Genetic Characterization of Zambian Native Cattle Breeds

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Thesis submitted to the Faculty of the Virginia Polytechnic Institute and State University in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

In

Animal and Poultry Sciences

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Date: September 9th, 2008

Blacksburg, Virginia

Keywords: Zambian indigenous cattle, RAPD, Genetic Variation

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ABSTRACT

Breed characterization is a primary step in designing appropriate management and conservation programs of livestock in developing countries. Since cattle represent a major food animal species in Zambia, its conservation is a major goal for both the government and non-governmental organizations. To support the conservation effort, the objective of this thesis research was to assess the phenotypic and molecular characteristics of indigenous Zambian cattle breeds including Angoni, Barotse, Tonga, and Baila based on body measurements and randomly amplified polymorphic DNA (RAPD) markers, respectively. A total of 100 animals, 25 from each of the four breeds associated with different tribes and region of Zambia, were used in the molecular analysis research. Additionally, 10 Holstein x Jersey crossbred animals were used as a reference and to test the extent of cross-breeding, if any, of the indigenous stock with exotic breeds. To further compare the Zambian indigenous breeds, morphometric measurements including body length, heart girth, and height at withers on 50 animals of each breed were measured. Blood was collected from animals at randomly selected farms and DNA isolated by standard protocols in Zambia. A total of 10 primers, of the 20 evaluated for informativeness, were used in the RAPD-PCR analyses. Differences among the four breeds for all the three

morphometric measurements were significant with the Barotse significantly higher than the other three ($P<0.05$). The average number of bands per primer was 7.1 and the percentage of polymorphic bands per primer ranged from 40 to 71.4 with an average of 64.8%. Breed divergence was highest between the Tonga and the Barotse and lowest between the Tonga and Baila breeds. Both the morphometric measurements and RAPD-based distance estimates suggest that the Barotse may be different from the other indigenous breeds while the Tonga and Baila were more closely related. In addition, the genetic distance estimates imply that the Holstein x Jersey crosses are different from the four Zambian indigenous cattle breeds evaluated. This thesis research provides, for the first time, the basic genetic information necessary for conservation of Zambian cattle breeds and the use of these populations for effective crossbreeding. The data suggest that though there is isolated by geographic distance and cultural differences among the tribes, two of the breeds are significantly related.

ACKNOWLEDGEMENT

I am deeply indebted to my advisor, Dr. E. J. Smith for according me the chance to pursue my MS. Degree program in Animal and Poultry Sciences at Virginia Tech. and for the timely advice he offered to me both within and outside academic circles.

I am thankful too to Dr. Bennett Cassell and Mr. David Winston for serving on my committee and for their helpful comments and suggestions in conducting this research.

I am thankful to all the staff in Dr. Ed Smith's laboratory for helping out in my laboratory work and for all the necessary advice that they offered. It is my sincere hope that this spirit will continue.

I would like to thank the Director of our Department in Zambia, Dr. Peter G. Sinyangwe, for allowing me to take leave of absence to pursue my studies. I am thankful to Dr. Benson Mwenya for all the advice and help that he rendered during and beyond my research activities in Zambia. I am thankful to my immediate supervisor, Mr. V. Simoongwe for the help he rendered during my research and for all the support he rendered to my family while I was away.

I would like to express my appreciation to Mr. and Mrs. Mweetwa who have been so valuable during the whole period of my studies at V.T. My deepest thanks and appreciation goes to my wife, Rosalyn, for her patience and for taking care of our family during the period I was away. Great thanks to Elina Banda, for being what she is, my mum, and for her patience and understanding. Last, but not the least, many thanks to all my family members for all the encouragement during my studies.

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CHAPTER 1

INTRODUCTION

Domesticated animals, especially livestock and poultry, are an important source of protein in African countries including Zambia. Increasing this protein resource requires the conservation of diversity among indigenous livestock. In order to cope with an unpredictable future, genetic reserves that are capable of readily responding to directional forces imposed by a broad spectrum of environments must be maintained. Maintaining genetic diversity is an insurance against future adverse conditions. In Africa, diversity among environments and nutritional standards as well as challenges from multiple infectious agents requires diverse breeds and populations. The breeds therefore act as storehouses of genetic variation which form the basis for selection and may be drawn upon in times of biological stress such as famine, drought or epidemics. The wide range of breeds and species that have evolved in various environments represent unique sets of genetic diversity.

It has been estimated that since domestication, over 6,379 documented breed populations from 30 species of livestock have been developed globally in the last 12 thousand years (FAO, 2000). It is generally accepted that the highest amount of genetic diversity in these populations of livestock is found in the developing world, where record keeping is poor but the risk of extinction is high and is increasing. Recently, loss of genetic diversity within indigenous livestock breeds has been a major concern. It is estimated that 35% of mammalian breeds and 63% of avian breeds are at risk of

extinction, and that approximately two breeds of livestock and poultry are lost each week (FAO, 2000). More particularly, it is estimated that 22% of known livestock breeds have become extinct in the last 100 years and another 27% are at varying degrees of risk (Rege and Tawah, 1999). The losses, as well as the risk to existing breeds, have been partially blamed on indiscriminate crossbreeding between exotic breeds and indigenous animals. Globally, this realization has led to efforts to study genetic diversity in livestock species in order to provide a foundation for conserving these potentially useful germplasms.

In addition to loss of diversity, a large proportion of domestic animal breeds in the world is believed to be in danger of extinction (Cardellino, 2004). According to Hanotte and Jianlin (2005), though it may be too late for many livestock and poultry breeds in Europe, optimism in the developing world about slowing down the loss of both diversity and indigenous animals is high. The most significant threat to domestic animal diversity in the developing world, it is believed, is the sustained importation of high performing animals from developed countries. This leads to crossbreeding or even replacement of local breeds. Conservation of indigenous animal resources has been proposed as a method for slowing down the loss in diversity in livestock breeds through extinction. Apart from preventing extinction, conservation of indigenous breeds is also important for the future health of the animal industry globally as they could be a resource for novel genes that can permit sustained genetic improvement as well as enable adaptation to changing breeding objectives and environments (Notter, 1999).

In order to ensure proper conservation and utilization of indigenous breeds, it is necessary to evaluate genetic variations that exist within and among breeds. A large proportion of indigenous livestock populations in the developing world have yet to be

characterized or evaluated at phenotypic and genetic levels (Hanotte and Jianlin, 2005). In the case of African livestock, genetic classification of animal breeds is still needed. Most existing classification of livestock breeds in Africa including Zambia is based on historical, anthropological and morphological evidence, that are most often not enough for the purpose of conservation (Mwacharo et al., 2006).

Relative genetic diversity can be determined using phenotypic characteristics and/or molecular markers. Phenotypic characteristics of livestock breeds as well as their adaptive characteristics are important in identifying breed attributes for immediate use by farming communities. Despite this importance, most African breeds have not been phenotypically characterized and adaptive attributes of most indigenous livestock breeds have remained unknown. In one of the few studies reported, Mwacharo et al (2006) phenotypically characterized two breeds of Zebu in Kenya. They used a total of 12 morphometric measurements to show that the Masaai Zebu was different from the Kamba Zebu in Kenya. The apparent wide within-breed and between-breed variations in linear body dimensions that were observed in the study were indicative of the large genetic diversity inherent in the small East African Zebu cattle, with clearly well-differentiated breeds.

The advent of molecular techniques has led to an increase in the studies that focus on the genetic characterization of domestic breeds using genetic markers (Giovambattista et al. 2001). As a tool used in evaluating genetic variation, markers can provide useful information at different levels and purposes such as structure of animal populations, levels of gene flow, phylogenetic relationships, patterns of historical biogeography, and parentage (Feral, 2006). In addition, genetic assessment is also of interest for the design

of genetic improvement programs including appropriate choice of breeds for crossbreeding (Mwacharo et al., 2006).

Though understanding genetic diversity is of importance to Africa, only a limited number of studies in livestock have been done in some African countries. For example, Gwakisa et al. (1994) described a study that involved characterization of Zebu cattle in Tanzania using randomly amplified polymorphic DNA (RAPD) markers. The cattle breeds included in their study were Tanganyinka Shorthorned Zebu, Boran, and the Mpwapwa. They reported that divergence between the Mpwapwa and Tanganyinka Shorthorned Zebu was on average lower than all other between comparisons. In a study of Ethiopian cattle breeds including Horro, Sheko, Arsi, Abigar and Guraghe, Hassen et al. (2007) reported that genetic diversity between Guraghe and Abigar were smallest followed by Guraghe and Arsi.

Despite these studies, our understanding of genetic variation and the relatedness among cattle in most African countries including Zambia remains poor. This thesis describes research that has begun to address this paucity. Specific objectives of this thesis are therefore to:

1) quantify the phenotypic differences among four indigenous Zambian cattle breeds based on body morphometric measurements,

   and

2) determine the genetic relatedness of four indigenous Zambian cattle breeds.

The data developed here will provide a resource for scientists interested in germplasm conservation and will add to previous reports of genetic variation in Tanzanian and Kenyan cattle.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Cattle in Africa

It is generally believed that domestication of cattle in Africa started in the Ancient East, the region with the most ancient civilization. According to Payne (1964, 1970) and Rouse (1970), the Semitic tribes of southern Arabia were the first to introduce Zebu and *Bos taurus* into Eastern, Southern, and Northern Africa. Cattle in Africa can be classified into four major groups: the humpless *Bos Taurus*; the humped *Bos indicus* (Zebu), distributed widely in Africa; *B. taurus* x *B. indcus* (Sanga), found mainly in eastern and southern Africa; and Sanga x Zebu types also known as Zenga (Reisti-Marti et al., 2003). With its unique set of genetic resources, Africa is known to be one of the centers of cattle diversity and domestication (Hanotte et al., 2002). However, the site of initial domestication of cattle in Africa is as yet without any consensus. The indigenous Zebu cattle accounts for about 90 % of the total cattle population in most African countries (Teale et al., 1993). However, indiscriminate crossbreeding of the Zebu with Taurine breeds has been a major contributor to the genetic dilution of this resource in most African counties. This has been accelerated by advances in new reproductive techniques, particularly artificial insemination and increased exchange of breeding stocks between regions (Moazami-Goudarzi et al., 1997).

