then a measure of potential or actual capacity to adapt in

different environments (Hoffmann, 2010). Adaptation traits are usually characterized by low heritability. In relatively stable environments, such traits have probably reached a selection limit; however, they are expected to respond to selection if the environment shifts, thus resulting in changing fitness profiles and increases in heterozygosity (Hill and Zhang, 2009). Empirical evidence strongly supports the expectation that the genetic basis of population differentiation for fitness traits will be nonadditive, with different adaptive gene complexes evolved in each breed. Genetic improvement programs therefore should start with an adapted population, with selection then for production traits (Barker, 2001). Extensive genetic variation exists between varieties/breeds in a species and amongst individuals within breeds. This variation has developed over very long periods of time. A major ongoing challenge is how to best utilize this variation to meet short-term demands whilst also conserving it for longer-term possible use.

Many animal breeding programs have led to increased performance for production traits but this has often been accompanied by reduced fitness (Rege and Gibson, 2003). In addition, the global use of genetic resources prompts the question whether introduced genotypes are adapted to local production systems. Phenotypic characteristics, including adaptive characteristics, are important in identifying breed attributes in ways that are relevant to the immediate farming community's needs and utility. Given the current emphasis on sustainable agricultural systems, adaptation of breeds to their environment is particularly important. Well-adapted animals are essential components of such systems, especially where genotype–environment interactions are important. Understanding the genetic nature of adaptation will enable us to better manage genetic resources allowing us to make efficient and sustainable decisions for the improvement of these resources.

1.3.2 The Challenges of High Altitude Environment and Cattle

High altitude environments are characterized by a lower partial pressure of oxygen (pO2) and lower ambient temperatures compared to low-altitude environments at similar latitudes thus, present a number of physiological challenges for endothermic animals. Thus, animals in high-altitude are subject to hypoxia which is defined as the reduced availability of oxygen at high altitude (Bouverot, 1985; Schmidt-Nielsen, 1997). The reduced pO2 at high altitude results in reduced oxygen loading in the lungs such that the blood may not carry a sufficient supply of oxygen to the cells of respiring tissues (Bouverot, 1985; Monge et al., 1991; Schmidt-Nielsen, 1997). Hypoxia at high elevation causes pulmonary vasoconstriction, increased pulmonary artery pressure (PAP), right ventricle stress, congestive right heart failure, and hydrothorax in the chest cavity and brisket disease (Will et al., 1962; Rhodes, 2005). This disease in domestic animals is named as high altitude disease, high altitude pulmonary hypertension or brisket disease and is an indicative of a classic genetic by environment interaction (Ahola et al., 2002). Historically, the bovine species provided the first (known since 1889) clinical indication of hypoxic pulmonary hypertension and became the first animal model for the study of the disorder. It is also the bovine, among mammalian species, that exhibits the most severe chronic hypoxic pulmonary hypertension and significant variability in the magnitude of this response among individual animals (Rhodes, 2005). It appears that no one breed is resistant to the effects of high-altitude hypoxia (Tucker and Rhodes, 2001; Holt and Callan, 2007), even though some breeds, and pedigrees within breeds, appear to be more naturally resistant.

The disease is one of the top causes of morbidity and mortality (5% annual death loss) and also accounts for significant loss in growth and reproductive performance of cattle raised at high altitude in some parts of United States (Holt and Callan, 2007). The hypoxia

at high altitude can be particularly debilitating for lowland species, such that even basal metabolism is a challenge to sustain. Multiple factors contribute to the variance in pulmonary arterial pressure in cattle, including breed, gender, body condition, concurrent illness, environmental conditions, elevation, and genetics. PAP testing is an effective diagnostic and management tool used to identify clinically affected and high-risk animals. High values (> 50 mm Hg) indicate high risk and low values (< 35 mm Hg) indicate resistance to the disease (Rhodes 2005; Holt and Callan, 2007). No consistently effective treatment or prevention is available other than movement of affected cattle to lower elevation or selection using an indicator trait.

Ethiopia is a high land country where highlands covers 44% of the country's total land area, 90% of human population and carries an estimated 70% of country's cattle population (CSA, 2010). Cattle are kept and used at altitudes as high as 4000 m and play significant social and economic roles in the subsistence production systems. Under such circumstances no information is available regarding the high altitude disease of cattle in Ethiopia. There fore, this part of the study is initiated with the view to capture information about epidemiology and genetics of the disease through conducting two consecutive, haemodynamics and quantitative histological, studies.

1.4 Objectives of the Study and Outline of the Thesis

The overall objective of this study was characterizing indigenous cattle populations of the study area as a contribution for designing appropriate breeding strategies to the rural farming communities. To meet this objective the study addresses two aspects, adaptation to altitude and molecular characterization, of cattle genetic resources of North Western Ethiopia. The first part of the thesis deals with the adaptation of cattle to high altitude environment. Efforts are made to document how the cattle genetic resources of the study area, indigenous and crossbred, are adapting themselves to hypoxia as measured through

PAP testing, percent arterial oxygen saturation (% SaO_2), haematological and histological parameters. The first exploratory study investigates pulmonary and hematological parameters in relation to high altitude adaptation, specifically on how the native high altitude cattle responded to hypobaric hypoxia. Following this the second investigation is conducted with the view to understand survival and functionality of animals under hypoxic environment. This part of the study, which is basically a comparative haemodynmic and histological experiment, explains the pulmonary circulation of low altitude indigenous and crossbred animals transported to high altitude hypoxic environment, measures the extent of medial wall thickness of muscular pulmonary arteries of these populations. Furthermore, it examines the pulmonary vasculature of adapted Simien cattle to make a comparison with its counterparts. The outcome of the two investigations are presented and thoroughly discussed in the papers in part 1 of the thesis. In part 2, molecular characterization of indigenous cattle genetic resources of the study area is treated. Evaluation of population genetic structure of the different cattle populations through the use of molecular markers, microsatellites, is conducted. Results from the genetic diversity study are presented and discussed. Finally important findings of the research are presented under the summary and conclusions part.

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PART I

CHAPTER 2

Assessment of physiological adaptation of indigenous and crossbred cattle to hypoxic environment in Ethiopia

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ABSTRACT

High altitude pulmonary hypertension is common in cattle at high altitude areas. The extent of proneness, epidemiology, and genetics of the disease is not, however, known in Ethiopia where a large proportion of the area is at altitudes above 2,700 m. To learn about adaptive characteristics of cattle towards altitude, a study of physiological adaptation, measured via PAP score from 218, hematological parameters from 672, and arterial oxygen saturation predicted by pulse oximeter from 241 animals was conducted in North Western Ethiopia. Local breeds and their crosses with Holstein Friesian and Jersey were investigated. Results showed that all PAP scores (21 to 47 mm Hg) fall under low to moderate risks. No sign of pulmonary hypertension was observed among all the cattle genotypes. Crosses of the local cattle with Holstein Friesian and Jersey were not more prone to the disease than local cattle. A statistically significant ($P < 0.05$) decrease in the % SaO_2 to approximately 82% was present in the high altitude animals. Crosses and locals at high altitude, ≥ 2,700 m, did not exhibit significant differences ($P > 0.05$) in % SaO_2. We report a new clinically relevant range of oxygen saturation, ≥ 68%, for the high altitude cattle which is far below the threshold value usually assumed for temperate cattle, > 80%. Hematological values of the studied genotypes lie within normal ranges set for temperate breeds despite suffering from heavy parasitic infestation. The significantly greater ($P < 0.001$) red blood cell counts, hemoglobin and hematocrit values of Simien cattle measured at 3,500 m compared to the other genotypes in this study were not different when compared to other breeds studied elsewhere at lower altitudes and around sea level. Simien cattle probably have unique adaptations of oxygen uptake and delivery that result in the

absence of hypoxemic stimulus to increase red blood cell production and hemoglobin concentration. We concluded that indigenous cattle of the Simien Plateau of Ethiopia are adapted genetically to high altitude by largely eliminating the hypoxic pulmonary vasoconstrictor response. The good adaptation is most likely due to natural selection. Understanding this adaptation model requires investigation of the biological mechanisms and the underlying genetics.

Key words: adaptation, **altitude**, **Brisket disease**, **cattle**, **hematology**, **oxygen saturation**

1. Introduction

Cattle in the North Western Ethiopia are kept at altitudes of up to 4000 m and down to 550 m. Their phenotypic differentiation along levels of altitude is strong (Wuletaw, 2004). Such a continuous distribution of breeds across altitudes provides the opportunity to study adaptive characteristics of animals to effects of altitude. Numerous investigations have shown that high altitude hypoxia leads to pulmonary hypertension and stimulates hematopoiesia. High altitude pulmonary hypertension or brisket disease of cattle (Tucker and Rhodes, 2001) is common at high altitude areas and a result of reduced blood oxygen saturation at high elevation that results in decreased transport of oxygen to the tissues. Consequently animals suffer from physiological stress resulting from hypoxia that inhibits diffusion of oxygen from the air into the lungs (Schmidt-Nielsen, 1997). The disease is heritable (Will et al., 1975; Enns et al., 1992; Shirley et al., 2008).

Blood parameters are also considered as important indicators in measuring adaptation of animals to altitude. Investigations have shown that high altitude hypoxia leads hematopoiesia, where the reduced oxygen tension in high altitude regions to an increase of erythrocytes as an adaptive mechanism to low oxygen level (Schalm et al., 1975; Coles, 1986; Jain, 1993; Hyun et al., 2007). As a result indigenous animals permanently dwelling in the high altitude areas are known to have significant differences in a number of important blood parameters to their lowland counterparts (Jain, 1986; 1993). Hematological values that are currently in use as references in Ethiopia for all species of animals are those from temperate breeds (Tibbo et al., 2005). However, differences in environmental variables and genetic differences between breeds induce differences in hematological values. The use of hematological values that are derived from exotic breeds for monitoring adaptive attributes of indigenous breeds could be misleading. The extent of

proneness, epidemiology, and genetics of the brisket disease and level of % SaO_2 are not, however, known in Ethiopia where a large proportion of the area is at altitude above 2,700 m.

The objective of the study is, therefore, to learn about adaptive characteristics of indigenous cattle populations of Ethiopia and their crosses with European types towards altitude. Adaptation is measured via PAP test, pulse oximetry and analysis of hematological parameters.

2. Materials and methods

2.1The Study Area

The investigation was carried out in North Gondar and some part of South Gondar which are located in North Western part of Ethiopia (Figure 1). The altitude extended from 4620 m in the Simien Mountains in the North-East to 550 m in the Western parts of the study area and rainfall varies from 880 mm to 1,772 mm. Temperature ranges from a minimum of -2.5°C to 4°C to a maximum of 11°C to 18°C in the North and 22°C to 43 °C in the western lowlands (MWER, 2008).

2.2 Animals

The cattle in the region are different types of Zebu, and Zenga, a mixture of Zebu x Sanga, with strong phenotypic differentiation in terms of body size along levels of altitude. Furthermore, relatively small numbers of crosses of local types with Holstein Friesian and Jersey, having exotic blood proportion of around 50%, are also available within the range of 1,730 to 2,700 m. All animals are kept under the traditional extensive system of management. Young animals having an age of one to three years from both sexes representing the different altitudes and genotypes were considered. The study was conducted during the dry season, December 2006 to February 2007.

2.3 Methods of Data Collection

2.3.1 High altitude pulmonary hypertension

To assess the proneness of animals to high altitude pulmonary hypertension, PAP test scores were taken from representative populations of indigenous and crossbreds of local with Jersey and Holstein-Friesian. PAP is an indicator of proneness to the disease using a machine called Hewlett Packard (model, 78354-A, California, USA). High values (> 50 mm Hg) indicate high risk and low values (< 35 mm Hg) indicate resistance to the disease (Rhodes, 2005). The animals were restrained in a manually operated squeeze chute without use of anesthesia or sedatives. A rope halter controlled each animal's head during PAP testing. An experienced veterinarian from Colorado State University took the records. 218 animals composed of three indigenous and three crossbred populations residing within the altitude range of 1,730 to 3,500 m had their pulmonary artery pressure measured via right heart and pulmonary artery catheterization. In this procedure, a flexible plastic catheter is passed through a needle inserted into the jugular vein. The catheter is advanced down the jugular into the right atrium through the right atrioventricular valve into the right ventricle, through the pulmonary value (semi-lunar) and into the main pulmonary artery segment. Wave forms on an oscilloscope via a pressure transducer are observed to assure the catheter is in the right location. Pressure readings including systolic, diastolic and mean pressures are taken in the jugular, ventricle and pulmonary artery (Holt and Callan, 2007).

2.3.2 Arterial Hemoglobin Oxygen Saturation

A pulse oximeter (Eickemeyer oxyvet**:** model, 4802585, Tuttlingen, Germany) with a corresponding reflectance probe was used in our study to collect % SaO_2 values in non-invasive way. A transmittance probe is typically applied to an easily accessible portion of an animal that is well vascularized, the tail, a site with stable and intense signal and

smallest bias (Coghe et al., 1999), to monitor % SaO_2 while the animal is standing. Pulse oximeter predicts arterial oxygen saturation of hemoglobin using two wavelengths of light, near infrared and red light (Shapiro et al., 1989; Tremper and Barker, 1989). The red light is absorbed by deoxygenated hemoglobin, whereas the near infrared light is absorbed by the oxygenated hemoglobin. To determine % SaO_2, the transmittance probe measured the amounts of each light type that were reflected from the tissue. Saturation levels were differentiated between an artery and a vein because of the ability of the pulse oximeter to sense a pulse, which is unique to arteries (Shapiro et al., 1989). The use of pulse oximeter in monitoring health situation of animals such as on equines (Whitehair et al., 1990), on calves (Coghe et al., 1999; Uystepruyst et al., 2000) and small animals (Hendricks and King, 1993) has been taken as a standard procedure. Its accuracy was examined by (Emery, 1987; Coghe et al., 1999; Uystepruyst et al., 2000). Severinghaus and Kelleher (1992) documented limitations on finding probe position, abnormal pulses, influence of skin pigmentation, as well as false alarms and false non-alarms that may influence readings. Similar approach was used by Coghe et al., (1999), Uystepruyst et al. (2000) and Ahola et al. (2006). Details of data sources, study sites and number of animals to the corresponding studies are depicted in Table 1.

2.3.3 Hematological Parameters

Three millilitres of blood were collected from the jugular veins of 672 animals, representing different altitudes and genotypes (Table 1) into EDTA coated vaccutainer tubes. The animals were restrained in a manually operating squeeze chute without use of anesthesia or sedatives. After collection the samples were transported to the hematology laboratory at Gondar University hospital, Ethiopia with appropriate transportation and handling condition. The blood cells and related parameters were measured with the aid of

an automated hematology analyzer (model- Sysmex-kx 21**,** Japan). White blood cells counts (**WBC**), red blood cell counts (**RBC**), hemoglobin (**HgB**), mean corpuscular volume (**MCV**) were measured directly by the machine. Hematocrit (**HCT**), mean corpuscular hemoglobin concentration (**MCHC**) and mean corpuscular hemoglobin (**MCH**) were derived values. Hematological analyses were carried out within six hours after sample collection.

