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RESEARCH ARTICLE
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Investigation of haemoglobin polymorphism in Ogaden cattle

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Abstract

Background and Aim: The Ogaden cattle is one among the tropical cattle breeds (*Bos indicus*) widely distributed in eastern and south eastern part of Ethiopia. The breed has been evolved in arid and semi arid agro-ecological setup, but later on distributed and adapted to the wide agro-ecological zones. Because of its multi-purpose role, the Ogaden cattle have been used for milk, beef, and income generation. Information on the inherent genetic diversity is important in the design of breeding improvement programmes, making rational decisions on sustainable utilization and conservation of Animal Genetic Resources. Limited information is available about genetic variation of Ogaden breed at molecular level. The present investigation was aimed to study the biochemical polymorphism at the Hemoglobin (Hb) locus.

Materials and Methods: Blood samples collected from 105 Ogaden cattle maintained at Haramaya beef farm by jugular vein puncture were subjected to agarose gel electrophoresis [pH range 8.4-8.5] to study the polymorphic activities of haemoglobin.

Results: Three types of phenotypes were detected i.e. a slow moving (AA) band, fast moving (BB) band and a combination of slow + fast moving bands (AB). The frequency of the fast moving band was less [13 (12.3%)] than the slow moving band [57 (54.2%)]. Both slow & fast moving phenotype was observed in 35 (33.3%) animals. The gene frequency of HB^A allele was 0.709 and that of HB^B allele 0.291.

Conclusion: The distribution of phenotypes was in agreement with codominant single gene inheritance. The Chi-square (χ^2) test revealed that the population is under Hardy-Weinberg equilibrium.

Keywords: Ethiopia, haemoglobin, Ogaden cattle, phenotype, polymorphism.

Introduction

Ogaden is an area found in the Somali region, southeastern Ethiopia, bordering on Somalia (Figure-1A). The Ogaden cattle are one of the tropical cattle breeds (*Bos indicus*) widely distributed in eastern and south eastern part of Ethiopia where it is used for milk and meat purpose by pastoral society [1]. The breed (Figure-1B) has been evolved in arid and semi arid agro-ecological setup, but later on distributed and adapted to the wide agro-ecological zones. This breed is also known to be drought tolerant [2].

The Ogaden Zebu, also known in the literature as the Lowland Zebu, is classified as Small East Africa Zebu breed [3], and described as a strain of the Borana occupying the Ogaden rangelands of southeastern Ethiopia [4], but it is not clear why they are classified as a small rather than Large East African Zebu. This breed is also associated with the Jigjiga breed of cattle occupying adjacent rangelands to the north of its habitat [5].

Phenotypic characterization studies conducted at university of Haramaya indicated that though some of the physical traits of Ogaden cattle are similar to Boran, they differ from Boran in many other traits [6]. The Ogaden have white grey to white coat color; whereas, the Boran coat color is light grey or fawn and some of them have patches. Horns of Boran are thick based; whereas, the Ogaden breed has normal based horns. The hump of Ogaden bulls is pyramidal in shape; whereas, Boran Bulls have humps hanging over one side. Borans have more pendulous sheath; whereas, the Ogaden breed has tied up sheath. Preliminary blood protein polymorphism studies [2] found that Ogaden exhibited high heterozygosity values.

However, very little information is available on biochemical genetic variations of Hemoglobin (Hb) locus of Ogaden cattle. Hence, the present study was carried out to study the haemoglobin polymorphism of this breed.

Materials and Methods

Ethical approval: Permission was obtained from the departmental head of the Animal & Range Sciences to carry out the present work. Standard protocol for animal care and welfare was followed during sample collection.

Study area: The study was conducted at Haramaya University located at 09.00 ^{0} N and 42.00 ^{0} E at an altitude of 1950 meters above sea level. The area receives a bimodal rain fall, long rainy season (July to