The expectation is that there is more genetic diversity among indigenous cattle breeds in Africa than in the rest of the world. One explanation for this diversity is that

breeds in the developed world have been highly selected while most cattle in Africa are free-roaming and indigenous and are under little or no artificial selection (Giovambattista et al., 2001). Mburu and Hannotte (2005) described a study that looked at genetic diversity and population structure in 52 populations of African cattle using 15 microsatellite markers. The study indicated a wide range of genetic diversity among African cattle based on mean number of alleles, effective number of alleles, and expected heterozygosity. There was high differentiation among African cattle breeds. Diversity was highest in the North African *Bos taurus* and lowest in the western cattle. The analysis supported isolation by distance in all, but the Southern African region, with extensive gene flow between populations. Within these, there is extensive gene flow between populations. These and other molecular studies have revealed that cattle in Africa are appropriately partitioned by origin into East, West, North, and South.

Though both *Bos Taurus* and *Bos indicus* can be found in West Africa, Red Bororo and White Fulani are the primary cattle raised in that region. It is also the only region of Africa that still maintains a large population of apparently pure taurine breeds. The recognized cattle breeds in West Africa include the N'Dama and Muturu. Other breeds that can be found in the West African region include the Baoule-Somba and various Zebu strains (Alderson and Bodo, 1992; and Belemsaga et al, 2005). It has also been reported that the Biu cattle of Nigeria have been restricted only to the confines of hilly volcanic area of Bornu province in Nigeria and that fewer than 1000 to 2000 cattle remain. Cattle account for 32% of the 200 million livestock reported to be found in East Africa (Herlocker, 1999). Almost all of the cattle, approximately 95%, are reported to be indigenous (Rege, 1994). Another characteristic of cattle in East Africa is that an

estimated 60% is found in the arid and semi-arid zones. East African cattle possess, as expected, valuable traits such as disease tolerance/resistance, high fertility, good maternal qualities, longevity, and adaptability to harsh conditions and poor-quality feeds—all of which form the basis for low-input and sustainable agriculture. Several cattle breeds in Africa have been reported to be near extinction or have already been lost. Though there are no recent updates, examples of both were previously reported by Epstein (1974). Though most North African cattle were reported to be long or giant horned and humpless types, currently, only the N'Dama and Muturu of West Africa, the Namji of Northern Cameroon and the Kuri of Lake Chad with these characteristics can be found. In the Republic of Sudan, the humpless Nuba short-horned cattle that were previously found in the mountain region of the southern Kardofan, are now shard to find even (Alderson and Bodo, 1992). In Nigeria, the Biu cattle normally restricted to a hilly volcanic region area of the Bornu province are becoming increasingly hard to find. In Ethiopia, the Sanga cattle maintained by the Danakil and the Balla Azebo people are also very hard to find. In Uganda, the giant-horned Ankole Sanga are said to have been restricted by the advances of *Glossina morsitans* to a few isolated enclaves. Also in Tanzania, only a few herds of Ankole cattle are said to be present on the eastern side of the Tabora belt. Aderson and Bodo (1992) have also reported that in Zimbabwe, the original Amabowe have become exceedingly rare owing to the disastrous effects of the rinderpest which destroyed a large proportion of African herds. In South Africa, the Sanga types of cattle are still present in the Zululand, Swaziland and a few enclaves of Northern Transvaal. However, in other parts of South Africa, Curson and Thornton (1936) reported that the Bantu Sanga cattle

have been hybridized with breeds of European origin and can no longer be described as indigenous cattle of the Cape, Orange Free State or Southern Transvaal.

Despite their importance, not much has been done to fully characterize African cattle breeds, including the clear understanding of their reproductive and productive characteristics. Knowledge of the genetic variation that can be effectively measured within and between populations is important and also required for breed characterization (Hetzel and Drinkwater, 1992). The relationship among most of the African cattle breeds has remained poorly understood. Often, the same breed may be known by different names and equally, two breeds may be known by the same name depending on the geographical locations of such breeds (Gwakisa et al. 1994). For example, in Zambia, most of the livestock species have no specific breed names but are known by the local or common names in different parts of the Country. Depending on the different geographical location and or tribe, goats are called Mbuzi, Mpongo, Imbushi; sheep are called Mbelele, Imbelele, Mpanga; and different chicken strains e.g Inkoko, Kunku. The different synonyms may represent the same or different breeds. In order to develop appropriate strategies to curb the threats of dilution and extinction it is important to quantify the genetic variation that is present among breeds.

Zambia, like most African countries, has witnessed an increased form of agriculture development and urbanization during the past few decades. To meet and sustain the increased demand for livestock products such as milk, importation of specialized cattle breeds from temperate regions has been the trend. This has placed an excessive selection pressure against indigenous cattle. In turn this has led to the reduction in genetic variability of indigenous cattle breeds through breed substitution.

Globally, there has been an increase in awareness about the importance of indigenous animal breeds and the need to properly utilize and manage these resources. Awareness of the value of genetic resources in livestock has stimulated the study of the genetic diversity of native breeds. Most of such studies have been done on European cattle breeds and very little information is available concerning the genetic diversity of cattle breeds native to Africa. However, recently there has been an increase in the number of programs and studies on livestock genetic diversity in several African countries. In Zambia for example, tremendous efforts are now being made to genetically characterize the major breeds of livestock, including cattle, for conservation. In most African countries including Zambia, much of this effort is attributable to the Food and Agriculture Organization (FAO, 1999) of the United Nations global program that addresses the needs of both development and conservation of animal genetic resources in different parts of Africa.

In the following sections, I will discuss as part of this review, specific East African countries, a region whose cattle has been the most widely investigated in Africa.

2.1.1. Tanzania

Tanzania, like most parts of East Africa, is rich in livestock diversity. According to Gwakisa et al. (1994), local cattle breeds in Tanzania consists of Bos indicus Boran and the Tanganyika short-horned Zebu and also a considerable range of ‘synthetic’ types that have been developed like the Mpapwa. The Tanzania Short horned Zebu is said to be the major type of indigenous cattle in the country and is comprised of a number of strains, which include Iringa Red, Maasai, Mkalama Dun, Singida White, Mbulu, Gogo, Chagga and Pare (Rege and Tawah, 1999). Mpwapwa cattle have been described (Syrstad, 1990)

as a synthetic dual-purpose breed that was developed at the Livestock Production Research Institute at Mpwapwa in Tanzania. They received about 60% of their inheritance from improved dairy breeds originating in the Indian subcontinent (Red Sindhi and Sahiwal), about 30% from African zebu breeds (Boran and Tanzania Shorthorn Zebu) and the remaining 10% from European breeds (mainly Ayrshire). According to a molecular DNA based study that was done to characterize these local breeds (Gwakisa et al., 1994) it was revealed that there was a higher degree of homogeneity within than between the three local Zebu breeds and that there is a considerable divergence between the three breeds. In Tanzania most Ankole sanga cattle have been swamped by the more virile Zebu cattle and only a few are found on the eastern side of the Tabora belt (Alderson and Bodo, 1992).

2.1.2. Kenya

The indigenous cattle population of Kenya is of the thoracic humped type, commonly referred to as the small and large East African Shorthorn Zebu (Mason, 1996). Like the majority of indigenous African animal genetic resources, the Kenyan Zebu breeds are currently at risk of extinction. Despite the increasing genetic erosion of the indigenous Kenyan cattle populations and the urgent need for their conservation, information about their genetic uniqueness is completely lacking. These cattle have not been well defined, classified or studied adequately in the past. Like in most African countries there is not even information on the number and population sizes of breeds/strains. In Kenya, like in most of the African countries cattle populations bear the names of communities that own them and/or locations in which they are found, and the distinction between various populations is not clear. Information on phenotypic (including performance figures and

adaptive attributes) and genetic/molecular characteristics for most of the indigenous breeds is either completely lacking or incomplete. This information is required before appropriate strategies for long-term maintenance and use of these breeds can be formulated. A study that was recently done to phenotypically characterize two breeds of Zebu cattle in Kenya, Maasai and Kamba Zebu, revealed that the two breeds can be classified as medium-sized breeds but with great variations in their body sizes within and among breeds.

2.1.3. Zambia

Indigenous cattle in Zambia are grouped into four breeds: Angoni, Barotse, Baila, and Tonga. Because of their adaptation to harsh local environmental conditions like high temperatures, drought, and tropical diseases these cattle are important resources. They also have important roles in socio-economic development. For example, cattle are sometimes used as dowry and may be sold to raise cash to pay the school fees of family members. The origins of these breeds form the basis for understanding and improving them.

The Angoni cattle are found in Eastern province of Zambia and the bordering regions of Malawi and Mozambique. In Mozambique, they are known as Angone. The Angoni cattle are descendants of the Zebu cattle brought into Zambia by migrating Ngoni tribe when they crossed the Zambezi River from the South around 1835 (DAGRIS, 2007). The Angoni breed belongs to the Shorthorn Zebu. Its habitat lies between 9^{o} and 14^{o} South latitudes and 30^{o} and 32^{o} East longitudes (Mason and Maule, 1960). Genetic analysis has indicated that the frequency of taurine allele is high in the Angoni cattle next to Kilimanjaro Zebu cattle relative to other breeds (Hanotte et al. 2000).

