Application of DNA markers in parentage verification of Boran cattle in Kenya

By

David K. Kios (Dr)

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Supervisor: Dr Este Van Marle-Köster

Co-Supervisor: Mrs Carina Visser

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DECLARATION

I, David Kios, do hereby declare that the research presented in this dissertation, was executed by myself, and apart from the normal guidance from my supervisor, I have received no assistance.

Neither the substance, nor part of this dissertation has been submitted in the past, or is to be submitted for a degree at this University or any other University.

This dissertation is presented in partial fulfilment of the requirements for the degree of MSc (Agric) in Animal Production.

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David Kios (Candidate) Dr E. Van Marle-Köster (Supervisor) Mrs Carina Visser

Date……..

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Jeremiah 33:3; Call unto me and I will answer thee and show thee great and mighty things which you know not.

'We cannot change the direction of the wind but can adjust the sails'

ABSTRACT

Boran cattle provide livelihood to thousands of households in the arid and semi arid lands of Kenya. The Kenya Boran cattle breeders' society (KBCBS) is actively involved in the improvement of the breed. Due to their superior adaptive and productive traits in comparison to other indigenous breeds of cattle, they have also become a popular choice for breeders in Eastern and Southern Africa. Their continued genetic improvement through progeny and performance testing is critical and accurate pedigree records are paramount. Pedigree records of four stud herds were evaluated for accuracy using 11 microsatellite markers on 178 samples. The microsatellite markers had a combined probability of exclusion (CPE) of 0.9997. The dam misidentification rate was 0 to 5% and that of the sires ranged from 4.3 to 80% between the four stud herds. 4,456 Boran pedigree records from Kenya stud book for the four participating stud herds were analysed for inbreeding. The average generation interval was 6.8 years and the estimated inbreeding coefficient was unexpectedly low (0.0023), probably due to incomplete records. The high rate of mispaternity will lead to low response to selection and increased inbreeding. The use of DNA markers for parentage assignment will improve the accuracy of the pedigree records. This will enhance the accuracy of selection, increase the rate of genetic gain and improve effective monitoring of inbreeding.

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LIST OF ABBREVIATIONS

ADG	Average daily gain
AI	Artificial insemination
ASAL	Arid and Semi Arid Lands
BLUP	Best linear unbiased prediction
CPE	Combined Probability of Exclusion
DADIS	Domestic Animal Diversity Information System
DAGRIS	Domestic Animal Genetic Resources Information System
DNA	Deoxyribonucleic acid
dd	Dam to daughters
dNTP	Deoxyribonucleotide triphosphate
ds	Dam to sons
EASZ	East African Shorthorned Zebu
EBV	Estimated Breeding Value
EPZA	Export Processing Zones Authority
F1	First filial generation
FABI	Forestry and Agricultural Biotechnology Institute
FAO	Food and Agricultural Organization
GDP	Gross Domestic Product
HEXP	Expected heterozygosity
Hobs	Observed heterozygosity
ILRI	International Livestock Research Institute
ISAG	International Society for Animal Genetics
KBCBS	Kenya Boran Cattle Breeders 'Society
KLBO	Kenya Livestock Breeders' Organization
KMC	Kenya Meat Commission
KSB	Kenya Stud Book
Ksh	Kenya shilling
LOD	Natural Logarithm of Likelihood ratio
mM	Milli molar
MOET	Multiple Ovulation and Embryo Transfer

MOLD	Ministry of Livestock Development
ng	Nanogram
nmol	Nanomole
PCR	Polymerase Chain Reaction
PE	Probability of Exclusion
PIC	Polymorphic information content
pmol	Picomole
RFLP	Restricted Fragment Length Polymorphism
SABCBS	South Africa Boran Cattle Breeders' Society
sd	Sire to daughters
SNP	Single nucleotide polymorphism
ss	Sire to sons
TA	Annealing temperature
TAE	Tris Acetate EDTA
µl	Microlitre

CHAPTER 1: INTRODUCTION

1.1 The Boran cattle in Kenya

Boran cattle are the major livestock breed kept in Kenya primarily for beef production, though some communities utilize them for subsistence milk production and traction. They constitute 35% of the East Africa Short horned Zebu (EASZ) population which forms 99% of the total number of beef cattle in Kenya (Rege &Tawah, 1999; DAD IS, 2010; MOLD, 2010). Boran cattle are kept in the arid and semi arid lands (ASAL) which accounts for over 80% of the land mass and is characterized by low, unreliable and poorly distributed rainfall with inadequate pastures (Mwangi & Omore, 2004; Kabubo-Mariara, 2009). Beef cattle facilitate the use of marginal lands of little use to crop production and only the adapted breeds can survive the environmental stress (Rege & Gibson, 2003). Selected for their ability to survive under harsh and unfavourable climatic conditions, Boran cattle are well adapted to their habitat. Despite their genetic merit, there is lack of adequate attention to characterize and improve these indigenous breeds (Dadi *et al*., 2008).