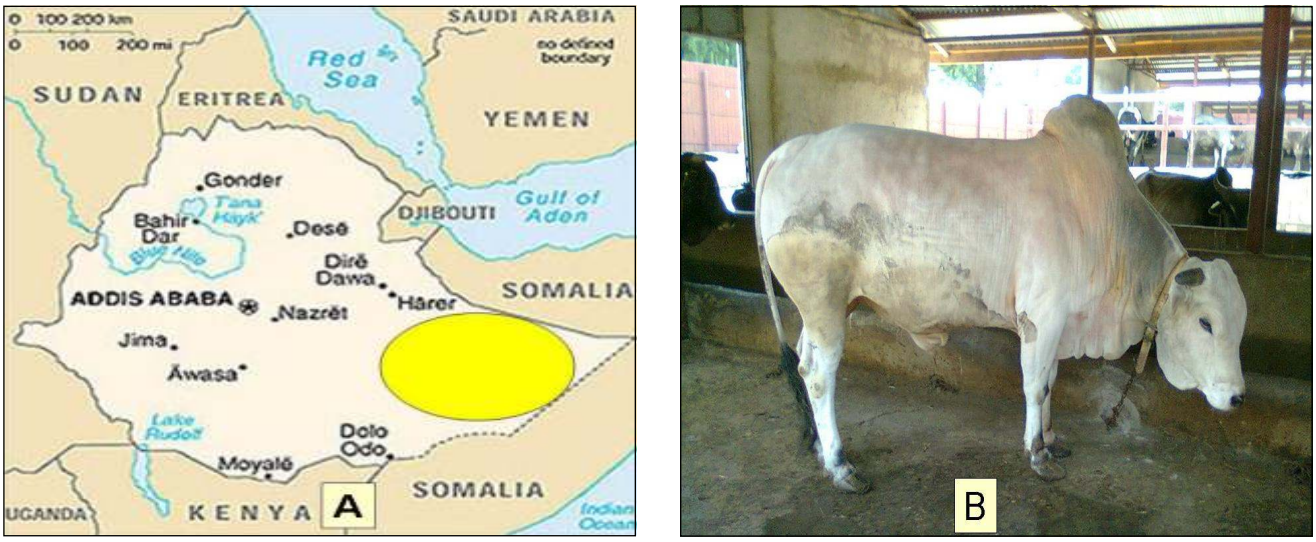

Figure-1: A. The Ogaden region marked in yellow; B. Ogaden bull at Haramaya farm

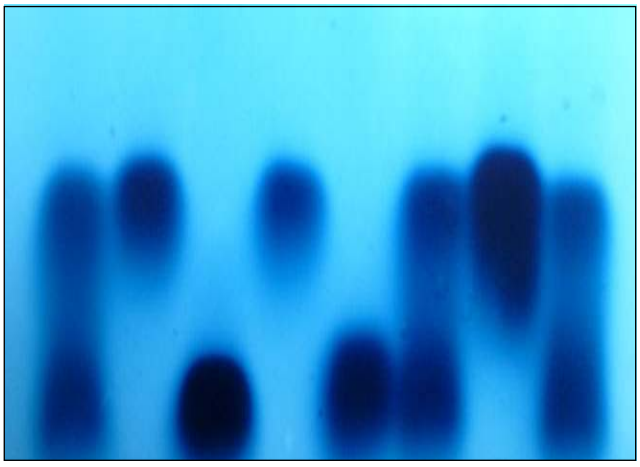

Figure-2: The observed haemoglobin polymorphism in Ogaden cattle

September) and short rainy season (March to June). The average rainfall is about 790 mm. The mean maximum and minimum temperature are 23.6^{0}C and 10.1^{0}C, respectively.

Experimental animals and Managemental conditions: Pure Ogaden cattle that are maintained at the beef farm of Haramaya University were used in the present study. The farm presently accommodated around 200 head of Ogaden cattle. The farm was established in 1990 with the aim to characterize and improve Ogaden cattle for beef production. Foundation stock and replacement were procured from different sites of Dhagaehabure, from Somali region which is the natural habitat of this breed. Natural controlled mating is practiced on the farm. Bulls are allowed to run freely with cows. One breeding bull was used for 20 to 25 breeding cows. Selections of breeding bulls were made based on performance on birth, weaning and yearling weight. Cows were selected based on normal conditions of the teats and health condition.

Blood collection and storage: Blood sample was collected by veno-puncture with 10 ml vaccutainer tube containing EDTA as anticoagulant. Red blood cells were separated from whole uncoagulated blood by addition of equal volume of normal saline (9.0g of NaCl in 1 litre deionised water) and allowed to stand for 30 minutes. The cells were finally lysed by addition of two volumes of deionised water, exposing the haemoglobin.

Analysis of samples: Haemoglobin genotypes were determined by agarose gel (1%) electrophoresis. The electrophoretic tank contained Tris-EDTA-borate buffer pH 8.4 - 8.5. A constant voltage of 200 volts was applied and allowed to run for about 2 hrs. After completion of the electrophoretic run the haemoglobin pattern could be read directly on the gel without staining.

Statistical analysis: Chi-square (χ^2) test was done to check if the population is under Hardy-Weinberg equilibrium.

Results

Electrophoresis showed one slow band for HbAA, a fast one for HbBB and two bands for the heterozygous HbAB (Figure-2). The HbAA and HbBB from Ogaden cattle were found to have similar electrophoretic mobility to those from Western cattle. The Hb allelic and genotypic frequency and expected

Table-1: Gene and genotypic frequency of haemoglobin in Ogaden cattle (n=105)

Alleles	Allele frequency	Genotypes	Genotype observed	Genotype frequency	Genotype expected	χ^2 df=2
A	0.709	Hb-AA	57	0.502	52.78	3.85 ns
B	0.291	Hb-AB	35	0.411	43.17	
		Hb-BB	13	0.084	8.86	

df = degree of freedom

genotypes of the Ogaden cattle population studied is given in Table-1. There was no sample that showed any abnormal phenotype. The observed and the expected genotype were calculated. Chi-square (χ^2) test revealed the population is under Hardy-Weinberg equilibrium for the Hb^A and Hb^B allele.