The Barotse are found in the western part of Zambia, the Barotseland and homeland of the Lozi tribe (Mason and Maule, 1960). It originated from the Sanga cattle, a crossbreed of the humpless taurine and humped Zebu. 'Sanga' is an Ethiopian word meaning 'bull' which relates to the origin and centre of dispersal of this group of cattle breeds. Sanga cattle were introduced into southern Africa when the Khoikhoi (Hottentots) first crossed the Zambezi River about 700 AD (DAGRIS, 2007). The Barotse breed appears to have expanded after the rinderpest epidemic of 1889 – 1897 (Felius, 1995). The breed has adapted to the Kalahari sand type of environment found in the Western and North-Western provinces of Zambia. They can also be found in Botswana where they have been classified as Tswana Curson and Thorntorn (1936)

Prior to 1950, the name Baila was used for all of the Ila-tonga peoples of the Southern province of Zambia (Mason and Maule, 1960). A synonym was Mashukulumbwe (or Bashukulumpo), the name by which the Lozi referred to the Ila (plural Baila). Hence the name Baila has been restricted to the cattle of the Ila people. Currently, the Baila breed is believed to be derived from crossings of the Barotse and the Tonga. The Ila tribe is one of the Tonga dialects in Southern province of Zambia.

The Tonga breed also has its origin from the Sanga. The original Tonga had been maintained by the Tonga tribes in southern Zambia and northern Zimbabwe. The Tonga breed is very similar to the Mashona of Zimbabwe (Mason and Maule, 1960).

2.2 Measures of genetic variation and differentiation in cattle

It is generally accepted that genetic variation is the raw material of evolution, without which populations cannot evolve in response to changing environments. The knowledge of how genetic variation is partitioned among populations may have important implications not only in evolutionary biology and ecology, but also in conservation biology. In order to understand the amount of genetic variation in a population, methods are required to quantify this information.

Although several measures have been used to describe genetic variation at a single locus or a number of loci, heterozygosity has remained the most widely used (Hedrick, 2005). Heterozygosity refers to the state of being heterozygous and it is believed to be a good predicator of chances for long-term survival of a population. This is because it reflects the number of genetic options available within a population. Another measure that has sometimes been used to measure genetic variation is the number of alleles observed at a given locus in a population. But again, according to Hedrick (2005), this measure is strongly influenced by the population size so that comparisons across populations with different sample sizes should be made cautiously. The effective number of alleles, n_e, which is the inverse of the expected homozygosity, is also sometimes used to measure the amount of genetic variation in a population. In a recent study in cattle, Zhou et al (2005) used all these three measurements to analyze genetic diversity in breeds native to China. Based on polymorphisms of 10 microsatellite loci, they examined the genetic diversity among five native Chinese cattle breeds and also estimated the genetic differentiation and relationship within and between breeds. From their results, the

number of alleles, n, heterozygosity, and effective number of alleles, ranged from 4-12, 0.51-0.86 and 2.48-5.41 per locus, respectively.

Distance based methods have been widely used to measure genetic differentiation among populations. These methods involve calculating pairwise distance matrices, whose entries give an estimate of the distance between pairs of individuals. Amongst distance methods, Nei's genetic distance (Nei 1972) has been the preferred approach. Recently, for example, MacNeil et al (2006) used Nei's genetic distance metric to calculate the relationships among cattle in Chirik Island, off the coast of Alaska, and commercial breeds in North America. However, for closely related breeds within which drift is important, particularly in the developing world the modified Cavalli-sforza distance is recommended (Nei and Yatano, 1983). Another measure which partitions the genetic variation and provides a description of differentiation was developed by Wright (1951, 1965). This approach consists of varying types of differentiation measurements called F coefficients. One commonly reported F coefficient is Fst, which measures the degree of genetic differentiation based on standardized variances in allele frequencies among populations (Balloux and Goudet, 2002). Metta et al. (2004), for example, genetically characterized two Indian cattle breeds, Ongole and Deoni, using coefficients Fst and Fis, the levels of differentiation and inbreeding, respectively.

Phylogenetic trees often are constructed to accompany distance measurements. For example, Edwards et al. (2000) constructed two Neighbor-joining trees, one in which they used the genetic distances to visualize the relationships between the endangered pustertaler-Sprinzer and three European cattle breeds and another in which they used the

allele sharing statistic suggested by Bowcock et al. (1994) with individual animals acting as operational taxonomical units (OTUs).

2.3 Tools to understand livestock diversity – molecular characterization

Genetic marker types that provide different estimates of genetic diversity information have been described. The choice of the marker to use for genetic diversity is quite often dictated by the power of the method used to generate a reproducible polymorphism that can either be tracked in a Mendelian fashion or can show segregation of a phenotypic trait in a predictable manner (Hannotte and Jianlin, 2005). The choice can also be influenced by the availability of specialized equipment, operating of equipment and assays, and technical competence.

The first biomarkers to be widely used in livestock characterization were protein polymorphisms known as allozymes (Queller et al., 1993). Several livestock breeds have been characterized for variations in different proteins (Di Stasio, 1997). Using published data on protein polymorphisms from approximately 1000 papers in about 216 breeds of cattle, Manwell and Baker (1980) analyzed differences among animals from different continents. Protein polymorphisms, although still used in population studies, are of limited value in the assessment of genetic variation at the level of cattle breeds. This is largely because of the relatively low levels of polymorphism found in protein loci, resulting in a lower taxonomic limit for the resolving power of protein electrophoresis.

Molecular DNA polymorphisms are now the tools of choice for the assessment of genetic diversity among livestock breeds. They have great potential for discovery of fundamental parameters or characteristics important in conservation, including past effective population size (Garrigan at al., 2002), past bottlenecks (Luikart and Cornuet,

1998), population origin (Cornuet et al., 1999), inbreeding status (Ellegren, 1999; Lynch and Ritland, 1999), and sex-specific gene flow. According to Hannotte and Jianlin (2005), important assumptions that are needed for the use of genetic markers include: (i) neutrality of the polymorphisms and (ii) the use of a relatively small number of independently segregating marker loci are a good predictor of the overall genomic diversity of a population.

One of the oldest molecular DNA markers used for assessing diversity is restriction fragment length polymorphisms (RFLPs). This method involves comparing the number and size of deoxyribonucleic acid (DNA) fragments produced by the digestion of DNA with various restriction enzymes. The banding pattern is generated by the presence or absence of restriction cutting sites and these in turn are produced by mutation. The fragments are blotted to membranes and probed with cloned radio-labeled DNA that binds to a single locus. This technique can be applied to nuclear DNA or to mitochondrial DNA (also to chloroplast DNA in the case of plants). It has applications in the study of genetic distance, population variation, gene flow, effective population size, patterns of historical biogeography and analyses of parentage and relatedness. For example, Theilmann et al. (1989), used nine RFLPs in six breeds of cattle to show that the Brahman (*Bos indicus*) differed significantly from the *Bos taurus*.

Amongst the most widely used molecular markers for assessing relatedness in livestock are microsatellites. In recent years, in addition to being the markers of choice for gene mapping, they have been used in population studies to assess diversity (Wall et al., 1993). Microsatellites are highly polymorphic, densely distributed in the genome, highly variable, and relatively easy to detect using the polymerase chain reaction. As

hypervariability is highly significant for detecting differences in a population and between individuals, microsatellite typing can reveal degrees of polymorphism that are easy to interpret. Microsatellites have been isolated in large numbers from most livestock species and FAO has recommended a list of microsatellite markers for genetic diversity studies that are now publicly available (http://www.fao.org/dad-is). The primary disadvantage of this technique is that a prior knowledge of the DNA sequence is required to allow the design of PCR primers.

Randomly Amplified Polymorphic DNA (RAPD) technique, like microsatellites, involves the use of PCR for genetic typing and to assess diversity (Williams et al., 1990). In one study involving indigenous African cattle, Gwakisa et al. (1994) used RAPD markers to characterize the local Zebu (*Bos indicus*) cattle breeds of Tanzania. Using RAPD markers, the relatedness among the three local breeds of Tanzania was quantified. One of the primers, ILO 1127, amplified a RAPD fragment in 61% of the Tanganyika Shorthorned Zebu animals but less than 6% in the other breeds. Another primer, ILO 1065, revealed a DNA segment common to 89% of the Boran animals and less than 30% in the other two breeds evaluated. Further, the study revealed that ILO 1065 primer could be a *Bos indicus*-specific Y-linked polymorphism. They also showed that RAPD analysis could detect introgression. In another similar study, Yu et al. (2004) used RAPD analysis to estimate genetic diversity and relatedness of two native cattle breeds from the Yunnan province of China (DeHong cattle and DiQing cattle) and four introduced beef cattle breeds (Brahman, Simmental, MurryGrey, and ShortHorn). Using 10 primers, it was shown that the Yunnnan DeHong cattle breed was closely related to the Brahman (Bos

indicus), and the Yunnan DiQing cattle breed was closely related to the Simmental, ShortHorn, and MurryGrey (*Bos taurus*) breeds.

Under the FAO recommendation, most of the livestock variation studies that have been done used microsatellite markers. The major drawback of RAPDs is that they are dominant markers and heterozygotes are typically scored as homozygotes, which decreases their information content. However, RAPD analysis could benefit most of African laboratories in the studies of livestock due to their simplicity, affordability and relatively low technical requirements.

CHAPTER 3

MORPHOMETRIC TRAIT COMPARISONS AMONG FOUR INDIGENOUS ZAMBIAN CATTLE BREEDS

3.1 ABSTRACT

Appropriate management and conservation strategies of animal genetic resources require assessment of genetic diversity both within and among populations. Breed characterization, either phenotypically or genetically, is the primary step in any conservation program. This study aimed at phenotypically characterizing the four indigenous cattle breeds of Zambia, which are associated with specific tribes and regions. Morphometric measurements made on the animals were heart girth, height at withers, and body length. Heart girth and body length of Barotse cattle were on average significantly higher than those of the other indigenous breeds. Wither height was highest in the Barotse and Tonga. The morphometric measurements suggest that the Barotse should be classified as a large-sized breed, the Baila and Tonga as medium-sized, and the Angoni, because it had the smallest measurement among the four breeds, as small-sized. This thesis research provides, for the first time, evidence that the breeds associated with specific regions and tribes in Zambia have not significantly diverged phenotypically and may differ in only a few primary characteristics associated with productivity. The data will be useful for the improvement of these breeds as well as for conservation.