2.4 Data Management and Statistical Analysis

For any measured values, data more than three standard deviations from the sample mean were excluded from the analysis. The normality of the distribution of analyzed variables was examined from residuals. In some instances due to lack of significant differences data collected from crosses of Jersey with indigenous and Friesian with indigenous genotypes at 2,700 m were merged and termed as 'high altitude crosses'. Similarly crosses of exotics with locals at 1,730 m were merged and represented as 'mid altitude crosses'. PAP scores were analyzed using the Generalized Linear Model (**GLM**) of SAS (2008). Age and sex were not significant and excluded from the model so that the final model only included a single fixed effect (type of breed or cross). For hematological and oxygen saturation data tests for normality were rejected, and nonparametric tests are employed. Differences between means were tested for statistical significance by the nonparametric Kruskal-Wallis test with Bonferroni-Holm correction for multiple comparison post test. A value of $P < 0.05$ was considered statistically significant for all tests.

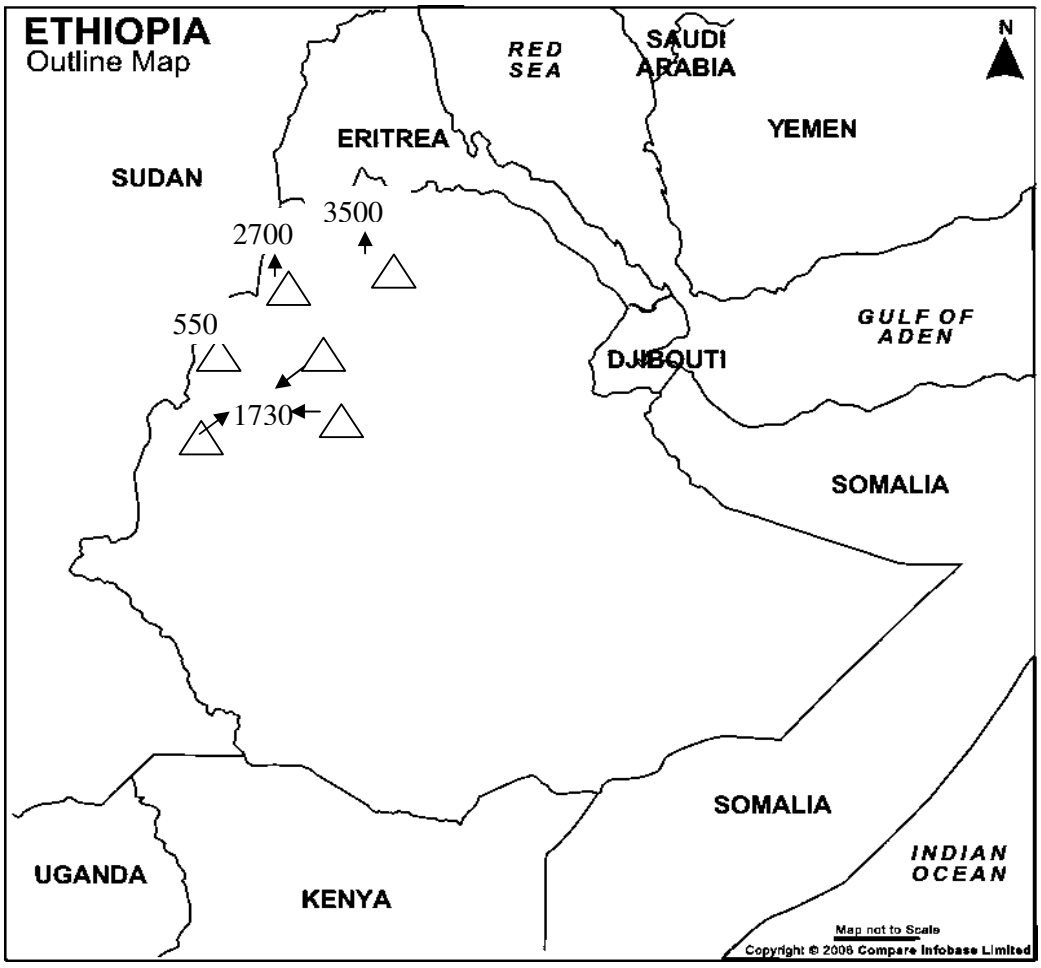
Table 1 Numbers of animals, stratified by breed and level of altitude, in different parts of the study

breeds	altitude, m	PAP[1]	[2]SaO_2	hematology parameters
Overall	550-3500	218	241	672
Indigenous	550-3500	126	168	490
Fogera	1730	55	-	37
Wegera	2700	39	81	54
Simien	3500	32	33	218
Metema	550	-	54	181
Crosses	1730-2700	92	73	182
Fogera x Friesian	1730	8	14	98
Wegera x Friesian	2700	64	36	43
Wegera x Jersey	2700	20	23	41

[1]Pulmonary artery pressure

[2] Arterial oxygen saturation

Figure 1 Map of Ethiopia showing the location of the study sites and their corresponding altitude



△ **Study sites**

3. Results

3.1 High Altitude Pulmonary Hypertension

The results in Table 2 indicate that no sign of brisket disease is observed among the populations studied. Animals found at high altitude (3,500 m) have PAP values comparable to that of the mid altitude (1,730 m) animals. All PAP scores (21 to 47 mm Hg) fall within the range of low to moderate risks. Differences in means were not significant for any pair of populations. Crosses of the local cattle with Holstein Friesian and Jersey were not more prone to brisket disease than local cattle measured at the same altitudes. However, there remains a considerable degree of individual variability within each of the populations.

Table 2 Studied populations, their respective location and corresponding PAP[1] scores (mean ± SD*)* measured in millimeters of Mercury (mm Hg)

breed	Altitude, m	Number of observations	PAP scores[2]	PAP scores Range
Overall	1730 - 3500	218	33.40 ± 3.94	21 – 47
Overall indigenous	1730 - 3500	126	33.08 ± 3.91	21 – 46
Fogera	1730	55	32.51 ± 2.95	27 – 42
Wegera	2700	39	34.41 ± 3.44	28 – 42
Simien	3500	32	32.47 ± 5.36	21 – 46
Overall crosses	1730 - 2700	92	33.84 ± 3.96	28 – 47
Fogera x Friesian	1730	8	34.50 ± 2.66	31 – 39
Wegera x Friesian	2700	64	33.42 ± 4.15	28 – 47
Wegera x Jersey	2700	20	35.00 ± 3.49	30 – 41

[1]Pulmonary artery pressure

[2]no significance differences (P > 0.05) within a column

3.2 Arterial Hemoglobin Oxygen Saturation

A statistically significant decrease in the % SaO_2 to approximately 82% was present in the high altitude animals. Arterial hemoglobin oxygen saturation tended to increase as the altitude decreases (Table 3). Differences for arterial hemoglobin oxygen saturation were significant ($P < 0.05$) between cattle populations residing at an elevation of 3,500 m (Simien) and 550 m (Metema).

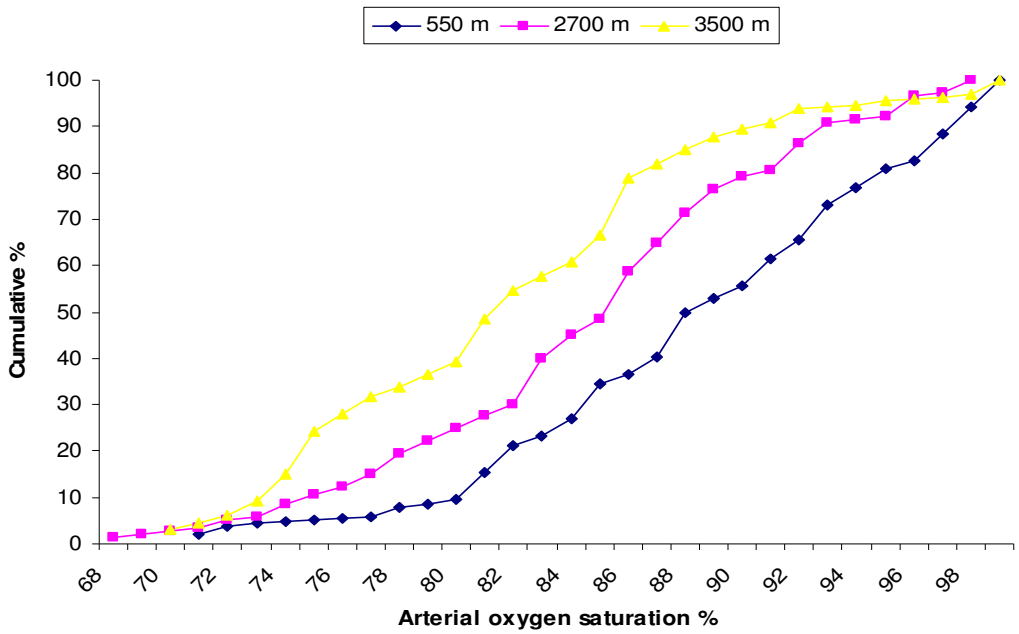


Figure 2 Cumulative frequency distribution of arterial oxygen saturation of Ethiopian cattle breeds residing at different altitude. Oxygen saturation distribution of cattle residing at 550 m, 2700 m, and 3500 m, mainly contrasting throughout the distribution curve and overlapping only at a very low and very high percent saturation levels. Significant proportions of cattle found at 2700 m and above have saturation level ≤ 80 %.

Further, there is also significant difference ($P < 0.05$) between Simien and crossbreds of the mid altitude areas. A range of variation in % SaO_2 among healthy individuals (68%

to 99%) was found which may indicate differences in physiological hypoxemia despite uniform ambient hypoxic stress.

Table 3 Arterial hemoglobin oxygen saturation (% SaO_2) values as predicted by the Pulse oximeter

breeds	altitude, m	Number of observations	[1]SaO_2, %	SaO_2 range, %
Overall indigenous	550-3500	168	85.00 ± 7.73	68 - 99
Simien	3500	33	82.45 ± 6.94[b]	70 - 99
Wegera	2700	81	84.17 ± 7.18[ab]	68 - 98
Metema	550	54	87.81 ± 8.26[a]	71 - 99
Overall crosses	1730-2700	73	86.22 ± 6.76	70 - 98
Mid altitude crosses	1730	14	87.28 ± 8.64[a]	70 - 98
High altitude crosses	2700	59	85.96 ± 6.30[a]	71 - 96
reference value			> 80 %[2]	

[1]within a column, means without a common superscript differ ($P < 0.05$)

[2]Coghe et al., 1999

3.3 Hematological Parameters

The effect of breed was significant ($P < 0.0001$) for all the blood parameters considered. Relatively greater within breed variation was noted for MCV, HCT, WBC counts (Table 4). Animals residing at 2,700 m and above have lower WBC than their counterparts found at 1,730 m and below**.** RBC, HgB, and HCT are significantly greater for the Simien cattle than for all other breeds. The differences between the other breeds are not significant. Except for WBC ($P < 0.001$), crossbreds at different altitudes do not differ significantly ($p > 0.05$). Similarly crosses and locals of the same altitude do not differ significantly ($P > 0.05$). This implies that the altitude effect is stronger than the effect of genotype. Comparison made between overall crosses and indigenous revealed no

significant difference (P > 0.05) for all the blood parameters studied. In general WBC categorized the studied populations in four altitude groups, MCHC in three groups, whereas RBC, HgB, HCT and MCV grouped populations in two.

Table 4 Hematological parameters[1] of the study animals expressed as mean and standard deviation (mean ± SD)[2]

breeds	altitude m	samples	WBC ($x10^3$/µl)	RBC ($x10^6$/µl)	HgB (g/dl)	HCT %	MCV (fl)	MCH (pg)	MCHC (g/dl)
Simien	3500	218	8.48±1.81^{e}	7.83±0.83^{a}	11.24±1.13^{a}	33.96±3.07^{a}	43.92±2.25^{a}	14.38±0.08^{a}	32.66±1.12^{a}
Wegera	2700	54	8.49±2.15^{e}	6.18±1.44^{b}	9.22±1.65^{b}	28.21±4.68^{b}	45.42±3.68^{b}	15.18±1.42^{b}	33.06±1.14^{bc}
Fogera	1730	37	11.28±3.34^{a}	6.62±0.98^{b}	9.60±1.31^{b}	29.20±4.59^{b}	44.27±3.24^{ab}	14.66±1.34^{ab}	32.97±1.04^{b}
Metema	550	181	10.73±2.62^{b}	6.60±0.98^{b}	9.61±1.31^{b}	29.20±4.16^{b}	44.58±2.95^{ab}	14.76±1.30^{ab}	32.92±0.90^{ac}
[4]MAC	1730	98	12.78±2.82^{c}	6.50±1.01^{b}	9.55±1.35^{b}	29.10±4.22^{b}	44.99±3.11^{ab}	14.76±0.98^{ab}	32.83±0.90^{ac}
[5]HAC	2700	84	8.82±2.45^{de}	6.53±1.41^{b}	9.71±1.70^{b}	29.04±5.40^{b}	45.20±4.28^{ab}	15.23±1.45^{b}	33.52±1.13^{ac}
[6]Ov ind	1730-3500	490	10.52±2.79	6.64±1.13	9.69±1.41	29.47±4.83	44.88±4.60	14.80±1.87	32.80±1.82
[7]Ov cr	1730-2700	182	11.02±3.40	6.05±1.20	9.61±1.51	29.05±4.78	45.12±3.72	14.94±1.23	33.13±1.07
[3]Reference			4 - 12	5 - 10	8 - 15	24 - 46	40 - 60		30 - 36

[1]Hematological parameters: WBC = white blood cells: RBC = red blood cells: **HgB** = hemoglobin: **MCV**= mean corpuscular volume: **HCT** = Hematocrit : **MCHC =** mean corpuscular hemoglobin concentration: **MCH** = mean corpuscular hemoglobin

[2]within a column, means without a common superscript differ ($P < 0.05$)

[3]Jain, 1993

[4]mid altitude crosses; [5]high altitude crosses; [6]overall indigenous; [7]overall crosses;

4. Discussion

Pulmonary hypertension from hypoxic pulmonary vasoconstriction threatens life at high altitude. There is evidence that certain high altitude species, apparently adapted to high altitude, have lost the hypoxic vasoconstrictor response and have a low pulmonary

arterial pressure (Anand et al., 1986). This paper described the characteristics of high altitude adaptation of indigenous cattle populations of Ethiopia and their crosses with European breeds. We collected pulmonary artery pressure, SaO_2, and hemtological data sets on a large number of cattle. Cattle in the Rocky Mountain regions of the United States are prone to Brisket disease, which is a form of severe pulmonary hypertension causing edema in the brisket and right heart failure (Weir et al., 1974; Will et al., 1962). Our goal was to learn/observe if the hematological and hemodynamic characteristics of cattle in the high-lands of Ethiopia (3500 m) showed evidence of brisket disease, such as occurs in the rocky mountain regions or were more adapted to high altitude.

4.1 High Altitude Pulmonary Hypertension

Pulmonary arterial pressure is an indicator of resistance to blood flow through the lungs and when measured at high altitude is a reliable predictor of susceptibility of an animal to brisket disease (Rhodes, 2005). We observed no significant changes in PAP between low and high altitude in the populations data sets. Crosses of the local cattle with Holstein Friesian and Jersey were not more prone to brisket disease than local cattle measured at the same altitudes. However, there remains a considerable degree of individual variability within each of the populations. Some of the readings (values < 28 mm Hg) for the Simien cattle group measured at 3,500 m are out of the range of readings of approximately 175,000 cattle that the veterinarian has taken in the Rocky Mountains in the course of 30 years. As reported by Holt and Callan (2007) such a variance in PAP readings of individuals within each of the populations might be explained due to multiple factors such as, gender, body condition, concurrent illness, environmental conditions, and genetic makeup of individuals.