The Boran breed has unique adaptation traits that make them suitable for production in a harsh environment. They are resistant to ticks, efficient in digesting poor quality forages and when exposed to heat stress, there is minimal effect on feed intake, growth rate, milk production and reproduction with the ability to withstand drought, water shortages and disease (Hansen, 2004; Zander & Drucker, 2008; KBCBS, 2010). Boran cows are well known for their ease of calving which is a positive attribute in ranching where calving supervision is difficult (KBCBS, 2010). This breed has been used in improvement programmes for other indigenous and exotic cattle for both beef and milk production (Wasike *et al*., 2009; KBCBS, 2010) and is the ideal breed for grass fed beef production (SABCBS, 2010).

Boran cattle are preferred to other *Bos indicus* and *Bos taurus* breeds due to their higher reproductive, productive and adaptability performance in similar harsh ASAL conditions (Wasike *et al*., 2009). Boran sired calves in F1 crossing weigh 1.7 to 3.1 kg more at birth

and up to 10 kg more at weaning compared to *Bos taurus* sired calves, but have lower post weaning average daily gain (Lunstra & Cundiff, 2003). Pedigree and performance recording is done on the Kenyan Boran subtype and the *Bos taurus* breeds (DADIS, 2010; KBCBS, 2010). There are a total of 1,841 pedigree, 13,993 pure bred and 18,548 foundation Boran cattle registered with the Kenya stud book (KSB) according to Musyoka (2009, D.M. Musyoka, Pers. Comm., Kenya Stud Book, Nakuru, Kenya, email musyokambai@yahoo.com). The traits measured include regular weighing during growth, calving ease and some adaptive traits (KBCBS, 2010).

1.2 The Livestock sector in Kenya

The livestock sector contributes 10 - 12% of the gross domestic product (GDP), which represents 47% of the Agricultural GDP of Kenya (Mwangi & Omore, 2004; Kabubo-Mariara, 2009). In the ASAL, the livestock sector accounts for 90% of the employment, 95% of family incomes and supports 25% of the total human population in Kenya (Kabubo-Mariara, 2009). The market opportunity for beef and beef products is enormous in Kenya. Breeding stock is in high demand from the neighbouring countries e.g. Tanzania, Uganda, Rwanda and Democratic Republic of Congo (DAGRIS, 2010; KBCBS, 2010; MOLD, 2010). The Kenya meat commission (KMC) is the largest single market for beef cattle with a capacity of 1000 head slaughtered per day and supplies beef to the local market and exports to United Arab Emirates, Kuwait, Qatar, Saudi Arabia, Egypt, Sudan, Tanzania, Uganda and Democratic Republic of Congo (KMC, 2009).

Kenya was exporting over 14,000 metric tons of meat by 1974 to Europe (Jasiorowski, 1979) but due to poor disease control the export licence was withdrawn in 1993. The total beef production currently stands at 362,815 metric tonnes valued at Kenya Shillings 34.4 billion (EPZA, 2005). Kenya has 63 abattoirs, of which 32 are privately owned while 31 are public property owned by the local authorities and together they process 1.7 to 2.9 million head of cattle annually (EPZA, 2005). Hides and skins are exported to Germany, United Kingdom, Netherlands and Italy with a total of 13,910 tonnes exported annually (EPZA, 2005). In 1947 some Boran cattle and embryos were exported from Kenya to Zambia and more recently some were introduced to Australia, Zimbabwe, the United

States, Brazil, Mexico and South Africa (Kios, 2008; KBCBS, 2010; SABCBS, 2010). Exportation to the listed countries was meant to improve beef productivity in the stressful tropical rangelands in both pure breeding and terminal crossbreeding schemes (Cherogony & Kios, 2008; KBCBS, 2010; SABCBS, 2010). This is an emerging market with prospects for expansion and generous earnings for the breeders with pedigree and performance records.

There are concerted efforts by the National Department of Veterinary services to enforce functional disease free zones. Beef trade suffers much due to trade barriers such as tariffs, licences, health and sanitary conditions imposed by importing countries. The legal and regulatory frame work guiding the beef industry includes: The animal disease ACT CAP 364, The Kenya stock traders licensing ACT CAP 498, the hides, skins and leather trade ACT CAP 359 and the stock and produce theft ACT CAP 355 of the laws of Kenya (EPZA, 2005; MOLD, 2010). The market is however opening up and productivity is expected to improve. Global output of the livestock sector particularly in developing countries is expected to double in the next 25 years through improved efficiency contributed by biotechnologies (Cunningham, 1999). The Kenyan livestock sector has the potential to contribute more to the global demand for livestock products.