3.2 INTRODUCTION

Management and conservation of animal genetic resources require assessment of genetic diversity. This is because it is difficult to design appropriate breeding programs for breeds that have not been adequately characterized either phenotypically and/or genetically. Phenotypic characteristics are important in breed identification and classification in ways that farming communities can relate to. For over half a century, it has been known that body measurements can be used to interpret growth and production factors, to describe size inheritance and types of breeds or strains, and to estimate weight in beef cattle (Wanderstock and Salisbury, 1946). Morphological descriptions have also been used to evaluate breeding goals, to assess type and function and to estimate the animals' value as potential breeding stock (Mwacharo et al., 2005).

Although indigenous cattle play a major role in the sustainability of livelihoods of the people of Zambia, there is very little published information concerning the physical characteristics of these breeds. The lack of information on the physical characteristics hinders the development of programs for improving these breeds. Though lacking in primary information, Zambian cattle have been described as belonging to four breeds: Angoni, Baila, Barotse and Tonga (Rouse, 1970).

The Angoni, a *Bos indicus* with a compact to medium size is found primarily in the Eastern region of Zambia. It has been estimated that a mature and healthy Angoni cow weighs on average 400 kg while a bull weighs 500 kg. Though its predominant color is dark red color, other colors have been observed including black and black with white markings (Rouse, 1970). The horns vary widely in size and shape and the hump is prominent on both males and females. Among local cattle breeds in Zambia, there are

more records on the Angoni than on any other. It is the only indigenous breed which performance records have been kept by the Herd Book Society of Zambia.

The Barotse is a Sanga derivative that is commonly referred to as the longhorned Sanga with an average beef-type conformation. The breed gets its name from the Lozi tribe of the Western Province of Zambia. It is mainly concentrated in Zambia though it can be found in border towns in Angola and Botswana, where it may have different names. According to the State of the World Animal Genetic Resources (SOWAnGR) report (Mwenya et al., 2005. Unpublished), a mature and healthy Barotse cow normally weighs about 455 kg while a bull of the same age and condition will weigh about 680 kg. The colors vary from dark red, black and brown, to a combination of these colors and sometimes with white markings. The hump is small and not noticeable in the females. The horns of bulls are generally large and spreading. The skin is fairly loose, dark pigmented, and of medium thickness.

The Tonga breed also has its origins from the Sanga. It is a shorthorn that is associated with the Tonga tribe of the Southern Province both in the Zambezi valley and on the Tonga plateau. While the horns are, like those of the Barotse, of average size and shape, they are reported to be smaller. Like the other three indigenous breeds in Zambia, the Tonga cattle do not have uniform colors. The colors include black, red, brown and a combination of these as well as with white markings. The hump is very small especially on the females. In conformation, the breed is much smaller than the Angoni and Barotse. The horns are shorter than the Barotse but longer than the Angoni.

This Baila breed of cattle is believed to be from a cross between the Barotse and the Tonga. It is named after the Ila tribe, which is one of the Tonga dialects in the

Southern province of Zambia. The Ila speaking people are found in Namwala district, the region with the highest concentration of the Baila breed. The Baila colors and hump are similar to the other breeds. To more fully utilize the Zambian native cattle for improvement, there is need for comparative quantitative data and for information on their adaptive and special genetic attributes. The purpose of this study was to compare physical characteristics of the four indigenous Zambian cattle breeds based on three morphometric measurements including body length, height at withers, and heart girth.

3.3 MATERIALS AND METHODS

3.3.1. Animal and phenotypic measurements

Data from 50 animals from each of the four breeds, Angoni, Barotse, Baila, and Tonga, for a total of 200, were used. The animals included in this study were sampled from regions where each breed is predominantly found. The locations from which sampling was done are shown in Figure 1. Thus, the Angoni cattle were sampled from Chipata, Katete and Petauke districts in the Eastern province of Zambia; The Barotse from Mongu, Senanga and Kaoma districts in the Western province; the Tonga from Gwembe, Monze and Pemba districts in the Southern province; and the Baila cattle were also sampled from the Southern province in the Namwala district and along the Kafue river plains. Typically, the animals included in the study were raised under similar conditions that involved an extensive production system with animals that are free grazing.

Measurements taken from each animal at about four years of age included body length, height at withers, and heart girth. Because farmers often did not have birth records, age was estimated by examining each animal's teeth as suggested by Pace and Wakeman (2003). Only animals with an eruption of the fourth pair of teeth, indicating maturity, were included in the study. Body measurements were taken according to the procedure described by Wanderstock and Salisbury (1946). Body length and height were measured using a ruler while a cloth graduated in centimeters was used to measure girth. The heart girth measurement was obtained by placing the cloth around the animal at the point of smallest circumference just behind the forelegs. It was pulled snugly around the animal, tight enough to keep the hair down but not indent the flesh. Body length was measured, with the animal in a "normal" position, as the length from the pin bone to the

prominence on the shoulder, located about one inch posterior to the point of the shoulder. All measurements were repeated three times with the animal being moved to a “normal” position for each measurement.

3.3.2. Statistical analysis

Data were analyzed with the General Linear Model (GLM) procedure of SAS (SAS, 1999-2001). In the analyses, breed and sex were included as fixed variables while body length, heart girth, and height were used as continuous variables. The significance of means of all variables were separated via protected Least Significant Difference (LSD) (SAS, 1999-2001). Further, data was subjected to more analysis using a multiple regression model of SAS. The proposed model using these factors was:

$$Y = \mu + b_i + s_j + (b_i + s_j) + e$$

Where,

Y = the vector of N observations on an animal of a given breed and sex for a given variable (body length, heart girth and wither height); μ = the average from a specific population and sex; b_i = i th breed (i = Barotse, Angoni, Tonga or Baila); s = jth sex (j = male or female); ($b_i + s_j$) is a two-way interaction effect between sex and breed; and e = residual effect, which is independently and identically distributed with mean = 0 and variance = σ^2.

Analysis of variance of SAS was used to determine the significance of all factors.

The correlation coefficients among the variables were computed on a within sex and within breed basis using proc corr procedure of SAS (SAS 1999-2001).

3.4 RESULTS AND DISCUSSION

A summary of the analysis of variance (ANOVA) for the three measurements evaluated is presented in Table 1. Results on the analysis of variance are presented in table 1. Breed and sex interaction had significant effects on body length and height at withers due. Breed had significant effects on heart girth.

As expected body measurements in males were higher than those in the females (Table 2). There were no significant differences between male and females in heart girth across all breeds. For body height and body length, Baila and Angoni males were significantly taller than the females. In the Tonga breed too, the males were significantly longer than the females. There were no significant differences between the males and the females for all measurements in the Barotse breed. For the heart girth and body length, the Barotse female cattle had significantly higher body dimensions than the other female breeds. The Angoni females were significantly smaller in heart girth than the other breeds. Generally the linear body dimensions for the Barotse breed were higher. The relatively large body frame may be an adaptation to dissipating large amounts of heat, typical to the Barotse land in the Western province. On the other hand, the Angoni were relatively smaller for all measurements. Whereas the body dimensions for wither height have relative higher measurements than body length, there appears to no difference in body length and wither height in the Barotse males. As indicated by the standard deviations, there are substantial inherent differences in body size within indigenous cattle breeds in Zambia. According to Kalmaldzadeh et al. (1998), skeletal measurements such as ulna length, body height and length, and chest depth are less affected by nutrition and

thus may indicate inherent size better than measures related to muscle and fat deposition, such as body girth. For most of the measurements, there were no significant differences among the Zambian indigenous breeds. This indicates that the Zambian indigenous have not fully diverged in body size, despite their geographical and cultural differences.

Body dimensions have been used to indicate breed, origin and relationship through the median of head measurements or to indicate size (Jewel, 1963). More recently, alternative body measurements and indices estimated from various combinations of conventional and non-conventional body parameters have been used as markers for weight and as indicators of type and function in domestic animals (Schwabe and Hall, 1989; Salako, 2006). Similarly, the results of body measurements obtained in this study could be useful identifiers of the indigenous breeds in Zambia. Although the Angoni is considered closely related to the small East African Zebu (Mason and Maule, 1960), the mean adult measurements for the Angoni females presented here (Table 3) are relatively higher than those of the small East African Zebu (Mwacharo et al., 2006). In a similar study, the mean measurements in the Masaai female adults for body length, heart girth and wither height were reported to be 116.7cm, 145.9cm and 111.3cm respectively, which are lower than those of the animals studied in the present work (Table 3). Rege (1999) reported wither height for the Angoni to be 119-127cm, suggesting a large variation in size.

Table 3 gives the correlation coefficient estimates among the three linear body measurements, body length, heart girth and withers height for female Zambian indigenous cattle. The coefficients in the Angoni were generally lower than in the other breeds. The coefficients for the three measurements of the female Zambian cattle were

similar to those reported by Mwacharo et al (2006) but higher than those reported by Okeyo et al (1996) on small East African cattle. All the correlation coefficients for each trait were significant across the breeds.