Rhodes (2005) and Shirley et al. (2008) reported increase of the severity and incidence of disease with altitude. However, such a trend is not observed in our study. The average

mean PAP of studied populations was lower than those of Hereford calves measured at 3,400 m but born at sea level (55 mm Hg) and high altitude (48 mm Hg) respectively (Bisgard et al., 1974), Hereford measured at an altitude of 3,870 m (Anand et al., 1986), mixed breeds measured at an altitude of 4,500 m (Tucker et al., 1975), Angus bulls at 2,200 m (Ahola et al., 2006), Angus calves at weaning measured at 1,981 m (Shirley et al., 2008) and of susceptible and resistant calves at an altitude of 3,048 m (Will et al., 1975). It is relatively greater than PAP of Himalayan cattle (Anand et al., 1986) measured at 1,700 m, Hereford steers (Reeves et al., 1962) and Lama (Banchero et al., 1971). With such a relatively low PAP record it seems likely therefore that the strain of indigenous cattle established in Simien Plateau of Ethiopia over many generations is adapted. We have also observed that these cattle are smaller and quite distinct in appearance from the other local breeds that are available in the mid and low altitude areas of region.

Another important aspect of the result from our study is that crosses of locals with exotics, measured at an altitude of 2700 m, were not more susceptible to brisket disease and also did not show significant differences in their PAP records. The 50% blood proportion from the indigenous cattle might have some contribution for lack of significance difference. In a study comparing PAP readings in yak, cattle and their crosses (Anand et al., 1986) the crosses had equally low PAP readings as the yaks. Yaks are known to be resistant to high altitude disease due to an adaptation of vascular system, indicated by thin-walled small pulmonary arteries. Evidence from previous studies (Anand et al., 1986; Sun et al., 1989; Durmowicz et al., 1993) also showed that species that are long-term residents at high altitudes, such as yaks, snow pigs, and llamas, maintain a low pulmonary arterial pressure with an absence of highly muscularized pulmonary arterioles, despite living at very high altitudes. This is suggested to be an adaptation to chronic hypoxia through genetic transmission. Tucker et al. (1975) found significant linear

relationship between the amount of medial smooth muscle cells and pulmonary hypertension (r = 0.88) and ventricular hypertrophy (r = 0.97) for the seven species in response to chronic hypoxia. The Wagenvoorts (1969) noted a diminution of the thickness of the pulmonary arterial walls in sea level cattle after the age of 1 year. Further, Durmowicz et al (1993) found that Yak pulmonary artery endothelial cells were much longer, wider, and rounder in appearance than those of domestic cows. Differences in both endothelial cells morphology between the yak and those reported in the domestic cow suggest the adaptation to high altitude may include changes not only in the amount of pulmonary vascular smooth muscle but in endothelial cell function and structure as well (Durmowicz et al., 1993; Philip et al., 2006). Given the moderate to high (0.3 to 0.5), heritability of brisket disease (Will et al., 1975; Enns et al., 1992; Shirley et al., 2008), the lack of animals with PAP beyond the normal range confirms that animals at 3,500 m are genetically adapted. This is in agreement with the findings of Weir et al. (1974), Will et al. (1975) and Holt and Callan (2007), who reported that some breeds and pedigrees within breeds appear to be more naturally resistant to the effects of high altitude. One possible adaptation mechanism to this sample of high altitude indigenous cattle from the Simien Plateau of Ethiopia, in addition to what is suggested by other scientists, may be the low level oxygen saturation requirement of the cattle (see discussion on oxygen saturation). To learn about the adaptive mechanism of the studied cattle genotypes a quantitative histological study is currently being undertaken.

4.2 Arterial Hemoglobin Oxygen Saturation

High altitude reduces the amount of oxygen initially available in the environment to the tissue of the animals. Consequently, less oxyhemoglobin is produced resulting in decreased transport of oxygen to the tissues (Schmidt-Nielsen, 1997). This situation is believed to elicit strong pulmonary vasoconstriction. In such a situation the capacity to take in

sufficient air by virtue of anatomical features, respiration rate and physiological response is clearly an important aspect of adaptation to life at high altitudes. However, in our study we found that the mean oxygen saturation records of animals kept above 3500 m were within the range of clinically acceptable threshold levels like that of their lowland counterparts. This is consistent with our finding of PAP values from the same animals where no animal was found having a record out of the normal range. Nonetheless we found a wider range of oxygen saturation. Such a big variation, despite uniform hypoxic stress, could account for differences in physiological hypoxemia of individuals.

All mean values of % SaO_2 in the present study are lower than those of Hereford steers reported by Reeves et al. (1962), but greater than the finding of Bisgard et al. (1974) who reported 79% saturation for calves at 3,400 m, but affected with brisket disease. Will et al. (1962) reported mean SaO2 value of 94%, 87% and 81% of saturation from cattle categorized as control at 1,500 m, moderate hypertension at 3,000 m and severe hypertension groups at 3,000 m, respectively. An animal with saturation of 68% developed congestive failure in that study. Norton et al. (1998) reported mean values of 67% to 84% saturation from healthy neonatal lambs measured after one, five and ten minutes of birth respectively. Sheep are known to be hypo-responders to hypoxia (Tucker and Rhodes, 2001).

Clinically relevant range reported for cattle is > 80% SaO_2 (Coghe et al., 1999). Coghe et al. (1999) also reported < 80% of saturation from animals with clinical signs of undifferentiated bovine respiratory and all died. In our study we found values far below this range, down to 68% saturation. Analyzed cumulative percentage on the distribution of percent hemoglobin oxygen saturation showed that 10% cattle living at 550 m, 25% at 2700 m and 40% at 3500 m have saturation values $\leq$ 80%. We observed that all these animals are healthy. This is in agreement with the finding of (Schmidt-Nielsen, 1997) who

reported the ability of animals to adapt to decreased oxygen availability by increasing the effectiveness of oxygen uptake, thus shifting the oxygen dissociation curve to the left. In our study saturation range might go further down if potential overestimation of the pulse oximeter's percent arterial hemoglobin oxygen saturation is considered. In evaluating the accuracy of pulse oximeter in neonatal calves, Uystepruyst et al. (2000) reported a bias of +2.1%. The bias tended to be greater for lower ranges of % SaO2. Precision was also lower when SaO2 values were low. Coghe et al. (1999) also found a small bias between the measurements of % SaO2 and arterial blood gas samples, with a tendency for pulse oximeter to underestimate greater values and to overestimate lower values. The precision of pulse oximeter decreased substantially with the values for % SaO2 < 80%. The clinically relevant range reported for animals elsewhere, of > 80% SaO2 is therefore not applicable to cattle populations living at high altitude.

In humans three successful pattern of adaptation models to high-altitude hypoxia (Andean, Tibetan and Simien Plateau of Ethiopia) have been reported (Beall et al., 2002). People living on the Tibetan Plateau (4,000 m) exhibit little or no elevation of hemoglobin concentration compared to sea level population and has very low oxygen saturation. A similar pattern was found in our study. High-altitude indigenous cattle from the Simien Plateau of Ethiopia had hemoglobin concentrations that did not differ significantly from other cattle populations studied elsewhere (eg Doxey, 1977; Jain, 1993; Silva et al., 1999; Olayemi and Oyewale, 2002; Omer et al., 2002) and have low arterial hemoglobin oxygen saturation.

4.3 Hematological Parameters

Some hematological parameters were analyzed as per standard procedures to examine the possible change along the range of altitudes. Though we observed no significant changes in PAP records of animals across the different altitudes, differences in

hemtalogical values were noted. We found that HgB content, RBC and PCV increased significantly with the increase in altitude, which could be taken as an adaptive feature of high altitude cattle. This is in agreement with other studies by Jain (1986, 1993), Omer et al. (2002) and Olayemi et al. (2007), who reported significant differences of hematological values due to altitude and breed. Simien cattle had significantly ($P < 0.001$) greater RBC, HgB, HCT values than the rest of the studied breeds. The effect of high altitude on erythrocytes has been studied (Schalm et al., 1975; Coles, 1986; Jain, 1993; Hyun et al., 2007). Reduced oxygen tension in mountainous regions leads to an increase of erythrocytes as an adaptive mechanism to low oxygen level. Greater values for RBC, HgB and HCT in relation to high altitude are also reported by Cueva (1967), Jain (1993), Claxton and Ortz (1996) and Kumar and Pachauri (2000). Tucker et al. (1975) reported a significant increment in HCT for calves at 4,500 m. Significant difference of WBC count across the different altitudes and in some instances among genotypes is noted which is in agreement with findings of Schalm et al. (1975) and Jain (1993).

RBC values obtained in this study are greater than the values reported for Nigerian N'Dama and Fulani (Olayemi and Oyewale, 2002), Ayrshire (Holman, 1955), Holstein (Olusanya, 1979), Guernsey (Wingfield and Tumbleson, 1973), and lower than those of crossbred dairy cows (Kumar and Pachauri, 2000). PCV, MCV and MCH values of our study results are lower than N'Dama and Fulani reported by Olayemi and Oyewale (2002). Kumar and Pachauri (2000) reported lower values for HgB, MCHC, MCH; greater values for MCV and PCV and comparable value for RBC from well fed and healthy Holstein Friesian X Sahiwal dairy cows. Silva et al (1999) reported slightly greater values for HgB, and PCV (HCT) and lower value for MCHC from Zebu Gobra and Maure cattle breeds. Similarly HgB values observed in this study are lower than for a population of Friesian cattle (Omer et al., 2002) and the value reported for White Fulani and N'Dama (Olayemi

and Oyewale, 2002), but greater than the value reported for crossbred dairy cows (Kumar and Pachauri, 2000).

Significant difference of WBC count across the different altitudes and in some instances among genotypes noted in this study is in agreement with findings of Schalm et al. (1975) and Jain (1993). A slightly greater mean value of WBC for crossbreds at 1,800 m is reported which can be associated with a sign of leukocytosis (Jain, 1986). A slightly greater mean value of WBC for crossbreds at 1,800 m and Lowland cattle is reported which can be associated with a sign of leukocytosis (Jain, 1986). An increase in the number of WBC, leukocytosis, could be either physiological (Physiological leukocytosis) or could be reactive (Reactive leukocytosis) (Jain, 1986; 1993). In our situation the revealed leukocytosis could be attributed to the reactive type as the environment is highly infected with disease like trypanosomiasis and various types of tick born disease than the high altitude environment. WBC of our study are greater than the value reported for Holstein Friesians (Omer et al., 2002) and for N'Dama and White Fulani (Olayemi and Oyewale, 2002), but lower than the findings of AL-Shami (2003) for Hassawi cattle.

The capacity to take in sufficient air virtue of anatomical features and physiological response is clearly an important aspect of Simien cattle adaptation. However, the evidence from RBC, HgB, MCV and HCT content of Simien cattle to adaptation to life at high altitude is not fully conclusive. For RBC a mean of 7.0, and a range from 5.0 to 9.0, are given as normal values for adult cattle (*Bos taurus*) at or around sea level cattle in a review article by Doxey (1977). The 11.24 g/dl of HgB value of Simien cattle is close to the 11.0 g/dl given as the normal values of cattle by Doxey (1977), but slightly lower than the value reported by Jain (1993). This is also true for HCT, MCV and MCHC, which all are on average, lower than the normal mean given by Jain (1993), and by Olayemi and

Oyewale (2002). This, then, may indicate that Simien cattle have smaller red blood cells with a lower surface area.

Animals with relatively high HCT tend to have low MCV values and thus low MCHC. On the other hand, lower RBC and HCT and greater MCV values for both crossbred and indigenous cattle residing at 2,700 m indicate that the cattle are affected by internal parasite infestation. Molina et al. (2006) reported presence of inverse relation ship between parasite burden and RBC and HCT. A number of scientists reported on the reduced values of blood parameters due to parasitic infestations (Omer et al., 2002; Hofmann-Lehmann et al., 2004; Çöl and Uslu, 2006). The trend we observed in these hematological values could be taken as an adaptive mechanism of infected animals in which they compensated hemoglobin content by increasing the size of the cells.

Wuletaw (2004) reported frequency percentages on presence of parasites of 58% for external, 59 % internal parasites, up to 34 % for tick born diseases and around 44 % for viral and bacterial diseases in the study area. Despite this fact mean hematological values of the studied genotypes lie within normal ranges set for temperate breeds. However, slightly lower RBC**,** HCT**,** HgB are observed which is compensated by greater MCV. Our results are in agreement with Ngole et al. (2003) who reported values of hematological parameters from cattle under high infestation similar to clinically healthy animals under tropical conditions. The possible reason might be related to enzootic stability as a result of long time exposure of animals to infestation. However, with the exception of WBC, and MCHC, the rest of blood parameters considered had values close to the lower limit of the normal range. This is attributed to the direct effect of heavy parasitic infestation that the animals are facing. Çöl and Uslu (2006) reported a reduction of RBC, WBC, HgB and HCT from the reference values by 48%, 46%, 48% and 37% respectively in the affected cattle than in the controls. Similarly (Omer et al., 2002) reported statistically significant (*P*

< 0.005 to 0.0001) reduction for the same blood parameters and significant increase ($P <$ 0.01) in MCV value for the affected cattle.

5. Conclusions

This comparative study has investigated pulmonary and hematological parameters in relation to high altitude adaptation, specifically on how the native high altitude cattle responded to hypobaric hypoxia. Given the moderate to high heritability of brisket disease the lack of observations of PAP beyond the normal range confirms that animals at 3,500 m are genetically adapted. Furthermore the evidence that high altitude cattle of the study area with little or no elevation of RBC and HgB compared to its counterparts studied elsewhere at and around sea level coupled with the low SaO_2 suggests one form of adaptation to hypoxic environment. Thus, the indigenous cattle of the Simien Plateau of Ethiopia are adapted genetically to high altitude by largely eliminating the hypoxic pulmonary vasoconstrictor response in the absence of hypoxemic stimulus to increase red blood cell production and hemoglobin concentration. The good adaptation is most likely due to natural selection. The crossbred (local x European) animals observed in this study are also adapted up to 2700 m. Understanding the biological mechanisms and the underlying genetics of Simien cattle that allow successful high-altitude adaptation with low level of arterial hemoglobin oxygen saturation requires future investigation.