Discussion

The present study is in agreement with the earlier study of Gezahegn [2], who found that Ogaden cattle exhibited high degree of heterozygosity for certain blood proteins studied by starch gel electrophoresis.

The chief function of the red cells in blood is respiratory – carrying oxygen from the lungs to the capillaries of the various tissues and to remove carbon dioxide from the tissue to the lungs [7]. The ability to carry oxygen is dependent upon its haemoglobin content. It is this pigment that give blood its characteristic colour [8]. Hemoglobin is a large complex protein, consists of four polypeptide chains and one heam group. Bunn [9] demonstrated that ruminant (including HbA from a Holstein cow) and cat haemoglobins show intrinsically low oxygen affinity and are weakly reactive to 2,3-diphosphoglycerate (2,3-DPG), the main haemoglobin function modulator of mammals. However, Weber *et al.* [10] comparing yak haemoglobins with cow Hb A, showed a not so weak 2,3-DPG effect in the latter.

Due to their polymorphic nature, large number and simple Mendelian pattern of inheritance, the biochemical systems are used in the study of breed origin, determining structure and relations between breeds, and for the correlations that can be established with the several traits such as production, resistance to diseases etc. [11]. Though after the wide spread use of molecular characterization techniques the enthusiasm of biochemical polymorphism studies has gone down considerable, however, it is still popular especially in the developing world where sophisticated lab facilities are not readily available. Recent blood protein polymorphism studies has been reported for goat [12,13], sheep [14,15], pig [16], fish [17], camel [18], duck [19], and chicken [20] .

The Haemoglobin polymorphism is mostly due to a β chain variant - βB which shows a Mendelian mode of inheritance, presenting 3 phenotypes Hb A, Hb B and Hb AB, though rare α chain variant are also reported in Podolian cattle [21]. The gene for globin β-chain was mapped at chromosome 15 in cattle and is closely linked to the beta subunit of the follicle-stimulating hormone and the parathyroid hormone loci [22, 23], as well as in synteny with myogenic factor 3 (MYOD1) an muscle specific gene. The last two genes play an important role in muscular development. Hb polymorphism is one of the most studied polymorphisms in vertebrate species since the infancy of both population and evolutionary genetics because of its accessibility and its obvious biological importance. However, owing to the close relationship between structure and function, this complex protein remains a fascinating subject from all points of view and especially in terms of its molecular, genetic and adaptive features. Accordingly, Hb has been recently defined as "an evergreen red protein" [24].

Since, Cabannes and Serain in 1955 determined three haemoglobin phenotype in cattle, attributing them to two codominant alleles (HbA and HbB), and many alleles have been identified at the Hb locus [25]. Haemoglobin polymorphism in cattle is global phenomenon and has been reported in cattle from Europe [26], America [27], Africa [28], India [29], Korea [30] and Iran [31]. However, a great deal of genetic polymorphism studies is carried in dairy cattle as compared with beef cattle [32]. The results of present study are comparable with the earlier studies where high prevalence of HbA was found in *Bos Taurus* cattle [33, 34]. It was also consistence with gene frequency reported for some Indian cattle breeds [35]; that of Somalian Boran and Dawara cattles [36], and Bunaji cattle of Nigeria [37]. Ahmed *et al*. [38] reported 4 Hb variants, with allelic frequency as HbA= 0.51, HbB=0.33, HbC=0.014 and HbD=0.01 in Egyptian cows. In an earlier study Schwellnus and Guérin [39] could identify similar haemoglobin polymorphism in Brahman cattle and seven Southern African cattle breeds.

Most of the studies on different species and breeds revealed the existence of blood biochemical differences (including Hb-type). Nevertheless, the mechanism of such polymorphism is not clear or constant [40]. If Hb-type is connected or has any adaptive significance, it should not be different in breeds and species living in the same geographical and climatic region, especially that Hb-physiology and role is the same in all animals. Some blood factors are related to the suitability of the breeds under particular environmental conditions have been repeatedly suggested [37, 41, 42]. It is claimed that Hb polymorphism would be maintained by natural selection. However, no direct evidence of differences in fitness among the three phenotypes has been found in cattle [43]. Evidence on cattle presented by Sengupta [44] found

Hb-B to be favoured in hot arid climate and Hb-A appeared to be less frequent in hot arid climate than in warm humid. It may be regarded that the Hb-B originated from the zebu type cattle in India and has expanded its distribution to East Africa and other areas. Migration of Indian zebu cattle in Africa has been studied thoroughly by historical, anatomical and genetic methods [45].

Conclusion

The distribution of phenotypes was in agreement with codominant single gene inheritance. The Chi-square (χ^2) test revealed that the population is under Hardy-Weinberg equilibrium.

Authors' contributions

SKP and YYM - Substantial contribution to conception and design of study. YYM - Lab studies, acquisition of data and drafted the manuscript, SKP- analyzed and interpreted the results, revised manuscript critically for important intellectual content. Both authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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