Within each breed, the simple regression of mean wither height against body length gave linear relationships expressed in the following equations:

Tonga: $y = 102.02 + 0.5314x$(Body Length),

Barotse: $y = 96.20 + 0.5932x$ (Body Length),

Baila: $y = 75.766 + 0.7568x$(Body Length,

Angoni: $y = -23.236 + 1.4855x$(Body Length),

For the females, the relationships between wither height and body length is presented for the breeds graphically in Figures 2. The relative differences in the levels of regression coefficients appear to be indicative of the proportionate differences in body height and length and therefore breed differences. The regression coefficient of the equation relating wither height and body length is steeper for the Angoni females compared to other Zambian indigenous breeds (Figures 2). This indicates that, as body length increases for the Angoni females wither height increases at a faster rate than it does for the other three breeds. Nutritional stress may be strong in the Angoni, with genetic potential for body length not being expressed while that of wither height, which is observed to be less subject to nutritional stress (Lawrence and Pearce, 1964), is much more expressed. This indicates that the Angoni is taller than it is long, relative to its proportionate size. According to Hall (1991) this type of body confirmation is an adaptation to dissipating heat than a short, squat body. The tall and leggy confirmation of the Angoni can be

considered an adaptation to free and wide-ranging mode of grazing in search of pastures and water.

The marked differences between male and female cattle were evidence of sexual dimorphism among the Zambian indigenous cattle breeds, with the males (as expected) having relatively greater measurements for all traits studied. The sex-related differences are the result of the usual between-sex differential hormonal effects on growth. There were wide variations in linear body dimensions both within-breed and between-breed among the Zambian indigenous cattle breeds observed in this study. These variations are indicative of the amount of genetic diversity that exists among the Zambian indigenous cattle.

It should be noted that comparisons discussed in this paper are based on three measurements and only on mature animals having four pairs of permanent incisor teeth. There is need to characterize the indigenous breeds in Zambia using additional morphological traits. It is important to characterize cattle phenotypically because information on the phenotypic traits is easily accessible by the communities. For example in Zambia, the Lozi people relate themselves with large, horned Barotse cattle. Other breeds with characteristics that are different are considered foreign. This indicates, as expected in these regions, that cattle have phenotypic attributes that fulfill cultural values. In addition to these physical attributes, cattle in developing countries are also valued for the reproductive prowess. According to Ndamu et al., (2006), while cattle must possess attributes that fulfill cultural values, (be beautiful as defined culturally), attributes that ensure productivity (fertility, fitness and production) are emphasized. Morphometric characteristics, like those evaluated here, can provide useful information about breed

relationships and size as well as its cultural value. In addition, periodic evaluations of morphometric characteristics have also been used to identify populations experiencing inbreeding depression. According to Kamalzadeh et al. (1998), skeletal measurements such as ulna length, body height and length, and chest depth are less affected by nutrition and more by genetics. Data presented here that provides a foundation for these uses of morphometric measurements in Zambian indigenous cattle.

CHAPTER 4

MOLECULAR DNA ANALYSIS OF ZAMBIAN INDIGENOUS CATTLE BREEDS

4.1 ABSTRACT

In this study, RAPD analysis was used to estimate genetic diversity and relationship within and among four Zambian indigenous cattle breeds including Angoni, Baila, Barotse and Tonga. Additionally, to test the extent of crossing with exotic breeds, animals from a Holstein x Jersey cross were also included in the analyses. Ten random primers, from 20 tested, amplified informative (polymorphic) fragments were used to assess diversity and relatedness. The average number of bands per primer was 7.1 and the percentage of polymorphic bands per primer ranged from 40 to 71.4 with an average of 64.8. The distributions of the 13 amplified polymorphic bands were significantly different among the Zambian indigenous and Holstein x Jersey crossbred cattle breeds. The genetic distance matrix was generated and the phylogenetic analyses were carried out using unweighted pair-group method using arithmetic averages (UPGMA) method. Based on genetic distances, the Zambian indigenous cattle showed higher genetic diversity both within and between the breeds. This is an indication that Zambian indigenous cattle breeds are generally outbred animals. Results from the phylogenetic analysis indicate that the Baila and Tonga are closely related while the Barotse and the Angoni are distinct breeds. The results also provide the basic genetic information for conservation of Zambian indigenous cattle and for designing appropriate breeding programs among these breeds.

4.2 INTRODUCTION

Indigenous cattle form the backbone of relevant and sustainable livestock production in Zambia because they are, compared to high performing exotic breeds, better adapted to survive and reproduce under harsh environments. Moreover, they require less input and management. They can survive and are able to utilize poor-quality feeds and thus sustain the livelihoods of most Zambian farmers, the rural poor. They are sometimes used for food, transport, as draft for animals in the cultivation of crops, and as a source of manure for crop production. In addition, animals play an important role in African culture as they are used for gifts, dowry, and in cultural ceremonies. Any effort to manage and improve indigenous livestock will have a positive impact to improve the livelihood of the majority of people.

Recently, there has been some concern about the potential loss of genetic variation among Zambian indigenous cattle from breed substitution, from indiscriminate crossbreeding, and from the absence of breed development programs (Zulu et al., 2003). Any reduction in the animal diversity of genetic resources affects a community's opportunity for response to changes in the environment, and to disease challenges or changes in consumer preferences. The gradual disappearance of indigenous breeds that are capable of surviving in extreme environments undermines food and livelihood security of the poor and reduces the ability of human populations to survive in marginal areas. In Zambia, as elsewhere, immediate steps must therefore be taken to conserve these cattle.

Characterization of indigenous breeds is a prerequisite for appropriate management and conservation of animal genetic resources. Breed characterization requires knowledge

of genetic variation that can be effectively measured within and between populations (Hetzel and Drinkwater, 1992). Methods considered appropriate for breed characterization vary from morphometric to molecular and genomic. Recently, the Food and Agriculture organization (FAO) of the United Nations proposed a global program for the management of genetic resources using molecular methodology for breed characterization (Bjornstad and Roed, 2001). This strategy strongly emphasized the use of molecular markers to assist the conservation and assessment of endangered breeds and to determine the genetic status of these breeds.

With the advent of molecular techniques, it has become possible to characterize breeds by determining genetic relationships among animals based on differences in DNA (Karp et al., 1996). The molecular DNA markers that have been used for breed characterization in both plants and animals include restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), microsatellites or simple sequence repeats, mitochondrial DNA (mtDNA), and randomly amplified polymorphic DNA (RAPD). Although they were the first DNA markers to be developed, RFLPs have continued to be useful in breed characterization of both plant and animal species. Recently, Marson et al. (2005) characterized a population of 370 European-Zebu composite beef heifers consisting of six different breed compositions, using RFLP markers or genes for luteinizing hormone receptor and follicle-stimulating hormone receptor.

AFLPs have also been used in both plant and animal breed characterization. AFLPs are DNA fragments (80–500 bp) obtained from digestion with restriction enzymes, followed by ligation of oligonucleotide adapters to the digestion products and

selective amplification by the PCR. AFLPs therefore involve both RFLP and PCR. AFLP are amongst markers that have been widely used in characterization of both plant and animal species. Recently, Marchi et al. (2006) assessed the genetic variation in four indigenous chicken breeds from the Veneto region of Italy using AFLP markers.

Microsatellites represent tandem repeats of genomic DNA consisting of repeating units of 1-6 base pairs in length. Recently, they have become markers of choice for genetic diversity studies. For example, Liron et al. (2006) used the polymorphisms from 9 microsatellite loci to assess genetic diversity and relationships within and among 4 Creole cattle breeds from Argentina and Bolivia, 4 European taurine breeds, and 2 American Zebu populations. The Creole populations displayed, relative to European breeds, high level of genetic variation as estimated by allelic diversity and heterozygosity. Microsatellites have also been used to determine the distribution and origin of livestock breeds. For example, MacHugh et al. (1997) used the genetic variation at 20 microsatellite loci to determine the evolutionary relationships and molecular biogeography of 20 different cattle populations from Africa, Europe and Asia. From phylogenetic reconstruction and multivariate analysis, the study highlighted a marked distinction between humpless (taurine) and humped (Zebu) cattle, providing strong support for a separate origin for domesticated Zebu cattle.

Mitochondrial DNA (mtDNA) polymorphisms are also markers that have proven valuable in the phylogenetic and genetic diversity studies of both plants and animals. The characteristics of mtDNA of non-recombination and maternal inheritance have enabled biologists to reconstruct relationships between and within species and breeds. In particular, the polymorphisms in the hypervariable region of the D-loop or control region

of mtDNA have contributed greatly to the identification of wild progenitors of domestic species, the establishment of geographic patterns of genetic diversity, and the understanding of livestock domestication (Bruford et al., 2003). In another study, Bradley et al. (1996) examined mtDNA displacement loop sequence variation in 90 extant bovine breeds from Africa, Europe, and India. Their results from the phylogeny estimation and analysis of molecular variance showed that the sequences clustered significantly into continental groups and that the Indian *Bos indicu*s samples were markedly distinct from the others.

RAPD, a random primer-based molecular technique, has also been used to assess diversity in plants and animals (Williams et al., 1990). For example, Hassen et al (2007) used RAPDs to assess genetic variability within and among five indigenous Ethiopian cattle breeds. The analysis revealed that within breed genetic variation was much higher than that between breeds. Because it is quick, less expensive and requires less expertise, the RAPD technique could be suitable in laboratories in Africa, including those in Zambia. The primary objective of this study is to assess the genetic diversity and relatedness among the Zambian indigenous cattle based on RAPD markers.

4.3 MATERIALS AND METHODS

4.3.1. Animals

Twenty-five (25) animals of each breed were sampled as described in chapter three and from areas shown in Figure 1. For Angoni cattle sampling was done from Chipata, Katete, and Petauke districts in the Eastern province. Sampling locations for Barotse cattle were Mongu, Senanga, and Kaoma districts in the Western province while those for the Tonga cattle were Gwembe, Monze and Pemba districts in Southern province. The Baila cattle were sampled from Namwala district and along the Kafue river plains in Southern province. Before sampling, each farmer was interviewed about the nature of the breeding schemes in order to determine the purity of the animals. Animals from farmers with clear knowledge of crossbreeding were not sampled. Thirteen Holstein x Jersey crosses (Courtesy of Dr. Bennet Cassell) were included in this study to compare with the Zambian cattle and to investigate the use of RAPDs to study closely related species.