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CHAPTER 3

Haemodynamic and histological study of the pulmonary circulation on Ethiopian indigenous and cross bred cattle:

A comparative study on cattle native to high altitude and their lowland counterparts transported to high altitude

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Abstract

A rapid development of severe pulmonary hypertension in the lowland cattle when they move to high altitude is associated with the unusually muscular pulmonary arteries normally present in those animals. With this hypothesis we conducted a comparative study to monitor the response in pulmonary circulation of low altitude local and crossbred animals transported to high altitude in North Western Ethiopia. We also measure extent of medial wall thickness of distal muscular pulmonary arteries of these populations in relation to their exposure to high altitude hypoxic environment. PAP, % SaO_2 and histological measurements of distal pulmonary arteries were collected from 10 animals from low to medium altitude (550 - 1730 m) but transported and 3 native high altitude animals at 3500 m. PAP scores taken after two months of testing period ranged between low to high risk (22 - 67 mmHg), but difference in means were not significant ($P > 0.05$) among the breeds as analysed from nonparametric Kruskal-Wallis test. However, the lowland cattle group (550 - 750 m) shows the highest mean value making it a high-risk candidate for use in high elevation environment. Analysis of histological data of muscular pulmonary arteries using General Linear Model revealed that all the experimental animals have thick percent medial thickness (**% MT**). Mean % MT varies between 14.63 - 16.42 % in a Simien animal, 17.04 - 20.48 % in a Lowland animal, and 18.96 - 20.06 % in a crossbred animal, confirming the progressive increment of wall thickness in the more distal small pulmonary arteries. Breed difference was apparent as well in lumen and total vessel external diameter where the Simien was significantly ($P < 0.01$; $P < 0.001$) different from crossbred and Lowland respectively. The Lowland cattle % MT was positively correlated with PAP recordings of the animal confirming that pulmonary vasoconstrictor response to alveolar hypoxia is higher. The level of % MT noted in Simien and crossbred did not match with their respective PAP values which are still within normal range. Our result, though needs further

verification with a larger sample size, suggests that Simien cattle (with thick medial wall thickness and wider lumen) have special mode of adaptation to high altitude hypoxia. Therefore, it is concluded that given to the presence of thick medial thickness without significant PAP value and the effect of wider lumen area on PAP of an animal the hypothesis that a "thin walled pulmonary vasculature is a feature of genetic adaptation" might need some rewording so as to accommodate our findings.

Key words: cattle, high altitude, histology, medial thickness, pulmonary hypertension, Ethiopia

1. Introduction

Pulmonary hypertension from hypoxic pulmonary vasoconstriction threatens life at high altitude. The disease is apparently the consequence of severe pulmonary hypertension which develops in some species in response to reduced blood oxygen saturation at high elevation. It is characterized by right ventricular hypertrophy and edema of the chest and brisket (Will et al., 1962; Hecht et al., 1959; 1962). Historically, the bovine species provided the first clinical indication of hypoxic pulmonary hypertension and exhibits the most severe chronic hypoxic pulmonary hypertension. Thus, became the first animal model for the study of the disorder (Rhodes, 2005). Cattle losses from this disease alone can result in 3% to 5% of the calf crop, but can be much higher (25%), if the lowland cattle are transported to high altitude environment (Holt and Callon, 2007). A characteristic feature of cattle is a muscular pulmonary vasculature which responds sensitively to alveolar hypoxia by constriction. The small muscular branches of the pulmonary arterial tree, in particular, the distal media of pulmonary arteries with a diameter of 1,500 - 100 composed of phenotypically uniform population of well-differentiated SMC (Stiebellehner et al., 2003) are thought to be the primary sites for constrictions at a reduction in partial pressure of oxygen (Hecht et al., 1962; Tucker et al., 1975; Moudgil et al., 1998; Tucker and Rhodes, 2001). Experiments from animal model evidenced that reduction of the alveolar oxygen pressure to < 70 mmHg (Barer et al., 1970), < 75 mmHg (Anand et al., 1994) elicits strong pulmonary vasoconstriction and pulmonary pressure. PAP is used as reliable predictor of susceptibility of an animal to the disease (Tucker et al., 1975; Ahola et al., 2002; Holt and Callon, 2007). The unusually muscular pulmonary arteries normally present in cattle residing at low altitude are associated with a rapid development of severe pulmonary hypertension when those animals are moved to high altitude (Tucker et al., 1975; Hanson et al., 2000). However, previous studies found evidence that certain high

altitude species, apparently adapted to high altitude, have lost the hypoxic vasoconstrictor response. For example, in the Andes, the llama *(Lama glama)* has thin-walled pulmonary arteries (Heath et al., 1969; 1974) and a low pulmonary arterial pressure (Harris et al., 1982). Similarly the Andean mountain viscacha *(Lagidium peruanum)* (Heath et al., 1981) and the yak *(Bos grunniens) (*Anand et al., 1986) have thin walled pulmonary arteries and low pulmonary pressure.

Wuletaw et al. (in press) described the characteristics of high altitude adaptation of indigenous and crossbred cattle populations of Ethiopia. In that study a total of 218 animals, found in the range of 1700 - 3500 m, were tested for their PAP, % SaO_2, and 672 animals for hematological parameters in relation to high altitude adaptation, specifically on how the native high altitude cattle responded to hypobaric hypoxia. Results evidenced that the indigenous cattle of the Simien Plateau of Ethiopia are adapted genetically to high altitude by largely eliminating the hypoxic pulmonary vasoconstrictor response in the absence of hypoxemic stimulus to increase red blood cell production and hemoglobin concentration. The finding of this study has initiated us to examine the pulmonary vasculature of the high altitude cattle and to undertake further study on pulmonary circulation of low altitude indigenous and crossbred animals transported to high altitude environment. Thus, in the present study we have examined haemodynamic of the pulmonary circulation and structural pattern of distal pulmonary arteries of these populations at 3500 m to meet the following specific objectives.

1. To monitor the response in pulmonary circulation of low and mid altitude indigenous and crossbred animals transported to high altitude hypoxic environment
2. To measure the extent of medial wall thickness of muscular pulmonary arteries of these populations in relation to their exposure to high altitude environment

3. To examine the pulmonary vasculature of adapted Simien plateau cattle and make comparison with its counterparts transported from low altitude.

2. Material and Methods

2.1 The study Area:

The study was conducted in North and South Gondar zones located in North Western part of Ethiopia. The altitude ranges from 4620 m in the Simien Mountain in the North-East to 550 m in the western parts of the study area and rainfall varies from 880 mm to 1772 mm. Temperatures ranges from a minimum of -2.5°C to 4°C to a maximum of 11°C to 18°C in the North and 22°C to 43 °C in the western lowlands (MWER, 2008)

2.2 Animals:

The cattle in the region are different type of Zebu, and partly Zenga, a mixture of (Zebu x Sanga) with strong phenotypic differentiation in terms of body size along levels of altitude. In addition, crossbreds of locals with Holstein Frisians and Jersey, having different level of inheritance are also available. All animals are kept under extensive system of production. For our experiment 13 young animals (<1 year of age) representing different breeds/altitude, ranging from 550 – 3500 m, were considered. Crosses with more than 75% of exotic inheritance and indigenous cattle representing 550 m and 1730 m were transported to the high altitude area, Semein Mountains, at 3500 m, where they were kept for two months of investigation period. During this time the animals were managed by contracted farmers. Prior to their transportation they were checked for their basic health situation and all found healthy were transported. Reports suggest the sensitivity of PAP to age and season in which PAP was measured (Holt and Callon, 2007; Shirley et al., 2008). Animals were tested for their adaptation during the coldest season of the year. Details of data sources, study sites and number of animals to the corresponding studies are given in Table 1

Table 1 Altitude ranges, cattle breeds and number of observations investigated for PAP, SaO_2 and histological measurements

breed	altitude m	number of observations
Overall	550 - 3500	13
Fogera	1730	4
Simien	3500	3
Lowland	550	3
Fogera x Friesian	1730	3

2. 3 Methods of Data Collection

2.3.1 PAP Recordings

PAP was obtained by right heart catheterization after the end of the testing period using Hewlett Packard (model: 78354-A, California, USA). In this procedure, a fine plastic tube is passed through a needle in the jugular vein, with blood flow into the upper right side of the heart (atrium), through a valve, into the lower right side (ventricle), through a valve, and into the pulmonary artery just short of the branches to the lungs. Pressure waves are observed on a heart monitor and the monitor gives a direct readout of the true average pressure. Details of the method are explained in Wuletaw et al (in press).

2.3.2 Arterial Hemoglobin Oxygen Saturation

A pulse oximeter (Eickemeyer oxyvet**;** model: 4802585, Tuttlingen, Germany) with a corresponding reflectance probe was used in our study to collect % SaO_2 values on non invasive way. Values from each of the animals were taken before PAP recordings were taken. Details of the procedure are described in Wuletaw et al (in press).

2.3.3 Histology Sample Preparation

After completion of the pressure and other measurements at an altitude of 3500 m, three animals which have the highest PAP value and representing the Simien, Crossbred and Lowland were slaughtered. The chest was then opened, and the heart and lungs were removed. Four blocks of lung tissue were taken from the right and left lungs and suspended in 10% formaldehyde solution for one week. The tissues were embedded in paraffin and cut into 5 μm thick sections with microtome. The sections were then stained with hematoxylin and eosin. Some 23 to 25 pulmonary arteries were examined in each of the three animals. The vessel diameter and wall thicknesses were measured by a digital microscope.

Assessment of Muscularization:

Computer-assisted image analysis for morphometric measurements were made of the external diameter of the media and average thickness of the media of pulmonary arteries up to 1000 μm in diameter, which had been sectioned as nearly as possible transversely. Efforts are made to measure vessels that were virtually circular in transverse section; this method of selection decreased the number of arteries included in each study but avoided error in the measurement of the vessel diameter. In total 105 small muscular pulmonary arteries (26 - 41 in each animal representing different breed) were measured. The external diameter was taken as the mean of two measurements, at right angles to each other, of the distance between diametrically opposite points on the external elastic lamina. The medial thickness was estimated as the mean of four measurements taken at points approximately equally spaced around the vessel wall. Taking into account the possible considerable variation in the preparation and fixation of the tissue from the lung which lead to the relatively collapsed or distended blood vessels compared to in vivo properties the most common assessment has been to determine percent medial thickness (% MT) as: thickness

of the media (μm) /external diameter (μm). A value of the average percentage medial thickness in each animal was obtained by totalling all the mean values for percentage medial thickness and dividing the sum by the number of vessels examined. This technique was employed by different workers (Heath and Best, 1958; Wagenvoort, 1960). The suggested range of external diameter of arteries classified as "muscular pulmonary" in cattle has been found variable. Alexander suggested the muscular artery to be 200 – 300 μm of in diameter. Best and Heath, (1961) indicated 30-90 μm and Stiebellehner et al (2003) suggested that the distal pulmonary arteries ranged between 50 -100 μm in diameter. After some debate about the site of hypoxic constriction in the pulmonary vascular bed it was demonstrated that hypoxia constricts pulmonary muscular arteries, vessels of <1 000 μm in diameter (Bergofsky et al., 1968; Glazier et al., 1971).

Therefore, in our study we considered arteries having an external diameter up to 1000 μm but grouped in three categories to optimize structural analyses. Category I included arteries with an external diameter between 100 and 1000 μm; category II contained arteries with an external diameter between 100 and 500 μm and category III included those arteries with an external diameter of 100-300. Some 105, 83 and 56 arteries were measured within each respective category.

2.4 Statistical Analysis

For data of PAP values, and oxygen saturation, tests for normality were rejected hence nonparametric tests are employed. Differences between means were tested for statistical significance by the nonparametric Kruskal-Wallis test with Bonferroni-Holm correction for multiple comparison post test. As tests for normality were accepted data of histological measurements were analyzed using General Linear Model (GLM) from SAS v 9.2 (2008). In this case multiple comparisons tests were accomplished using Tukey's studentized range test. A value of $P < 0.05$ was considered statistically significant for all tests.

3. Results

3.1 Pulmonary Hypertension

PAP scores taken at the end of testing period at 3500 m ranged between low to high risk (22 - 67 mmHg), but difference in means were not significant ($P > 0.05$) among the breeds (Table 2). Similarly, no significance difference was observed between indigenous and crossbreds. The lowland cattle group (550 m) shows the highest mean value making it a high-risk candidate for use in high elevation environment. The mean value of this group was heavily influenced by one animal for which the PAP recording was 67 mmHg suggesting the presence of individual susceptibility. One animal from the mid altitude group, 1730 m, had a very low % SaO2 value with out any symptoms for brisket disease and other health complications as measured in terms of PAP value, body temperature (37.7oc), heart beat (55/minute) and respiration rate (18/minute). We noted a non-significant negative correlation between PAP values and altitude and proportion of exotic blood level inheritance. The wide range o f PAP values observed in this study suggests individual variability in susceptibility to the disease.

3.2 Arterial Oxygen Saturation

Measure of % SaO_2 level of investigated animals taken after the testing period at 3500 m is presented in Table 2. No significant reduction in the saturation level was seen for cattle transported to the high altitude compared to the native high altitude Simien cattle. Similarly lack of significant difference between crossbred and indigenous is evidenced. All groups except Fogera, have comparable saturation level, Simien cattle show the highest saturation.

Table 2 Studied populations, their respective source location and corresponding PAP scores in millimeters of Mercury (mmHg) and percent SaO_2 measured at 3500 m altitude after two months of testing period

breed groups	altitude m	animals	SaO_2 mean ± s.d	SaO_2 range	PAP scores mean ± s.d	PAP scores Range
overall	550 - 3500	13	77.38±6.40	63 - 87	34.46±11.33	22 - 67
Fogera	1730	4	72.75±7.21	63 - 78	29.25±4.51	23 - 35
Simien	3500	3	81.33±5.86	72 - 87	27.33±5.10	22 - 33
Lowland	550	3	78.67±2.08	77 - 81	46.66±17.61	36 - 67
Fogera x Friesian	1730	3	78.67±5.86	74 - 85	36. 00±1.00	35 - 37

The relatively lower SaO_2 value of Fogera cattle than the low altitude cattle and crossbred that live on altitude range similar to Fogera is difficult to interpret. We observed a range of variation in oxygen saturation (63% - 87%) among this limited number of healthy individuals as was the case for PAP values. This might indicate differences in the physiological hypoxemia despite uniform ambient hypoxic stress and management system employed to all experimental animals.

3.3 Histological Measurements

Quantitative histological measurements of the three groups of animals sorted into three categories are presented in Table 3. Average % MT of each breed group varies between 14.63 - 16.42 for Simein, and 17.04 - 20.48 for Lowland, and 18.96 - 20.06 for crossbred animals for all categories (Table 3). Significant difference ($P < 0.05$) in the medial thickness of Simien with the Lowland and cross bred animals is noted.. All figures from % MT confirmed that the animals considered have thick muscular pulmonary arteries. There is also a marked increment of the % MT of muscular pulmonary arteries with progressive diminution in arterial diameter in all the animals studied. The higher figures of medial

thickness were calculated from measurements on the smaller arteries. Significant difference ($P < 0.05$) was observed among Simien and the transported crossbred and Lowland cattle. In all the three categories Simien have the smallest medial thickness (significantly different, $P < 0.05$ at category II and III) and have wider lumen area, significant at category II and III ($P < 0.05$) as well.

Table 3 Measurements of histological parameters at 1000, 500 and 300 µm external diameter (Mean± S.E) of investigated animals taken at 3500 m after two months of testing period

breed	n	Simien	Lowland	Crossbred
Category I: 1000-100 µm		41	38	26
External diameter	105	435.84±38.03^{b}	401.19±39.50^{b}	216.15±47.76^{a}
Lumen diameter	105	288.66±32.16^{a}	265.88±33.40^{ba}	141.65±40.38^{b}
Medial thickness %	105	14.63±0.81^{b}	17.04±0.85^{ba}	18.96±1.02^{a}
Category II: 500-100 µm		32	26	25
External diameter	83	365.47±24.87^{a}	230.76±27.59^{b}	170.73±28.14^{b}
Lumen diameter	83	255.20±26.02^{a}	133.73±28.87^{b}	106.86±29.44^{b}
Medial thickness %	83	15.33±0.93^{a}	18.81±1.03^{b}	19.35±1.05^{b}
Category III: 300-100 µm		13	20	23
External diameter	56	226.39±14.04^{a}	180.87±11.51^{b}	151.19±10.73^{b}
Lumen diameter	56	102.06±14.15^{a}	95.10±11.32^{b}	90.25±10.55^{b}
Medial thickness %	56	16.42±1.71^{a}	20.48±1.26^{b}	20.06±1.18^{b}

The highest medial thickness of the Lowland cattle is very much correlated with the PAP value recorded for this animal. We noted a remarkable difference in lumen and total vessel external diameter for most of the measured muscular pulmonary arteries. Breed

difference was apparent at category II where both lumen diameter and total vessel external diameter of the Simien was significantly ($P < 0.01$; $P < 0.001$) different from crossbred and Lowland respectively. The observed significance difference ($P < 0.05$) in lumen diameter was also evident at category III. The present study shows differences in pulmonary artery remodelling in different segments of the pulmonary artery trees. This suggests that mechanisms other than pressure contribute to pulmonary artery remodelling.

Figure 1 presents the graphical display of the distribution of percent medial thickness of the sample muscular pulmonary arteries.

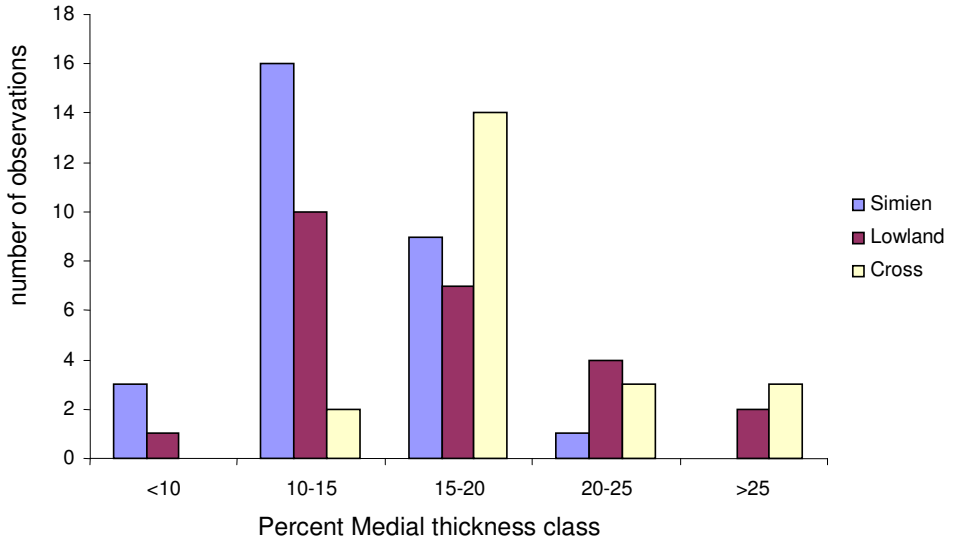

Figure 1 Frequency distribution of % MT of the sample muscular pulmonary arteries computed from category I showing wall thickness of Simien, with relatively lower thickness, have distribution skewed to the left while Lowland and Crossbred, respectively, dominated the right side of the distribution.

The range of % MT of each muscular pulmonary artery of the three groups of animals as computed from category I vessel diameter was variable. Simien cattle relatively have the

least variable range with a value ranging from 7.28 – 20.26%, crossbred 12.38 – 28.97% and Lowland 9.61 – 26.65% with an average % MT of 14.63%, 18.96% and 17.04% respectively.

4. Discussion

4.1 Pulmonary Hypertension

Several investigators (Best and Heath, 1961; Tucker et al., 1975; Kay, 1983) have reported the amount of smooth muscle in muscular pulmonary arteries of animals from several species and noted a considerable inter-species and intra-species variability. These observations and other investigations led to the hypothesis that the amount of inherent muscularization of small pulmonary arteries appears to be a determinant of this hypertensive response, as does the presence or absence of collateral ventilation (Tucker et al., 1975; Tucker and Rhodes, 2001).

This paper investigates the pulmonary circulation of low and mid altitude indigenous and crossbred animals transported to high altitude hypoxic environment, monitors the extent of structural changes of muscular pulmonary arteries of these populations in relation to their exposure to high altitude and examined the pulmonary vasculature of adapted Simien plateau cattle. PAP and percent SaO_2 of each experimental animal was collected. The hypothesis that a thin walled pulmonary vasculature is a feature of genetic adaptation was further tested by examining representative from a family with a naturally muscular pulmonary arterial tree living at high and low altitudes. One of the objectives of this experiment was to ascertain if normal cattle taken to high altitude develop pulmonary hypertension, and if so, to determine the extent of structural remodelling of the muscular pulmonary arteries. Therefore, histological investigation of the muscular pulmonary arteries was carried out. No specific interest was placed to elaborate some parameters like SaO_2 as this had already been discussed in detail in Wuletaw et al. (in press).

Overall there was high variability in PAP scores that ranged from very low to high risk (22 – 67 mmHg) category. This lends support to the hypothesis that genetic factors play a role in high altitude disease. Importance of genetic factors in high altitude disease is reported by many authors (see e.g Weir et al., 1974; Fagan and Weil, 2001; Holt and Callon, 2007). The variation in Simen cattle (22 – 33) in the PAP records, though within the normal range, confirms that vasoconstriction is largely reduced. The mean PAP values of Simien were within the values reported for sea level animals. It was lower or comparable to cattle measured at low altitude, 1590 m (Anand et al., 1988) who reported a value of 28.2±4.4 mm Hg, mean PAP of the Himalayan cattle 27mm Hg, (Anand et al., 1986) and Hereford steers at an altitude of 1520 m (Reeves et al., 1962). PAP values recorded below 28 mmHg in this study are considered far below the range of PAP readings of approximately 175,000 cattle measured in the Rocky Mountains (Holt, personal communication). On the other hand, the crossbreds manifest only a very slight rise, below 5% increment, in PAP above that shown by their counterparts measured at 1730 m Wuletaw et al (in press). The PAP score of the cross animals, 37 mm Hg, is still considered as fairly low suggesting only the importance of retesting before the use of the animal for breeding purpose. However, the variation in the case of Lowland cattle, with a mean value of 46.66, is high (36 - 67) suggesting the susceptibility of these cattle that have moved to high altitude. Despite such a huge discrepancy both in mean values and standardized variation, no statistical differences were detected among the different groups, probalby due to the small sample size caused by extreme experimental conditions.

The study further illustrates that Lowland cattle has an increase of over 43% in pulmonary artery pressure compared to cattle measured at 1730 m in our previous study (Wuletaw et al. in press). Reports on PAP readings of low altitude cattle but transported to high altitude are variable suggesting genetic difference but over all confirmed that cattle

transported to high altitude have been affected. PAP from yearling steers taken to 3885 m from 1600 has increased by 180% after six weeks (Grover and Reeves, 1962). Tucker et al. (1975) reported a much higher PAP (300%) for a calf after 19 days of exposure to 4500 m. Newborn calves (born at 1524 m) exposed at 4300 m for up to 14 days (Stenmark et al., 1987) exhibited marked pulmonary hypertension, 80 mmHg. Tucker et al. (1975) reported baseline PAP values of 19 mmHg in dogs, 20 mmHg in sheep, 25 mmHg in calf and 27 mmHg in pigs. These values increased respectively to 22, 23, 75 and 72 mmHg after sustained exposure to a simulated 4,500 m altitude. Such a rapid development of severe pulmonary hypertension when those animals are moved to high altitude is believed to be associated with the unusually muscular pulmonary arteries normally present in cattle residing at low altitude (Tucker et al., 1975; Tucker and Rhodes, 2001; Rhodes, 2005; Holt and Callon, 2007).

Effect of Crossbreeding on High Altitude Hypoxia

Another important aspect of the result from our study is that crosses of locals with exotics, with 75% exotic blood inheritance, were not more susceptible to brisket disease and also did not show significant differences in their % SaO_2 values. Only few reports are available regarding the effect of crossbreeding on high altitude hypoxia. In a study comparing PAP readings in yak, cattle and their crosses (Anand et al., 1986) reported that the crosses had equally low PAP readings as the yaks. Anand et al. (1988) observed that PAP values of the crossbred goats at high altitude was intermediate between that of the indigenous high and low altitude goats tested at high altitude, but with no significant differences between the various groups. On the same study no significant differences in PAP records were obtained between the three groups of sheep studied, indigenous, 50% cross and 75% cross at high altitude (Anand et al., 1988). Wuletaw et al. (in press) had also reported similar findings on cattle claiming that the crosses at high altitude, 2700 m, were

not more prone to the effect of high altitude than the indigenous ones. Similarly in our present study we observed a stable normal PAP records for the entire cross animals considered. These limited but informative studies suggest that crossbreeding may have positive impact on the effect of high altitude hypoxia at least when measured in terms of PAP.

4.2 Histological Measurements of Distal Pulmonary Arteries

As a family characteristic, cattle have exceptionally thick-walled muscular pulmonary arteries and arterioles (Alexander, 1962; Wagenvoort and Wagenvoort, 1969) and are unusually susceptible to high altitude pulmonary hypertension causing "brisket disease" (Will et al., 1962; Hecht et al., 1959, 1962). The result of our experiment is in conformity with this. All the experimental animals have thick medial thickness. One of the objectives of the present study was to see whether differences in PAP records were reflected in anatomical changes. Tucker et al. (1975) showed a much higher PAP (300%) after 19 days of exposure to 4500 m and an increase in % MT from 7.3 to 12.7%. On the other hand Best and Heath (1961) reported range of values (5 - 22%) % MT for cattle measured at sea level and Wagenvoort and Wagenvoort (1969) reported 15.1% of % MT. In a study by Heath et al. (1969) the pulmonary arteries of cats and dogs living at high altitude were thicker compared to representatives of the same species living at sea levels (Heath et al., 1969) which are known to be moderate and hypo responders to high altitude respectively. In the same study they found that the pulmonary arteries of a llama were much thinner and suggested that this might be of evolutionary significance in respect of survival at high altitude. Banchero et al. (1971) illustrated a thick walled muscular pulmonary artery and a muscularized pulmonary arteriole in a llama taken to high altitude in the Colorado Rockies. Llama is thought to have thinner muscular pulmonary arteries as a result of long time

survival at high altitude (Heath et al., 1969; Harries et al., 1982; Anand et al., 1986; Anand et al., 1988).

In the current study it was the Lowland cattle where the thickest medial coat was positively correlated with PAP recordings of the animal. It would seem, therefore, that the pulmonary vasoconstrictor response to alveolar hypoxia is higher in the lowland cattle. The level of % MT noted in the muscular pulmonary arteries of Simien and crossbred did not match with their respective PAP values which are still within normal range. Such thickenings were striking, and if seen in human would undoubtedly indicate hypertensive vascular disease. Thick medial thicknesses without any sign of high altitude disease were also reported by some investigators. Jones (1969) reported 15 % MT on Monkey and Hanson et al. (2000) documented 16.6% MT for Coati at low altitude.

Robert et al. (1998) reported increment of the media cross-sectional area from chronic hypoxia without a change of the lumen area. Pak et al. (2007) also noted instances where remodeling of pulmonary resistance vessels occurs in the absence of significant pulmonary hypertension. We have noted a remarkable difference in the lumen diameter of these experimental animals. The lumen diameter of Simien (adapted to high altitude) was significantly wider than that of the crossbred and the Lowland cattle at all categories of vessel diameter studied. But no apparent difference was observed between the indigenous, Lowland (affected by pulmonary hypertension) and Crossbred animals (normal). The case of crossbred animal, however, is somehow different. Despite normal PAP value the lumen diameter was narrow. Whether this narrow lumen diameter is a characteristic feature of taurus cattle, thus inherited, or emerged from vasoconstriction needs further (taurus versus indicus) histological studies. No literature is available on comparative histological study of taurus and indicus cattle. Therefore, the apparent differences between changes in pulmonary vascular structure and hemodynamic function of Simien animals may be

explained by the fact that vasoconstriction, rather than structural remodelling of pulmonary arteries, plays a pivotal role in the rise of PAP. The Simien might have not vasoconstriction because of its wider lumen size.

5. Conclusions

This comparative histological and haemodynamic study investigated different cattle genotypes, native and transported, at high altitude 3500 m. Our experiment revealed that all the experimental animals have thick medial thickness. However, it was in the Lowland cattle that the thickest medial coat corresponded with PAP recordings of the animal. It would seem, therefore, that the pulmonary vasoconstrictor response to alveolar hypoxia is higher in the lowland cattle. On the other hand Simen and crossbred cattle did have thick muscular pulmonary artery which did not reflect in their PAP recordings. Our results suggest that Simien cattle have a special mode of adaptation to high altitude hypoxia due to wider lumen.

Therefore, given to the presence of thick medial thickness with out significant PAP value and the effect of wider lumen area on PAP of an animal, the hypothesis that a "thin walled pulmonary vasculature is a feature of genetic adaptation" might need some reconsideration so as to accommodate our findings. However, to further verify our result repetition of the investigation with large sample size including more parameters is suggested as well as a comparative histological study on *Bos taurus* and *Bos indicus.*

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PART 2

CHAPTER 4

Genetic diversity and differentiation study of indigenous cattle populations of North Western Ethiopian based on microsatellite markers

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Abstract

The genetic variability and extent of population differentiation/substructures in five indigenous cattle breeds of North Western Ethiopia (Monastery, Dembia, Fogera, Wegera and Lowland), were studied by investigating 22 microsatellite markers. The monastery cattle, currently estimated around 3000 heads, is established some 350 years ago from religious donations of breeding cattle from the area as well as far off places, thus experienced a unique genetic evolution. Our investigation showed that all the breeds showed considerable genetic variability in terms of number of alleles and heterozygosity. Some 268 alleles were scored of which 24% are private alleles. The mean expected heterozygosity across loci within populations ranged from 0.62 to 0.77. We detected low but statistically significant ($P < 0.01$) levels of inbreeding (mean $F_{IS} = 0.175$) for all loci and populations with out evidence for recent bottleneck ($P > 0.05$). The analyses confirmed little but significant ($P < 0.05$) genetic differentiation ($F_{ST} = 0.029$). The very high rate of gene flow among the breeds ($Nm = 6.28$) is thought to have exerted significant influence on level of population differentiation. Further analyses of molecular data revealed only two major clusters, which did not confirm earlier phenotype based classifications, rather evidenced the admixed nature of the populations. The Monastery cattle was well differentiated with 91% bootstrap value suggesting its evolutionary adaptation and genetic uniqueness. These breeds are an important economic resource for the farming community of the area and their high variability makes them suitable candidates for conservation and improvement. Controlling gene flow between breeds, through adopting effective breeding and management practices to maintain variability and overcome within-breed substructures is suggested as a means to facilitate the conservation and utilization of each breed.