4.3.2. DNA Samples

Blood was collected in vacutainers containing EDTA and stored under refrigeration (-80 ^{o}C) until DNA extraction. Genomic DNA was extracted from whole blood using standard procedures as recommended by the manufacturer (Qiagen Inc., Valencia, CA). After isolation, the DNA was stored at 4 ^{o}C until ready for use.

4.3.3. RAPD-PCR Reaction

RAPD-PCR were performed in the same programmable thermal controller, PTC100 (MJ Research). Each reaction was in a final volume of 15 ul containing 5x PCR buffer, 25

mM $MgCl_2$, 10 mM dNTPs, 5 pmol primer (Table 4), 50-100 ug/ml template DNA, and 20 units of Taq DNA polymerase. Following an initial denaturation step at 95^{o}C for 2 minutes, the reaction was subjected to 44 cycles of denaturation at 92^{o}C (1 minute), annealing at 35^{o}C (30 seconds), and extension at 72^{o}C (3 minutes) followed by an additional extension at 72^{o}C for 7 minutes. The amplification products were separated by electrophoresis on 2% agarose gels in 0.5x TBE buffer in the presence of SYBR green for 2.5 hours. The RAPD patterns were visualized by UV illumination and the images of each gel were photographed using a Kodak Edas 290 digital camera.

Amplified products were classified as present (denoted by 1) or absent (denoted by 2) as suggested by Yu et al. (2002). Polymorphic or informative fragments (bands) were also labeled according to the protocol of Yu et al. (2002).

4.3.4. RAPD analysis

Analysis of the RAPD patterns involved first combining the total number of bands for each animal and for all the primers. The number of bands common between any two breeds formed the basis of calculation of relatedness by the Dice algorithm (Nei and Li, 1979). The DNA profiles ware analyzed using a RAPDistance software package (http://www.anu.edu.au/BoZo/software/). Genetic distances were calculated according to the method by Nei and Li (1979). Thus calculations proceeded as follows:

1. $F = Nxy/Nx + Ny$

Where F is the similarity coefficient, Nxy is the number of bands shared between individuals x and y and, Nx and Ny are the number of bands in individuals x and y respectively.

2. D = 1 –F,

Where D is the genetic distance.

The patterns were normalized with the bands that were uniformly present in all patterns, and a binary matrix containing the presence or absence of the major bands was recorded. Very faint bands were excluded from the analysis.

A Neighbor-joining tree was constructed using the NJTREE and TDRAW programs in RAPDistance software (L. Jin and J. W. H. Ferguson, University of Texas Health Science Centre, Houston) and visualized in the program Mega2.0.

Using the Pearson's Chi square test, the numbers of each polymorphic band was compared to determine the significance differences across the breeds.

4.4 RESULTS AND DISCUSSION

Preliminary studies involved testing 20 primers and, from these, primers were selected that yielded reproducible and polymorphic products (Table 4). The GC content of the primers ranged from 60 to 90% for primers OPN 15 and ILO 1127, respectively. Though primer ILO 1127, which had the highest GC content amplified the most fragments, there was no relationship between primer GC content and the number of bands amplified by the primer. The number of bands amplified by the primers ranged from 5 to 10 for a total of 71. Approximately 65% of these bands were polymorphic. Two examples of gel patterns showing polymorphic products amplified by primers OPA 19 and ILO 525 are presented in Figures 3A and 3B, respectively. The polymorphic fragments ranged in size from 0.25 to 1.80kb (Table 4).

Thirteen of the 38 polymorphic bands had significantly different frequency distributions across breeds ($P \leq 0.05$, Table 5). These bands could be investigated further by first cloning and conversion to sequence characterized regions (Gu et al., 1995). Since cattle breeds used for crossbreeding in Zambia include the Holstein and Jersey, the proportion of the ILO1204-350 among the Zambian indigenous cattle could be useful for inferring the level of introgression of these exotic populations into the native animals. In an earlier study primers ILO 1127, ILO 1065 and ILO 525 amplified breed specific bands in East African cattle, but here they did not. This could be due to several factors including differences in the breeds evaluated and/or inconsistencies that are typical of RAPDs as a marker system.

Within breeds, genetic distance was highest in the Tonga breed (Table 6) and lowest in the Holstein x Jersey crossbreds from the United States. This suggests that

genetic variation within the Zambian indigenous cattle may be higher than in the Holstein and Jersey. In the United States, genetic diversity in poultry and dairy cattle have already been described as limited (Notter, 1999).

Between breeds, the genetic distance was highest between the Baila and the Holstein x Jersey crossbreds and lowest between the Tonga and the Baila (Table 7). As shown in Figure 1, the proximity in distance among these breeds may account for their close relationship.

As shown in figure 4, the dendrogram shows the Baila and Tonga breeds to closely related while the Barotse, Angoni and Holstein x Jersey crossbred cattle are shown to be distinct.

The Neighbor-joining tree presented in Figure 5 shows relationships among the individual animals included in the study from each breed. Though as expected the animals clustered mostly into their breeds, there were some overlaps. This is consistent with the breed survey reported by Zulu et al. (2003) that indicated some admixture among the four Zambian breeds. It appears there has been extensive gene flow among Zambian cattle populations, involving both local and exotic breeds, mainly in the peri-urban areas. Currently, it is hard to find a pure bred Tonga in the urban or peri-urban areas of Zambia.

Results reported here indicate that there is greater genetic variation among the Zambian indigenous cattle than those reported for other African cattle. The magnitude of the genetic distances, as shown in Table 7 for the Zambian indigenous cattle breeds, were higher than those for East African Zebu, 0.005-0.194, reported by Gwakisa et al. (1997). They are also higher than the range reported by Hassen et al. (2007) for Ethiopian cattle

breeds using RAPD markers amplified by 3 reproducible primers. This is an indication that there is higher heterogeneity among the Zambian indigenous cattle compared to those of the East African cattle. The diversity among the Zambian indigenous cattle is most likely a result of relatively low natural selection undergone by these breeds for generations. For example, the Boran breeds of Kenya and Tanzania, as well as the N'dama and Sahiwal have been reported (Maule, 1990) to have undergone strong artificial selection resulting in less genetic diversity.

CHAPTER 5

SUMMARY, CONCLUSIONS AND FUTURE RECOMMENDATIONS

This thesis research investigated the relatedness and genetic variation among four Zambian indigenous cattle breeds. The first objective was to compare the four breeds based on phenotypic characteristics and the second to determine relatedness among these using randomly amplified polymorphic DNA markers. The 3 morphometric measurements evaluated included body length, heart girth, and height at withers. On comparison of the females, the measurements for the Barotse were higher than the other breeds. Among the Zambian indigenous breeds, the Barotse can be considered to be a large sized breed while the Angoni can be considered to be a small sized breed.

In the second objective of the study, the four Zambian indigenous breeds were analyzed using RAPD markers. Ten primers that were shown to be polymorphic and informative were used in the analysis. The Tonga and Baila were shown to be closely related while the Barotse and Angoni were different.

Specific conclusions from this research are:

1. The Barotse is a relatively large sized breed while the Angoni is a relatively small sized breed. Phenotypic differences among the Zambian indigenous breeds are small.
2. At the molecular level, the Tonga and Baila breeds appear to be closely related and different from Angoni and Barotse.

Some recommendations can be made from the present study. Since the Barotse appears to be the largest of the indigenous breeds, it should be given priority for

conservation. For cost effectiveness, the Baila and Tonga breed should be managed as a single management unit in a conservation program. These two breeds appear, probably due to their geographic closeness, to have been extensively crossed. More research needs to be done on the Zambian native cattle breeds and the choice of breeds for conservation must take into account any available information on productivity traits of economic value, specific adaptive features (tolerance to heat, low quality feeds, disease etc.), presence of unique genes or phenotypes, local or regional importance of a breed in production systems, and the availability of resources and infrastructure in Zambia for animal production.

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Table 1. Summary of analyses of variance in morphometric measurements on Zambian indigenous cattle breeds.

		Mean Squares[†]		
Source of Variation	DF	BL	HG	WH
Breed	3	404.3	557.6*	150.5
Sex	1	257.4	175.7	701.7
Breed x Sex	3	132.0*	22.1	140.5*
Residual	192	31.9	36.8	36.9

[†]BL, HG, and WH are morphometric measurements for body length, heart girth and withers height, respectively and DF is Degrees of freedom.
*Factors with superscript were significant

Table 2. Means ± standard errors of morphometric measurements in Zambian indigenous cattle.

Breed	N		**BL[†] (cm)**		**HG[†] (cm)**		**WH[†](cm)**	
	Male	Female	Male	Female	Male	Female	Male	Female
Angoni	13	37	$127.1 \pm 4.7^{a,1}$	$122.9 \pm 3.8^{c,2}$	$167.3 \pm 7.1^{b,1}$	$163.4 \pm 10.5^{c,1}$	$135.2 \pm 5.3^{b,1}$	$131.4 \pm 5.0^{b,1}$
Baila	8	42	$132.4 \pm 3.8^{a,1}$	$126.8 \pm 2.3^{b,2}$	$171.9 \pm 5.1^{ab,1}$	$169.5 \pm 9.8^{b,1}$	$142.2 \pm 6.6^{a,1}$	$131.5 \pm 2.2^{b,2}$
Barotse	8	42	$136.4 \pm 6.7^{a,1}$	$130.0 \pm 3.0^{a,1}$	$174.8 \pm 3.2^{a,1}$	$174.5 \pm 4.4^{a,1}$	$135.5 \pm 7.3^{ab,1}$	$134.8 \pm 5.0^{ab,1}$
Tonga	13	37	$127.0 \pm 6.1^{a,1}$	$122.8 \pm 3.7^{c,2}$	$171.5 \pm 4.6^{ab,1}$	$168.9 \pm 7.6^{b,1}$	$139.3 \pm 5.2^{ab,1}$	$136.4 \pm 5.2^{a,1}$

[a-c] Means within each column with differing alphabetic superscript are significantly different ($P < 0.05$).