Key words: microsatellite, genetic diversity, indigenous cattle, Ethiopia

1. Introduction

Given its diversified ecology and very large number of animals (CSA, 2010), Ethiopia is considered a centre of diversity for animal genetic resources. It is also considered as the home of most important cattle breeds for eastern and southern Africa (Payne, 1997; Beyene and Bruke, 1992; Rege, 1999; Hanotte et al., 2002; Ayalew et al., 2003). The indigenous breeds, as explained by Epstein (1957; 1971), originated from the migration of Hamitic Longhorn and Shorthorn from Egypt along the Nile Valley and the humped Zebu from India through the horn of Africa. Interbreeding between the Zebu and Hamitic Longhorn resulted in a third group called Sanga, which spread to the southern part of the continent. The present day Ethiopian cattle are classified in to four main groups and a variety of breeds or types (Alberro and Haile- Mariam 1982; Beyene and Bruk, 1992; Ayalew et al., 2003). These are the Humpless (taurine), Zebu, Sanga and Zebu-Sanga (intermediate). Currently 32 Ethiopian indigenous cattle breed types are registered (DAGRIS, 2009). There is a general concern that the genetic variation within Ethiopia cattle is declining through indiscriminate crossbreeding, absence of breed development programmes, accelerated admixtures and interbreeding among breeds (see Ayalew et al., 2003; FAO, 2007; Dadi et al., 2008). This situation has been worsened by neglect, feed shortage and livestock disease epidemics resulting in an estimated annual mortality of 4.5 million cattle (CSA, 2010). Any reduction in the diversity of genetic resources narrows the scope to respond to changes in the environment, disease challenges or demand patterns.

The current state of knowledge on characterization of farm animal genetic resources in Ethiopia shows that there is inadequate breed level characterization information (Ayalew et al., 2003; Wuletaw, 2004; Rowlands et al., 2006). However, some reports described surveys of the genomic polymorphism of some Ethiopian cattle breeds using protein polymorphism (Sisay, 1996), RAPD (Hasson et al., 2007) and Y chromosome (Zarabruk et

al., 2007), microsatellite markers (Dadi et al., 2008) that do not allow comparative analyses across all these studies. Moreover, none of these addressed cattle populations of the present study area where their current classification is based on available historical and phenotypic data (Wuletaw, 2004). Such information is subjective and inaccurate, making implementation of rational and effective conservation and utilisation strategies difficult (Rege, 1999).

The five indigenous cattle breeds addressed in this study are with different morphological and production characteristics. The populations belong to the two major cattle breed groups of the country, Zebu, Zenga and adapted to a wide range of environment ranging from 550 m to 2700 m. Particularly one of the sample populations, Mahibere-Silassie Composite (Monastery), is believed to have experienced a unique genetic evolution mainly due to its isolation for more than 350 years. The breed was established at the initiation of the Monastery in the middle of the 17th century (about 1630), from religious donations of breeding cattle from the area as well as far off places. Currently the population is estimated about 3000 heads. The breed is also known to have its own management, ranching production system, natural selection, and adaptation. These cattle is reputed to be heat-resistant, relatively rich in milk and growth performance under the existing ranching production system and have inherent longevity from living in the region of Metema district of North western Ethiopia in the territory of Orthodox church monastery known as Mahibere-Silassie Andnet Gedam. Earlier work from phenotypic measurements revealed that the cattle is different from the rest of the cattle breeds of the region which are supposed to be its constituents (Wuletaw, 2004; 2007). However, outcomes of multivariate morphological surveys need to be verified by complementary genetic characterization (FAO, 2007).

The evaluation of the population genetic structure of Mahibere-Silassie and the neighbouring, constituent cattle breeds is, therefore, of major interest to obtain the necessary elements for supporting breed conservation and the improvement of breeding programmes. Currently, microsatellites are the most popular markers in livestock genetic characterization studies (Sunnucks, 2000). Their high mutation rate, abundant distribution, polymorphic nature, suitability for amplification by polymerase chain reaction (PCR) (Bruford and Wayne, 1993) and codominant nature permit the estimation of within and between breed genetic diversity, and genetic admixture among breeds even if they are closely related. They are also highly sensitive to genetic bottlenecks (Hanotte and Jianlin, 2005) and effective in evaluating differences within a breed of cattle and determining population substructure (MacHugh et al., 1994, 1998)**.** The objectives of this study were to assess the levels of genetic diversity, estimate level of genetic differentiation, test for recent bottleneck effects and provide/generate genetic information for future conservation decisions of the studied indigenous cattle populations.

2. Materials and Methods

2.1 Cattle Populations

The study included five indigenous cattle breeds of North Western Ethiopia that belong to three major cattle breed groups of the country: Wegera which is the highland Zebu , Fogera and Dembia categorized as Zenga, Zebu x Sanga, Lowland cattle , grouped under Zebu with Sanga blood and the Mahibere-Slassie composite (Monastery cattle) constituted from various cattle populations around the area. These cattle breeds were reported to have adaptive advantages in their respective production environment. For example, The Monastery and lowland cattle are known for their resistance to tick infestation; Dembia and Fogera, to internal parasites and water lodging effects and Wegera cattle is adapted to gazing on mountainous area. Furthermore they all are suitable for draught power and have

the ability to cope with seasonal feed and water shortages (Wuletaw, 2004). Blood samples using FTA cards were collected from 203 animals which are supposed to represent these five local cattle populations. In the case of Fogera blood samples were collected from the two Government ranches where pedigree records were checked not to sample related animals. Similarly for the Monastery cattle monks were consulted and samples were collected from three herds. For the other populations blood samples were collected from animals found in different localities. Details of sample populations are given in Table 1 and sampling location is presented in Figure 1.

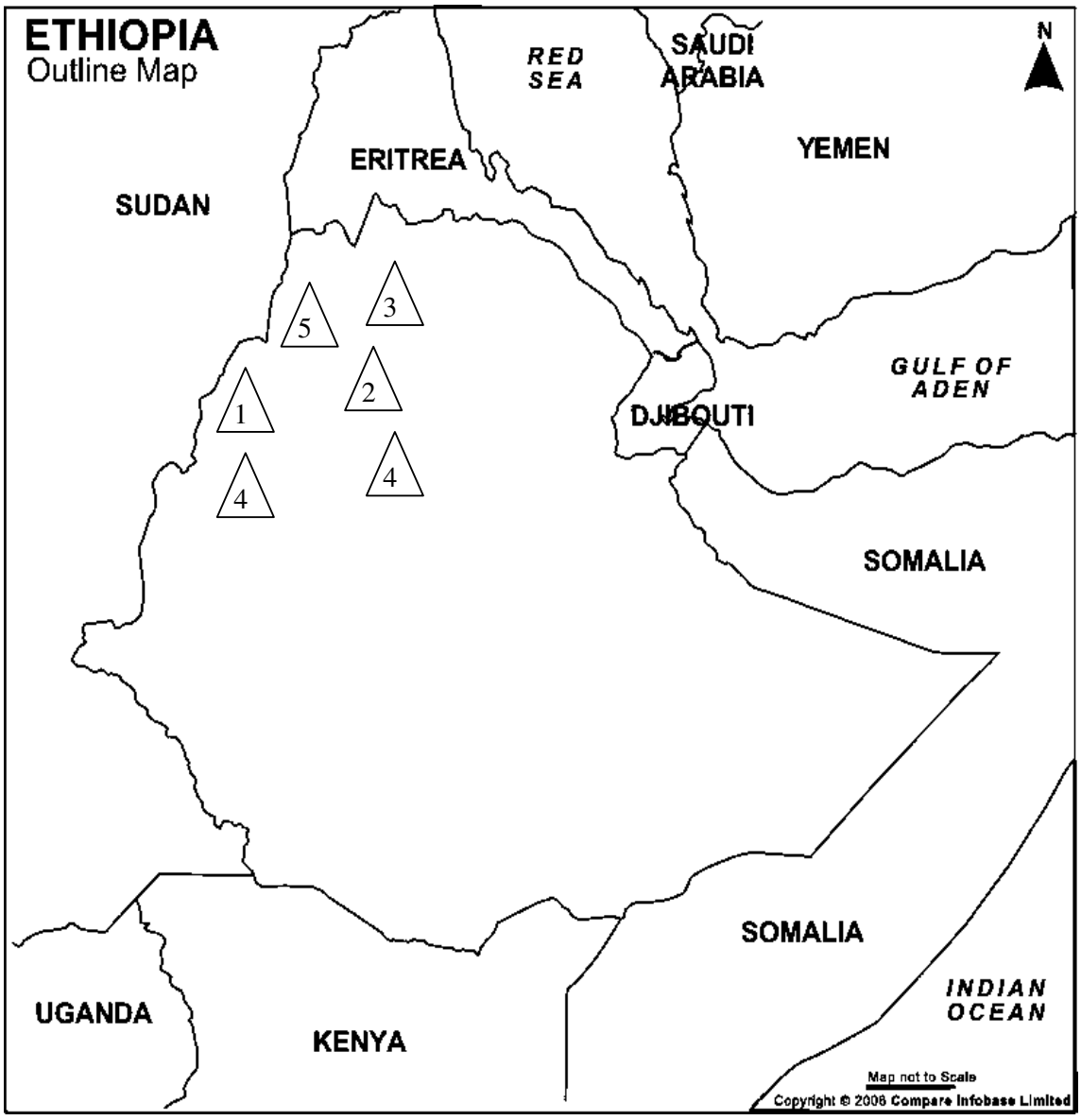
Table 1 Description of sampled animals

Populations	Class/category	Altitude, m	Sample size
Wegera	Highland Zebu	2700	40
Dembia	Zenga	1800 -2200	40
Fogera	Zenga	1700	44
Lowland	Zebu dominated Sanga	550 -1000	38
Monastery	Composite	550	41

2.1The Study Area

The study was conducted in North and South Gondar zones located in North Western Ethiopia (Figure 1). The altitude extended from 2700 m in the North-East to 550 m in the Western parts of the study area and rainfall varies from 880 mm to 1,772 mm. Temperature ranges from a minimum of -2.5°C to 4°C to a maximum of 11°C to 18°C in the North and 22°C to 43 °C in the western lowlands (MWER, 2008).

Figure 1 Map of Ethiopia showing the location of the study sites



△ **Study sites: 1 = Monastery; 2 = Dembia; 3 = Wegera; 4 = Fogera; 5 = Lowland**

2.2 Microsatellites

All the animals were genotyped for 22 microsatellite markerse chosen for their reproducibility, position on the chromosome, polymorphism and absence of null alleles. All markers belong to the panel recommended by the FAO/ISAG Advisory Committee for genetic distance studies (FAO/ISAG, 2004). The list of 22 microsatellite loci included in the study is: BM1824, BM2113, BM1818, INRA023, INRA032, ILSTS005, ILSTS006, HEL1, HEL5, HEL9, HEL13, ETH3, ETH10, ETH185, ETH225, CSSM066, TAGLA53, TAGLA126, TAGLA122, TAGLA227, HAUT24, and HAUT27.

2.3 DNA Extraction and PCR Amplification

Peripheral blood samples were dripped and dried on FTA cards (Whatman Inc., Middlesex, UK). Two or three circles of 2 mm diameter were punched from the FTA® cards (2.0-mm Harris Micro Punch; Whatman) and washed three times in 200 lμ of FTA Purification Reagent FTA (Whatman) and once with distilled and deionized water to isolate the DNA. The washed FTA pieces were used directly for polymerase chain reactions (PCR). Polymerase chain reactions (PCR) were carried out in 20 µl reaction volumes containing 10 ng of genomic DNA, 8.05 µl of PCR grade water, 2 µl of dNTP (2 mM), 1.60 µl of 25 mM $MgCl_2$, and 3.20 µl 10X PC Buffer B (magnesium free, 0.8 M Tris-HCl, 0.2 M $(NH4)_2SO4$, 0.2% w/v Tween-20) and, 0.5µl of each primer, 0.15 U firepol red lid/taq (*Taq)* DNA polymerase. PCR profiles were: initial denaturation for 5 min at 95 ^oC, followed by 35 cycles each at 95 ^oC of 1 min, at 55 ^oC for 1.5 min, at 65 ^oC for 3 min (annealing), at 65 ^oC for 5 min (elongation) and a final extension step at 4 ^oC for 10 minutes.

The PCR product was then diluted 1/10 in distilled water. From the eluate 2 µl were mixed with 3 µl diluted ET400-ROX MegaBACE size standard (0.25 µl of ET400-ROX in 2.75 µl). With a total volume of 5 µl per sample, genotyping was performed on

MegaBACE^{TM} 500, fluorescence-based DNA system utilizing capillary electrophoresis. The alleles were called and scored under MegaBACE^{TM} Genetic Profiler Software Suite v2.2 system.

2.4 Statistical Analysis

Each population was tested for Hardy-Weinberg equilibrium and linkage disequilibrium using GENEPOP Version.4.0.10 available on http://genepop.curtin.edu.au/index.html. Exact tests (Guo and Thompson, 1992) were applied using a Markov Chain Monte Carlo simulation (100 batches, 5,000 iterations per batch, and a dememorization number of 10,000) as implemented in GENEPOP version 4.0.10 (Raymond and Rousset, 1995). To assess within-population genetic diversity, the mean number of alleles (MNA), the observed (HO) and the expected heterozygosity (He, Nei's unbiased gene diversity) were calculated using the Microsatellite Toolkit (Park, 2001), available at http://animalgenomics.ucd.ie/sdepark/ms-toolkit/. Number of alleles per locus, allelic frequencies, and observed and expected heterozygosity were as well calculated. Number of private alleles was derived by the software package, CONVERT (Glaubitz, 2004).

The FSTAT 2.9.3 software (Goudet, 2001) was employed in calculations of allelic richness (an estimation of mean number of alleles per locus corrected by sample size), gene diversity (Nei, 1987), and estimation of Wright's fixation index ($\mathbf{F_{IS}}$, $\mathbf{F_{IT}}$, $\mathbf{F_{ST}}$) using the variance-based method of Weir and Cockerham (1984). A significance test on the estimates of Wright's F-statistics (F_{IT}, F_{IS} and F_{ST}) for each microsatellite locus were obtained by constructing 95% and 99% confidence intervals based on the standard deviations estimated by jackknifing across populations using FSTAT. The effects of migration and gene flow on the genetic structure of the analyzed populations were

estimated using the private allele method (Slatkin, 1993) as applied in genepop program (Raymond and Rousset, 1995).

Two methods were used to detect the genetic relationships and population structure among the five cattle breeds. Reynolds least square genetic distance matrix (Reynold et al., 1983) was calculated using Population software (Langella, 1999), available from http://bioinformatics.org/~tryphon/populations/. The Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm (Sneath and Sokal, 1973) was used to construct a phylogenetic tree with the Populations software. Tree robustness was evaluated by bootstrapping over loci (1000 replicates) and generated tree was viewed using the software TREEVIEW v. 1.6.6 software (Page, 1996). To better understand the level and effect of admixture between populations, population structure and degree of admixture, analyses were performed using a Bayesian clustering-model as implemented in STRUCTURE (Pritchard et al., 2000). The program estimates, using multi-loci genotypes to assign individuals to populations, individual admixture proportions and infers the number of parental populations (K) for a given sample. The parameter of Ln Pr(X|K) is an indicator of the posterior probability of the K clusters, as suggested by Pritchard et al. (2000). To obtain a representative value of K, we preformed 10 independent runs for each K ($2 \leq K \leq 5$) and the burn-in time and replication number were both set to 10,000.

Bottleneck events were tested by three methods. The first method consisted of two excess heterozygosity tests developed by Cornuet and Luikart (1996), standardized difference test and Wilcoxon sign-rank test. The second method was the graphical representation of mode-shift indicator originally proposed by Luikart et al (1998). Loss of rare alleles in bottlenecked populations is detected when one allele class have a higher number of alleles than the rare allele class (Luikart et al., 1998). This test was rescaled so that frequency distribution of the allele frequency class would be based on equal 0.05

increments. These three methods were tested using Bottleneck v. 1.2.03 (Piry et al., 1999) software. The probability distribution was established using 1000 simulations under two models, Step wise Mutation Model (SMM) and Two Phase Model of mutation (TPM).

3. Results

3.1 Tests of Hardy-Weinberg Equilibrium and Microsatellite Polymorphism

Using estimation of exact P-Values by the Markov chain method the number of microsatellite loci showing deviations from Hardy-Weinberg equilibrium (HWE) in each of the populations is shown in Table 2. At a 95% confidence level, the number of loci deviating among the individual breeds ranged from 5 (out of 22) in the Dembia population to 10 in the Fogera. In the pooled sample, 6 loci deviated. This is much more than expected by chance (< 2 loci at 5% error probability). In the majority of the cases deviations were due to heterozygote deficiency.

All microsatellite markers showed high diversity content in all breeds. A total of 256 alleles were detected over all loci in the 176 animals assayed, 64 alleles were private and the rest were shared alleles. All 22 microsatellite loci showed sufficient polymorphism for evaluating genetic diversity. Polymorphic information content (PIC) ranged from 0.253 to 0.848 and observed and expected heterozygosity from 0.281 to 0.818 and 0.531 to 0.867 respectively. Except at locus BM1824, in all the loci observed heterozygosity values were nominally smaller than the expected ones (Table 2). Each of the microsatellites has private alleles (1 to 8). Allelic richness varied from 4.11 in BM1824 to 9.47 in HEL5.

Table 2 Genetic variability of 22 microsatellite markers, consolidated data across breeds: number of alleles at each locus (k)**,** number of individuals typed for each locus (N)**,** mean heterozygosity observed (Ho)**,** mean heterozygosity expected (He), Private alleles (PA), and Allelic richness (AR)

Locus	N	K	Ho	He	PA	AR
eth225	195	10	0.457	0.614	3	5.99
cssm06	178	16	0.281	0.531	8	5.76
tglaA122	182	14	0.735	0.852	3	9.32
inra23	185	12	0.766	0.791	5	6.75
inra32	192	13	0.65	0.79	4	8.16
bm2113	174	16	0.818	0.847	5	9.01
haut24	181	12	0.649	0.779	1	8.44
bm1824	184	5	0.741	0.706	2	4.11
haut27	184	9	0.593	0.727	2	5.91
hel1	186	12	0.488	0.777	2	8.07
hel13	187	8	0.48	0.71	1	6.33
eth3	186	9	0.445	0.56	2	5.2
hel5	172	13	0.388	0.775	1	9.47
ilsts006	169	11	0.694	0.798	1	8.02
eth10	160	11	0.63	0.719	2	7.22
tgla53	152	18	0.285	0.699	6	9.8
bm1818	162	14	0.7	0.84	5	8.48
tgla126	175	9	0.705	0.751	2	6.12
ilst005	168	6	0.755	0.779	1	5.11
tgla227	168	13	0.623	0.681	5	7.5
eth185	160	13	0.593	0.818	2	9.06
hel9	168	12	0.791	0.867	1	9.32
Overall	176	256	0.603	0.746	64	7.42

3.2 Genetic Diversity Within Breeds

Diversity of each breed showed considerable difference when measured in mean number of alleles per locus per breed which fluctuates between 6.55 and 8.36. Furthermore, average observed and expected heterozygozity varied from 0.580 and 0.652 and 0.682 and 0.754 respectively. In all breeds observed heterozygosity values were nominally smaller than the expected ones (Table 3). However, locus–breed analysis revealed that observed heterozygosity was higher (six loci in Monastery and Dembia, three loci in Wegera and Lowland and two loci in Fogera) than the expected heterozygosity and in some cases significant ($P < 0.05$). On breed level low to moderate level of inbreeding (F_{IS}) ranging from 0.122 to 0.215, is observed. A relatively high number of unique microsatellite alleles (private alleles) ranged from 5 in the Monastery to 19 in the Lowland cattle populations were observed.

Table 3 Breed level genetic diversity: observed heterozygosity (Ho), expected heterozygosity (He), mean number of alleles per locus per breed (MNA), private alleles (PR), within breed heterozygosity deficit (F_{IS})**,** and number of loci deviated from Hardy-Weinberg equilibrium (HWE)

populations	Ho	He	MNA	PR	F_{IS}	HWE
Monastery	0.580 ± 0.017	0.682 ± 0.033	6.55	5	0.151	7
Dembia	0.652 ± 0.018	0.741 ± 0.019	7.41	13	0.122	5
Wegera	0.612 ± 0.018	0.710 ± 0.029	7.55	11	0.140	6
Fogera	0.656 ± 0.017	0.754 ± 0.019	8.18	14	0.215	10
Lowland	0.614 ± 0.018	0.749 ± 0.026	8.36	19	0.132	8

The indicated diversity indices suggested the availability of sufficient genetic variability within each of the studied populations with the Lowland cattle being the most diverse and Monastery the least diverse breed.

3.3 Breed Differentiation

Population differentiation of studied populations, by pairwise F_{ST} coefficients, and corresponding P-value are presented in Table 4. The F_{ST} coefficients ranged from 0.0066 (between Dembia and Lowland) to 0.0621 (between Monastery and Fogera). Thus, from <1% to 6.21% of the microsatellite variability is explained by the subdivision of populations while the remaining variability is attributed to within population variation. As expected from the breeding history pair wise F_{ST} values between Monastery and the rest of the subpopulations were higher (Table 4). The global deficit of heterozygosity (F_{IT} = 0.199±0.035) and global differentiation (F_{ST} = 0.029±0.007) were both highly significant ($P < 0.01$) (Table A1 in Appendix).

Table 4 Population differentiation: F_{ST} (below diagonal) and adjusted P- value (above diagonal)

populations	Monastery	Dembia	Wegera	Fogera	Lowland
Monastery		0.005*	0.005*	0.005*	0.005*
Dembia	0.0327		0.035	0.035	0.040
Wegera	0.0572	0.0153		0.005*	0.280
Fogera	0.0621	0.0152	0.0156		0.005*
Lowland	0.0466	0.0154	0.0066	0.0184	

* $P < 0.05$; significantly different

3.3.1 Structure of the Populations

According to Pritchard et al (2000) the most likely K is that where ln Pr(G|K) is maximized. In our case it was at K = 2. One cluster was mainly constituted by the

Monastery cattle population and the rest of the studied populations were grouped as second cluster. Estimated membership probabilities of the populations is given in Table 5 with prior and with out prior information.

Table 5 Estimated membership probabilities to different genetic clusters and assignment of individuals to their true cluster (K = 2)

	With out prior information		With prior information	
Populations	Cluster 1	Cluster 2	Cluster 1	Cluster 2
Monastery	0.161	0.839	0.047	0.953
Dembia	0.542	0.458	0.742	0.258
Wegera	0.633	0.367	0.972	0.028
Fogera	0.687	0.313	0.957	0.043
Lowland	0.684	0.316	0.972	0.028

Graphical representation of the clusters is displayed in Figure 2. At K = 2 and K = 3, the Monastery cattle was relatively distinct and other populations found admixed. In general, both of the analyses performed in STRUCTURE supported the presence of a two clusters. However, this number is less than the number of significant populations inferred from FSTAT.

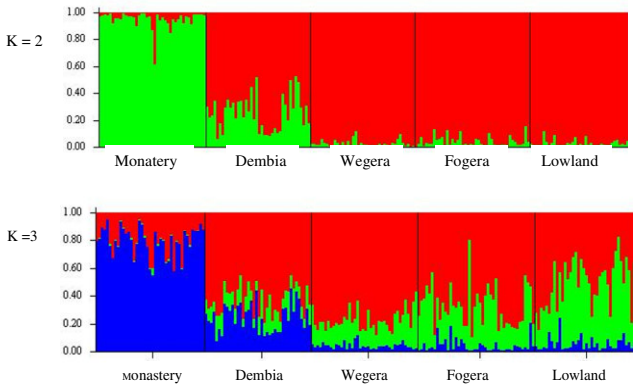


Figure 2 Clustering assignment of North Western Ethiopia cattle breeds obtained by STRUCTURE analysis at *K* = 2 and K = 3. Individuals are represented by a thin vertical line that is divided into segments whose size and colour correspond to the relative proportion of the animal genome corresponding to a particular cluster.Breeds are separated by thin black lines.

Allele frequencies from 22 microsatellites were used to generate the D_{rl} genetic distance for each pair of cattle breeds (Table 6). The D_{rl} genetic distances ranged from 0.0114 (between Wegera and Lowland breeds) to 0.0737 (between Monastery and Fogera breeds). These D_{rl} genetic distances were used to construct a phylogenetic tree using UPGMA (Sneath and Sokal, 1973) algorithm (Figure 3) revealing the clustering of the five populations into two major clusters.

Table 6 Pair wise estimate of genetic differentiation and genetic distance among all five studied cattle breeds. F_{ST} estimates above diagonal and Reynolds least square genetic distances (Reynold et al., 1983) (D_{rl}) below diagonal

populations	Monastery	Dembia	Wegera	Fogera	Lowland
Monastery		0.0327	0.0572	0.0621	0.0466
Dembia	0.0393		0.0153	0.0152	0.0154
Wegera	0.0704	0.0219		0.0156	0.0066
Fogera	0.0737	0.0229	0.0223		0.0184
Lowland	0.0546	0.0128	0.0114	0.0233	

As indicated in the Figure 3, bootstrapping values indicated the presence of two major clusters. The further differentiation of Fogera from the second cluster is only supported with very low bootstrapping value.

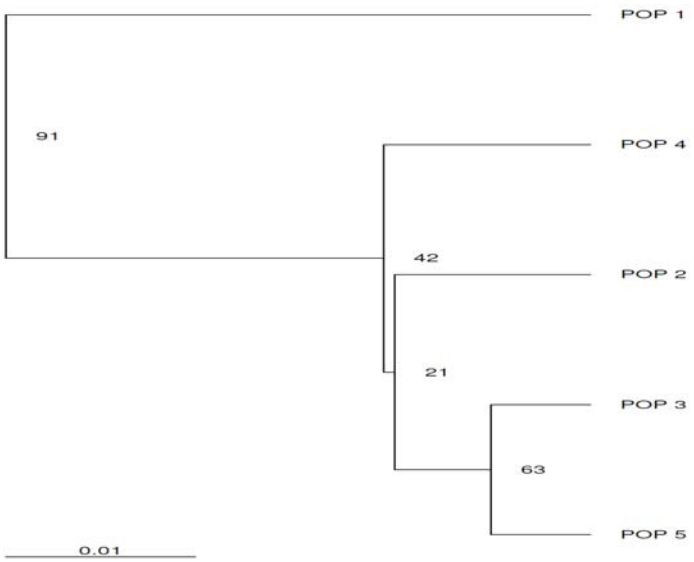


Figure 3 Genetic relationship among five North western Ethiopia cattle breeds (POP 1= Monastery; POP2= Dembia; POP 3 = Wegera; POP 4 = Fogera; POP 5 = Lowland) using UPGMA (Sneath and Sokal, 1973) algorithm based on Reynolds least square genetic distances (Reynold et al., 1983) estimated with 22 microsatellites. The numbers on the nodes indicate the percentage bootstrap values generated from 1000 replications

3.4 Bottleneck Detection

From measures of genetic bottleneck tests none of the calculated P- values from the Wilcoxon sign rank test (Table 7) was significant ($P > 0.05$), demonstrating that populations do not exhibit statistically significant heterozygosity excess (H_e) relative to that expected in an equivalent population at mutation-drift equilibrium (H_{eq}). Standardized difference test which measured population expansion in both models provided significant to highly significant ($P < 0.05$; $P < 0.0001$) gene diversity deficit. The second approach, a qualitative geographical method, also detected no mode shift in the frequency distribution of alleles and a normal L-shaped curve was observed, where the alleles with the lowest

frequencies (0.001–0.1) observed most abundantly (Appendix, Figure A1). There is, therefore, no statistical evidence of a recent bottleneck event in these populations.

Table 7 Results of the bottle neck detection tests of the five analysed populations

	Standardized differences test (T^2 values)				Wilcoxon sign rank test	
	SMM		*TPM		SMM	*TPM
Populations	P value	T^2 value	P value	T^2 value	**P value	P value
Monastery	<0.001	-4.519	0.026	-1.948	0.989	0.855
Dembia	<0.001	-5.888	0.001	-3.004	0.994	0.943
Wegera	<0.001	-7.890	<0.001	-4.624	0.992	0.931
Fogera	<0.001	-4.826	0.016	-2.130	0.996	0.869
Lowland	<0.001	-6.418	<0.001	-3.900	0.998	0.943

**P < 0.05 rejection of null hypothesis of mutation drift equilibrium

*90% SMM: 10% IAM; 12% variance

4. Discussion

This study presents a genetic analysis of microsatellite markers in five Ethiopian cattle breeds which as spatially close but have different morphological and production characteristics. Various diversity indices suggested the availability of sufficient genetic variability within each of the studied breeds. This was supported with assessment of demographic bottleneck with three different tests revealing no allelic loss in the recent past, 50 - 250 generations (Cornuet and Luikart, 1996). The discovery of 64 private alleles in the studied populations adds weight to the diversity harboured by these populations. Some authors (e.g. Petit et al., 1998) argue that private or rare alleles are of adaptive or evolutionary significance, perhaps representing possible loci of adaptive value, that is, a reservoir for adaptation to unusual conditions.

Historically Ethiopia, due to its geographical location, is considered as a home for most important cattle breeds of South and Eastern Africa (Epstein, 1957; 1971; Payne, 1997;

Rege, 1999; Hanotte et al., 2002). Thus, the present population genetic diversity might be attributed to this phenomenon. Furthermore long-term natural selection for adaptation and current interbreeding as a result of the transhumance system largely employed in the area are thought to contribute at large.

Several markers displayed a significant deficit of heterozygotes due to within-population inbreeding in all the breeds and in the combined analysis. Such result has been commonly observed in surveys of bovine breeds in Brazil (Egito et al., 2007), in Ethiopia (Dadi et al., 2008) in Eastern Africa (Ndumu et al., 2008) and in Mozambique (Bessa et al., 2009). The average number of alleles and levels of gene diversity were similar for all breeds (except Monastery which has relatively lower values), suggesting that there are no appreciable differences in the level of genetic variability among the studied cattle breeds. The amount of genetic diversity in these breeds was comparable to those reported for other cattle breeds (Rege et al., 2001; Ibeagha-Awemu and Erhardt, 2005; Egito et al., 2007; Ndumu et al., 2008).

The current study shows significant but low genetic structuring (FST = 0.029) in the five indigenous cattle breeds. Although rather low, it is within the range of values of (Rege et al., 2001; Ndumu et al., 2008; Sun et al., 2008; Bessa et al., 2009), but much lower than values reported by other authors (see e.g. Jordana et al. 2003; Ibeagha-Awemu and Erhardt, 2005; Sodhi et al., 2005; Egito et al., 2007; Sun et al., 2008). Migration rate between the five breeds was high (Nm = 6.28), indicating an extensive gene flow which exerted significant influence on lack of population differentiation. Trexler (1988) showed that if Nm>1, gene flow is enough to reduce the genetic differentiation between populations. However, a significant differentiation ($P < 0.01$) between the cattle breeds is still perceptible. Moreover, the model-based clustering analysis of the breeds with prior

population information (Pritchard et al., 2000) assigned individuals to their rightful populations, indicating that the breeds still conserve some of their genetic identity.