[1,2]Means within each row for each measurement with differing numeric superscript are significantly different ($P < 0.05$).

[†]BL, HG, and WH are body length, heart girth and wither height, respectively.

N represents number of animals.

Table 3. Correlation coefficients among the morphometric measurements in indigenous female Zambian cattle*.

Measurement	Body length	Wither height	Heart girth
Body length			
Wither height	0.62/0.94/0.91/0.92		
Heart girth	0.88/0.89/0.86/0.89	0.78/0.86/0.89/0.86	

*The coefficients for each trait represent Angoni/Baila/Barotse/Tonga cattle breeds.

Table 4. Random oligonucleotide primers used to detect polymorphism, number of bands, and percent polymorphism among Zambian native cattle breeds.

*Primer	Sequence 5' → 3'	Number of bands	% polymorphism	Fragment size (kilobases)
ILO 1127	CCG CGC CGG T	10	40	0.32-0.70
ILO 1204	GAC GGC GCA A	7	57.1	0.30-0.50
ILO 1065	CCG GTC TGG G	6	85.7	0.28-0.55
ILO 525	CGG ACG TCG C	6	85.7	0.25-1.70
ILO 526	GCC GTC CGA G	8	62.5	0.30-0.90
ILO 1212	GCG GCC GTA A	7	71.4	0.25-0.50
OPA 19	CAA ACG TCG G	8	62.5	0.30-1.80
OPN 5	ACT GAA CGC C	7	71.4	0.58-0.80
ZM 10	GCT GCT CGA GT	7	71.4	0.50-1.50
ZM 12	AAC CGC GGT CT	5	40	0.20-0.35
Total		71		
Average per primer		7.1	64.8	

* Polymorphic primers, from 20 tested, used in the analysis of four Zambian indigenous cattle populations. The column for fragment size shows the range of size of fragments amplified by each primer.

Table 5. Chi square significance of differences revealed by random primers in Zambian indigenous cattle breeds.

Primer	Fragment (bp)	P-value
ILO 1127	320	0.27
	420	0.89
	550	0.00*
	680	0.02*
	700	0.35
ILO 1204	300	0.00*
	400	0.02*
	450	0.01*
	500	0.20
OPA 19	300	0.90
	375	0.24
	625	0.94
	1400	0.17
	1700	0.00*
ILO 1065	280	0.94
	500	0.07
	550	0.84
ILO 525	250	0.22
	350	0.00*
	875	0.11
	1750	0.56
ILO 526	300	0.30
	350	0.01*
	500	0.65
	900	0.22
ILO 1212	250	0.11
	350	0.91
	500	0.84
ZM 12	250	0.00*
	350	0.61
	500	0.56
	650	0.05*
ZM 10	500	0.56
	700	0.75
	1000	0.84
	1200	0.87
OPN 5	300	0.01*
	400	0.05*
	500	0.02*
	700	0.84

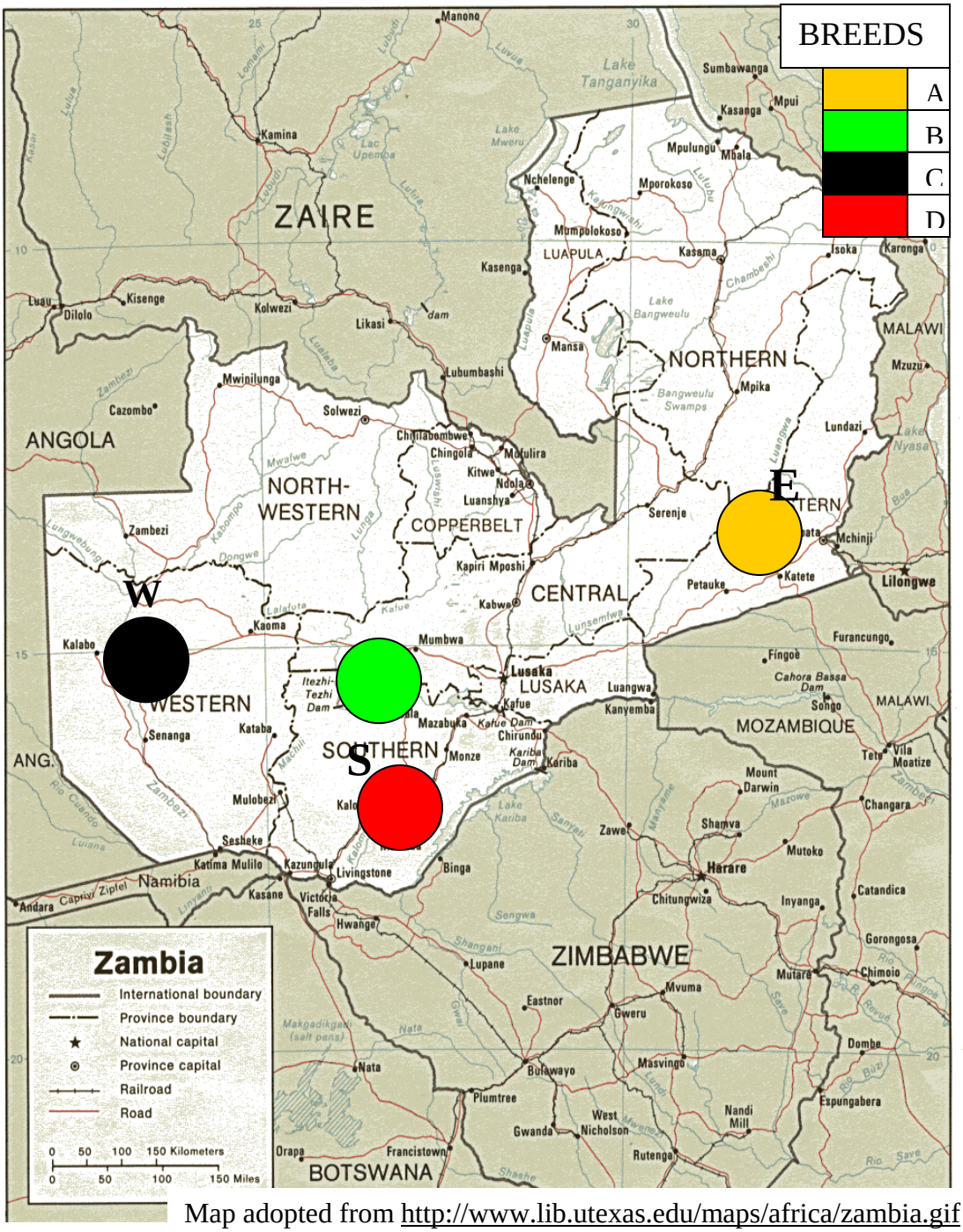
*Number of bands significantly different ($P \leq 0.05$) among four Zambian indigenous and United States Holstein x Jersey crossbred cattle.

Table 6. Average pairwise genetic distances among the indignous Zambian and U.S cattle breeds.

	Barotse	Angoni	Tonga	Baila	U.S
Barotse	0.38 ± 0.11*				
Angoni	0.38 ± 0.09	0.37 ± 0.09*			
Tonga	0.40 ± 0.10	0.39 ± 0.09	0.39 ± 0.09*		
Baila	0.37 ± 0.37	0.37 ± 0.09	0.36 ± 0.09	0.36 ± 0.09*	
†U.S	0.40 ± 0.09	0.40 ± 0.07	0.41 ± 0.13	0.41 ± 0.10	0.29 ± 0.07*

* Represent within breed genetic distance.

†U.S represents Holstein x Jersey crossbred animals from Virginia, U.S (Courtesy of Dr. Bennet Cassell, VT Dairy Science Department).



Map adopted from http://www.lib.utexas.edu/maps/africa/zambia.gif

Figure 1. Sampling sites in Zambia. Where E, W, and S represent eastern province, western province and southern province respectively.
A, B, C and D indicate region where Angoni, Baila, Barotse and Tonga animals were sampled, respectively.

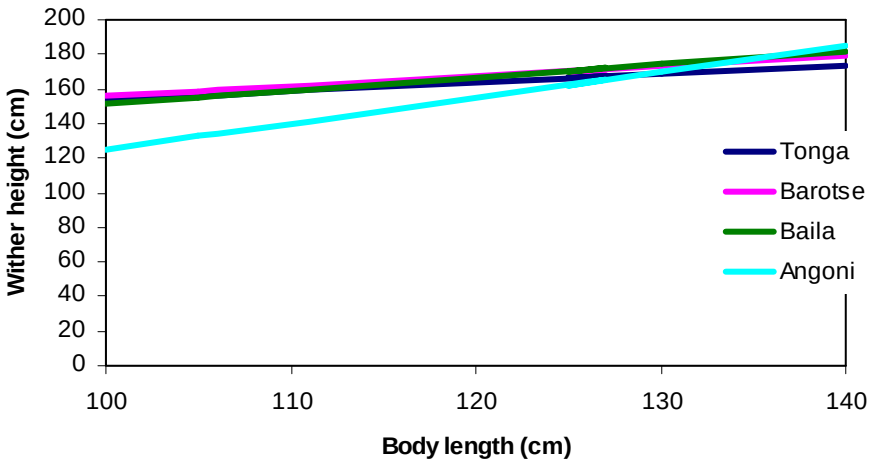


Figure 2. Relationship between body length and wither height within the Zambian indigenous female cattle.