Disagreement on the number of populations differentiated by FST and clustering analysis is noted. This might be attributed to the lack of capacity of STRUCTURE software to infer the number of clusters similar to what FSTAT has done. Latch et al. (2007) suggested that FST must be at least 0.05 for STRUCTURE to reach an assignment accuracy of greater than 97%, even though it has the ability of inferring the correct number of subpopulations and assigning individuals appropriately when genetic differentiation among groups is as low as 0.02. This suggestion is in agreement with our result where the Monastery cattle which have FST value of 0.0327 - 0.0621 is well differentiated from the rest of the group.

Summarized evolutionary relationships among populations described by UPGMA with different branch length suggests different evolutionary time span that these populations experienced. Based on the branch length information it is the Monastery cattle which is separated long ago from the rest of the population. However, documented information regarding the origin of Monastery (Wuletaw, 2004; 2007) cattle does not support this interpretation. A major drawback of phylogenetic tree reconstruction is that the evolution of lineages is assumed to be non-reticulate, i.e. lineages can diverge, but can never result from crosses between lineages (FAO, 2007). This assumption will rarely hold for livestock, where new breeds often originate from cross-breeding between two or more ancestral breeds, like the monastery cattle. Thus, the evolution of these studied breeds provided by phylogenetic trees must be interpreted cautiously.

The further differentiation of the second cluster into Fogera and the rest was only supported with very low bootstrapping values (42%) suggesting the admixture genetic background of the cattle. Even the four decade long conservation effort of the government

could not bring significant change on the genetic identity of Fogera cattle. The presence of Wegera (highland Zebu group) in the same cluster with Fogera (Zenga group) and the low land cattle might be the result of historical cross-breeding and recent interbreeding from transhumance system. The STRUCTURE analysis (Pritchard et al., 2000) designed to characterize parental populations taking into account the level of admixture of individuals has also revealed (bar plot and estimated membership probability) the admixed nature of the populations.

Traditional populations are thought of as distinct types evolved as a result of geographical isolation and cultural separation of the communities keeping the animals (Rege et al., 2001). However, in our study analysis of microsatellite data does not fully lend support to the traditional classification of the populations, which is mainly based on physical characteristics. Lack of genetic distinctness among the different cattle breeds of Ethiopia categorized under Zebu, Sanga, and Zenga was also reported in earlier studies (Li et al. 2007; Dadi et al., 2008). Therefore, as analysis of molecular data of these populations did not conform with earlier phenotypic based classifications, revisiting the basis of classification might be important.

5. Conclusion

This work provides an extensive analysis of the genetic structure of North Western Ethiopia cattle breeds. Although the genetic diversity of these breeds has been affected by gene flow and, therefore, little differentiation exists among them, it appears that they retain some of their genetic identity with out recent bottleneck effects. The Monastery cattle was well differentiated, suggesting its genetic uniqueness. All these breeds are an important economic resource for the farming community of the area and their high variability makes them suitable candidates for conservation and improvement. Adopting effective breeding and management practices to control between breeds gene flow and within population

substructure is suggested to facilitate the conservation and utilization of these genetically diversified indigenous cattle breeds.

Acknowledgment

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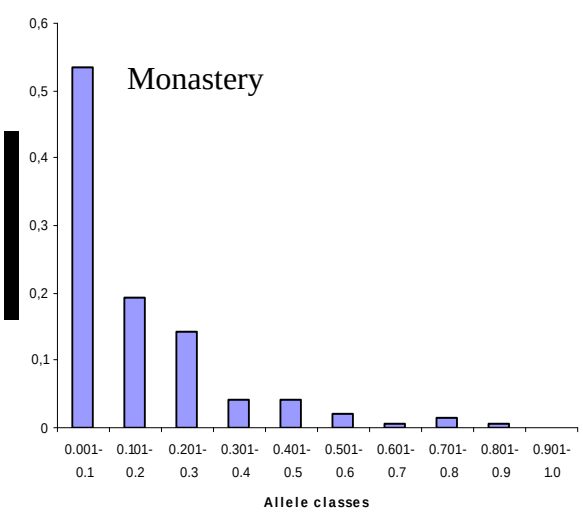
Appendix

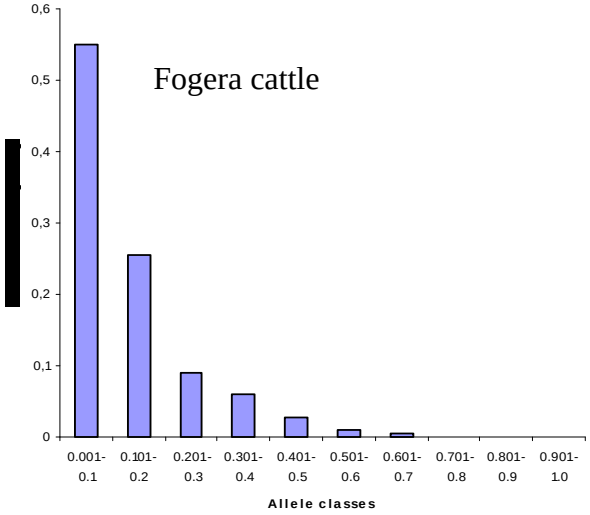
Table A1 Results of F-statistics (Weir & Cockerham 1984) and their standard errors for each locus across the five North Western Ethiopian cattle populations analysed

Locus	F_{IT}	F_{ST}	F_{IS}
eth225	0.288 (0.084)	0.063 (0.053)	0.239 (0.058)
cssm06	0.501 (0.169)	0.067 (0.040)	0.461 (0.162)
tglaA122	0.135 (0.050)	0.001 (0.003)	0.135 (0.050)
inra23	0.038 (0.019)	0.020 (0.027)	0.019 (0.028)
inra32	0.189 (0.044)	0.020 (0.005)	0.172 (0.044)
bm2113	0.040 (0.057)	0.008 (0.005)	0.032 (0.060)
haut24	0.168 (0.054)	0.009 (0.013)	0.160 (0.046)
bm1824	-0.038 (0.060)	0.040 (0.036)	-0.080 (0.058)
haut27	0.188 (0.092)	0.014 (0.011)	0.176 (0.090)
hel1	0.378 (0.068)	0.019 (0.014)	0.367 (0.074)
hel13	0.354 (0.087)	0.160 (0.053)	0.229 (0.067)
eth3	0.207 (0.075)	0.045 (0.055)	0.168 (0.041)
hel5	0.511 (0.060)	0.061 (0.027)	0.478 (0.052)
ilsts006	0.139 (0.077)	0.016 (0.007)	0.124 (0.079)
eth10	0.124 (0.044)	0.004 (0.008)	0.120 (0.041)
tgla53	0.601 (0.040)	0.044 (0.026)	0.583 (0.045)
bm1818	0.185 (0.045)	0.023 (0.027)	0.167 (0.055)
tgla126	0.074 (0.042)	0.019 (0.020)	0.057 (0.054)
ilst005	0.027 (0.022)	0.001 (0.007)	0.026 (0.020)
tgla227	0.077 (0.036)	0.010 (0.010)	0.068 (0.039)
eth185	0.297 (0.072)	0.049 (0.023)	0.262 (0.083)
hel9	0.090 (0.026)	0.003 (0.005)	0.087 (0.024)
Over all loci	0.199 (0.035)**	0.029 (0.007)**	0.175 (0.033)
95% CI*	0.136-0.270)	0.017-0.045	0.116-0.243
99% CI	0.119-0.292)	0.015-0.051	0.100- 0.266

* Confidence interval

** $P < 0.01$





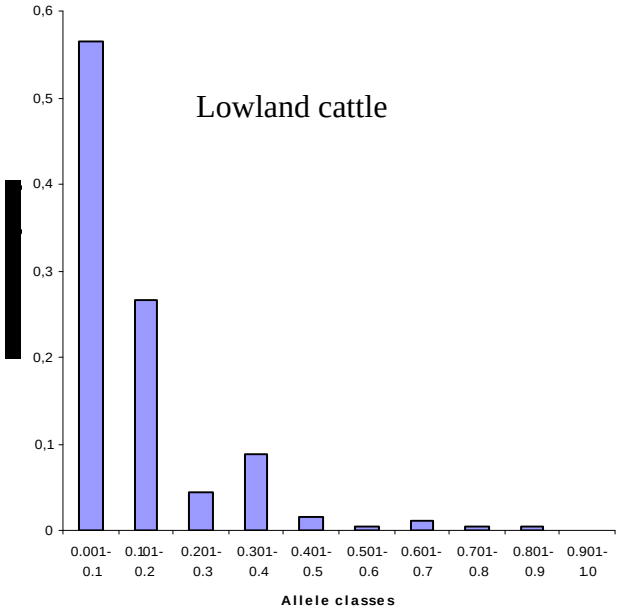


Figure A1 Graphical representation of the distribution of allele classes' frequency of Monastery, Fogera and Lowland cattle alleles with the lowest frequencies (0.001–0.1) observed most abundantly showing normal L-shaped graph

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General conclusions and recommendations

1. High altitude cattle of the North Western Ethiopia are not susceptible to high altitude disease. Reduced % SaO_2, absence of hematopoiesia (RBC and HgB) and thick medial wall thickness but wider lumen were some of the characteristic features of this cattle population. Presence of wider lumen coupled with other features might attribute to adaptation of these cattle to hypoxic environment. Thus, it is concluded that the indigenous cattle of the Simien Plateau of Ethiopia are adapted genetically to high altitude by largely eliminating the hypoxic pulmonary vasoconstrictor response. However, to further verify our result redoing of the investigation with larger sample size including more parameters is suggested, to be complemented with a comparative histological study on *Bos taurus* and *Bos indicus*.

2. Our molecular genetics study revealed two major clusters (Monastery and the rest of the populations) which is not in full agreement to their traditional classification. The differentiation of Monastery cattle with very high bootstrap value reflects the historical evolutionary adaptation of the breed, thus represents a unique component of the regional domestic animal biodiversity that deserve priority for conservation. All these breeds, however, are an important economic resource for the farming community as they are fitting well to quite different and specific production environments. In conservation decisions for farm animal species, emphasis should be given to variation in special traits and current merits of breeds. It is, therefore, concluded that adopting effective breeding and management practices while maintaining their adaptive attributes is the most rational and sustainable way to facilitate conservation and utilization of these adapted indigenous cattle resources.

Summary

Given its diversified ecology, geographyical location, and its large population size, Ethiopia can be considered a centre of diversity for cattle genetic resources. North Western Ethiopia is known for its cattle genetic resource but with little or no information on their adaptive attributes and level of within and between breed diversity. Cattle in the region are kept at altitudes of up to 4000 m and down to 550 m. The ability of concise classification, together with the continuous distribution of breeds across altitudes provides the unique opportunity to study adaptive characteristics of different types of animals to altitude. Here an effort is made to investigate cattle populations in terms of their adaptive characteristics in addition to genetic distance study as a contribution for designing appropriate breeding strategies to the rural farming communities.

Numerous investigations have shown that high altitude hypoxia leads to pulmonary hypertension, and stimulates hematopoiesia. High altitude pulmonary hypertension or brisket disease of cattle is common at high altitude areas and is a result of reduced blood oxygen saturation at high elevation that results in decreased transport of oxygen to the tissues. The extent of proneness, epidemiology, and genetics of the disease is not, however, known in Ethiopia where a large proportion of the area is at altitudes above 2700 m. Thus, Chapter 2 of this thesis described the characteristics of high altitude adaptation of indigenous cattle populations of Ethiopia and their crosses with European breeds. Adaptation was measured in terms of PAP testing, pulse oximetry and analysis of hematological parameters. We collected pulmonary artery pressure, % SaO2, and hemtological data sets on a large number of animals. Result revealed that no sign of pulmonary hypertension was observed among all the cattle genotypes. Crosses of the local cattle with Holstein Friesian and Jersey were not more prone to the disease than local

cattle. We report a new clinically relevant range of oxygen saturation, ≥ 68%, for the high altitude cattle which is far below the threshold value usually assumed for temperate cattle, > 80%. The crossbred (local x European) animals observed in this study are also adapted up to 2700 m. Given the moderate to high heritability of brisket disease the lack of observations of PAP beyond the normal range confirms that animals at 3,500 m are genetically adapted. Furthermore the evidence that high altitude cattle of the study area with little or no elevation of RBC and HgB compared to its counterparts studied elsewhere at and around sea level coupled with the low % SaO_2 suggests one form of adaptation to hypoxic environment.

The finding of this study has initiated a compararive haemodynamic and histological study presented in **Chapter 3**. Here we examined the pulmonary vasculature of the high altitude cattle and studied on pulmonary circulation of low and medium altitude indigenous and crossbred animals at high altitude environment (after transporting to 3500 m and observed for two months). Under this investigation the response of pulmonary circulation of these transported animals to the effect of hypoxic environment was monitored and structural changes of distal muscular pulmonary arteries of these populations assessed. Further more, distal muscular pulmonary vasculature of adapted Simien plateau cattle was examined and compared with its lowland counterparts. Our experiment revealed that all the experimental animals have thick medial thickness. Breed difference was apparent in lumen diameter where Simien was significantly different from the rest. In the Lowland cattle the thickest medial coat coincided with PAP recordings of the animal. It would seem, therefore, that the pulmonary vasoconstrictor response to alveolar hypoxia is higher in the lowland cattle. On the other hand Simen and crossbred cattle do have thick muscular pulmonary artery which is not reflected in their PAP recordings. Our result suggests that

Simien cattle have special mode of adaptation to high altitude hypoxia probably due to its anatomical feature of distal pulmonary arteries (wider lumen).

In **Chapter 4** molecular characterizations of the cattle genetic resources whose metabolic adaptation was assessed is treated. The current classification of indigenous cattle of the study area is based on historical and anthropological evidence, and phenotypic data, which are influenced by the environment. Molecular genetics via the use of microsatellite markers allows concise allocation of individuals to populations. We investigated five indigenous cattle breeds using molecular (22 microsatellite) markers. These five indigenous cattle breeds which belong to the two major cattle breed groups of the country, Zebu and Zenga are with different morphological and production characteristics and adapted to a wide range of environment ranging from 550 m to 2700 m. Particularly one of the sample populations, Monastery, is believed to have experienced a unique genetic evolution mainly due to its isolation for more than 350 years. Major objectives of the invcestigation were; to assess the levels of genetic diversity, estimate level of genetic differentiation, test for recent bottleneck effects and provide/generate genetic information for future conservation decisions We detected low but statistically significant levels of inbreeding (mean F_{IS} = 0.175) for all loci and populations with out evidence of recent bottleneck. The analyses confirmed little but significant genetic differentiation (F_{ST} = 0.029). The very high rate of gene flow among the breeds (Nm = 6.28) is thought to have exerted significant influence on level of population differentiation. However, they still maintained high level, 71%, of within population diversity. Further analyses of molecular data through the use of Phylogenetic and STRUCTURE statistical software revealed only two major clusters. The Monastery cattle was well differentiated, suggesting its evolutionary adaptation and genetic uniqueness. No attempt was made in this study to define conservation precedence based on our neutral genetic diversity study result alone.

All the local breeds are important economic resource for the farming community of the area and display peculiar traits that fulfil specific requirements with respect to local terrain and climate.