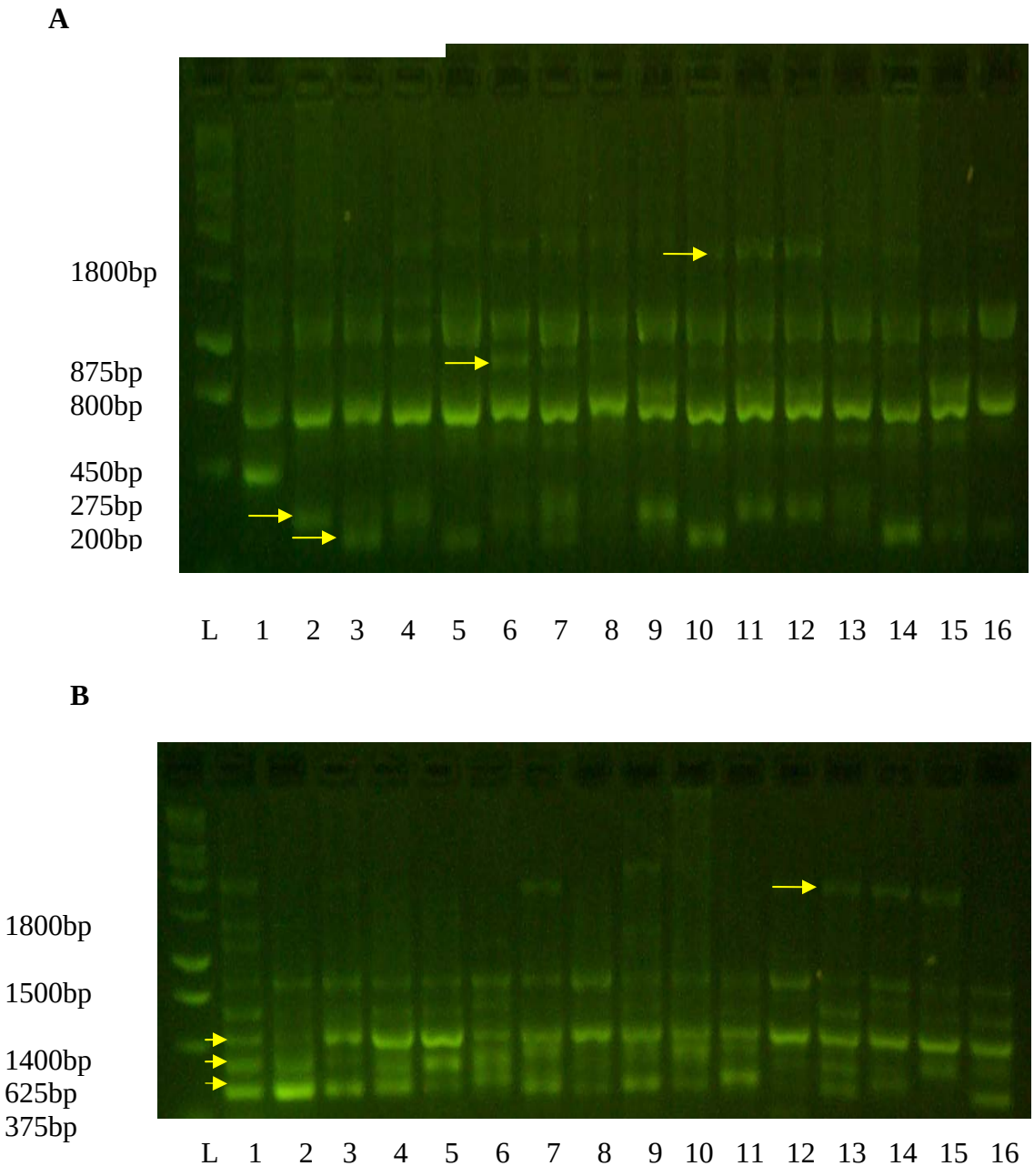


Figure 3. Examples of agarose gel patterns of random primer amplified products.

A. Primer OPA 19; B. Primer ILO 525.

Lanes identified as L represent 1 kb ladder; 1-4, Baila cattle; 5-8 Tonga cattle; 9-12, Barotse cattle; 13-16, Angoni cattle. Arrows indicate polymorphic bands.

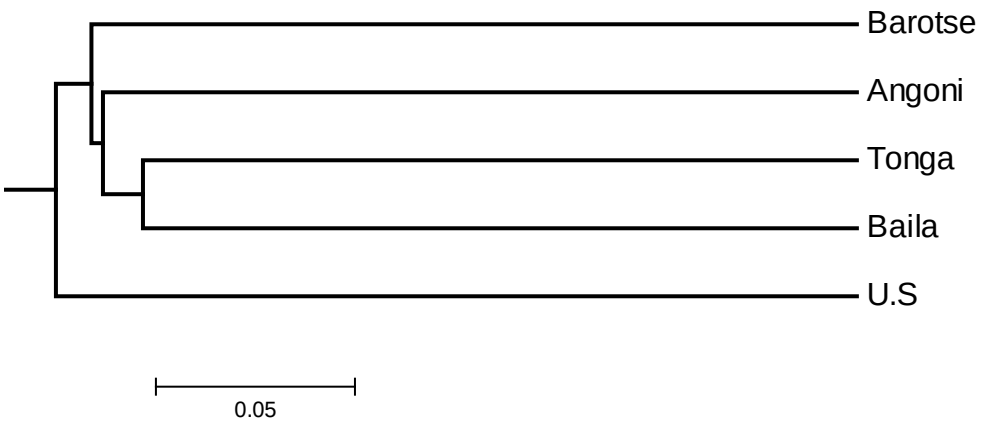


Figure 4. Genetic relatedness among four indigenous Zambian breeds based on the unweighted pair-group method using arithmetic averages (UPGMA).

U.S represents Holstein x Jersey crossbred animals from Virginia, U.S (Courtesy of Dr. Bennett Cassell, VT Dairy Science Department).

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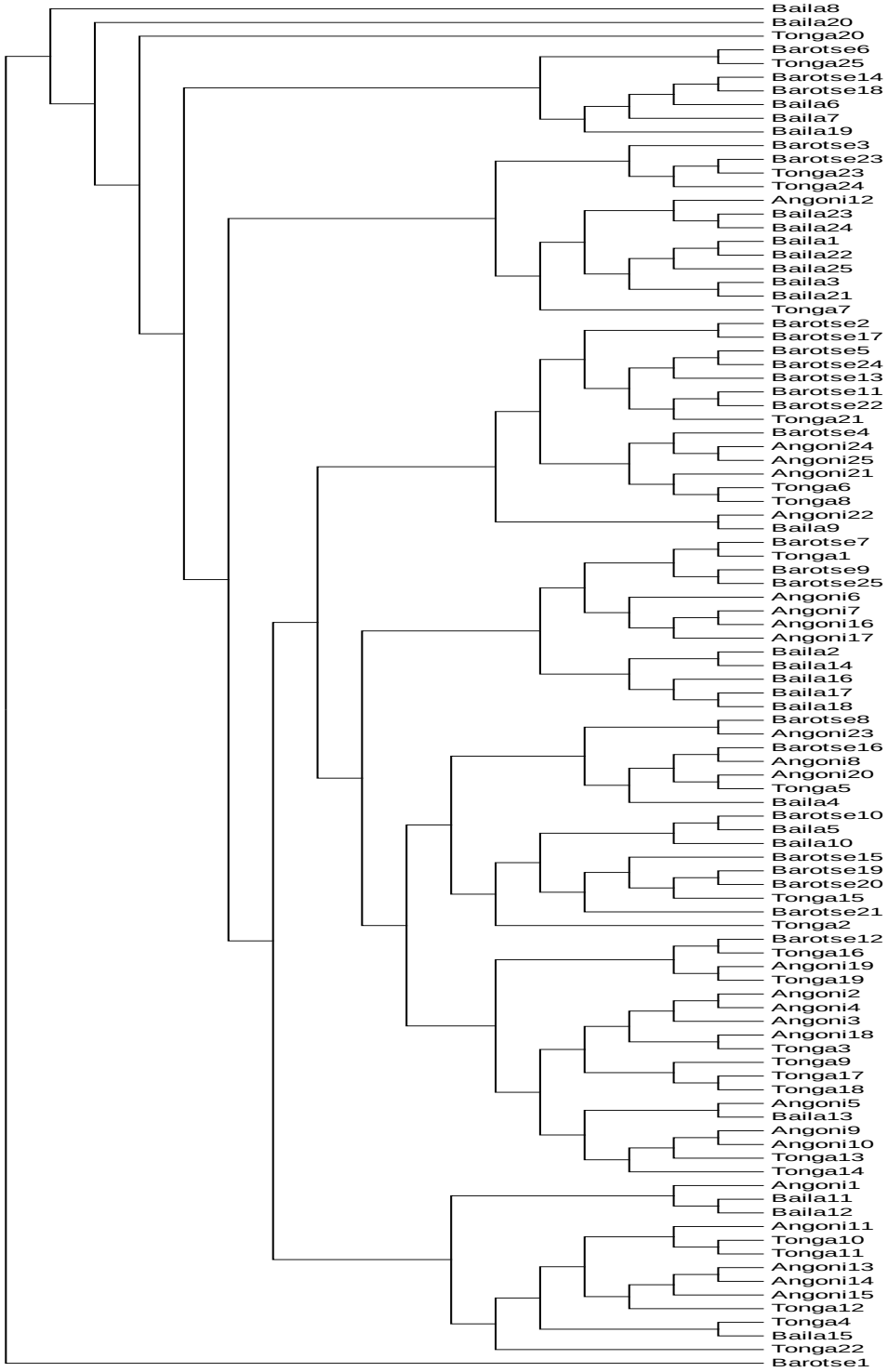

Figure 5. Un-rooted Neighbor-joining tree showing the relationships among animals from four indigenous Zambian cattle breeds based on pairwise genetic distances.