1.3 Limitations and challenges of genetic improvement of Boran

Most Boran cattle herds in Kenya have developed without the use of herd books and technical interventions (Zander *et al.*, 2008). The majority of the EASZ cattle are not recorded or genetically evaluated due to large variability between farms, farming systems and seasons, low reproductive efficiency and communal grazing systems (Rege, 1994; Rege & Gibson, 2003). The low level of registration of Boran stud herds contributes to low selection intensity resulting in relatively low genetic gain and increased inbreeding levels. The systems for animal recording, data analysis and the use thereof for evaluating genetic merit for growth and fertility require improvement in most of the African countries (AFRA, 2003). The animal records are kept manually at the Kenya stud book and it's almost impossible to obtain complete pedigree and performance records of past generations. The computerization of pedigree data began in 2008 and will aid in the

improvement of breeding of Boran and other cattle breeds in Kenya (KBCBS, 2010; KLBO, 2010; Rewe *et al*., 2010).

Livestock keepers in Kenya have become less food secure over the last 20 years due to high population growth, diminishing land resources, and low livestock productivity. Improving the productivity of their livestock production systems is a crucial poverty alleviation strategy (Scarpa *et al.,* 2003). The annual growth rate of beef cattle declined from 3.3% in the 1980 to 1990 period to -1.6% in the 1990 to 2000 period and meat production stagnated at a growth rate of 2.2% during the two periods (Kabubo – Mariara, 2009). These may have been caused by poor growth rates, inefficient reproductive efficiency and sub optimal selection due to lack of records. Many of these local livestock, though extremely well adapted to the harsh environment, are relatively unproductive (Rege & Gibson, 2003). The harsh conditions of the ASAL have deterred the widespread use of exotic breeds and the local breeds should be improved (Jasiorowski, 1979).

Boran breeders usually practice a closed nucleus breeding system providing their own female replacements and breeding bulls with minimal introduction of animals from commercial herds (Rewe *et al*., 2010). This practice will increase the rate of inbreeding if proper pedigree record keeping and planned mating is not done. There are efforts to develop a breeding programme for Boran cattle with the major obstacle being the inadequate pedigree and performance records (Rewe *et al*., 2006; Wasike *et al*., 2009). The available pedigree records have not been verified for accuracy. Boran breeders select their livestock based on visual appraisal which is highly subjective. Though the accuracy of selection using breeding values can be increased, information from relatives is required. The unavailability of this information in many Boran cattle populations makes the attempt impracticable. It is impossible to trace the sire of the offspring when random mating is done under free grazing systems, unless molecular techniques are used (Abeygunawardena & Dematawewa, 2004). Cow herds reared in groups of 150 – 200 heads are exposed to a bull for ten weeks and allowed to rest for two weeks before introduction of the next bull (Rewe *et al*., 2010). This was meant to aid in identification

of the sire of the offspring but the two week rest period is not sufficient unless pregnancy diagnosis is done prior to introduction of the next bull, which is seldom practiced.

1.4 The aim of the study

In large commercial ranches multiple sire mating strategies are often practised. These beef producers are unable to determine the sire of each progeny and therefore cannot estimate the relative performance of their progeny (Van Eenennaam *et al.*, 2007). In Kenya most ranches have no internal paddocks to separate mating groups and depend on the herders to accurately record the mating and calving at pasture. This is an important limitation, especially for stud breeders. The problem is aggravated by herders that have to record the parentage and only a few have formal education, which leads to potential inaccuracies in the pedigree records. Although records are available for stud Boran cattle, there is no official mechanism in place to determine their accuracy. The accuracy of pedigree records of Boran cattle has therefore not previously been determined and the level of parentage misidentification and inbreeding is unknown in the Boran stud herds registered at KSB.

Boran cattle play an important role in the enhancement of the livelihoods of most livestock keepers in Kenya and increasingly in other parts of the world. Due to the growing importance of the Boran breed, the expensive procedure of embryo transfer and the threat of inbreeding there is an urgent need to increase the accuracy of both pedigree information and performance recording. Accurate pedigrees will have a positive effect on the response to selection and assist to control inbreeding. The genetic improvement of this breed is paramount for continuous sustainability of the superior qualities it's associated with.

The use of DNA testing to confirm parentage for application of estimated breeding values (EBV's) in multiple sire breeding groups have been shown to be of value (Dodds *et al*., 2005b; Van Eenennaam *et al*., 2007; Gomez-Raya *et al.*, 2008). Genetic progress can be achieved using fractional parentage based on a relatively small panel of genetic markers (Dodds *et al*., 2005b). The availability of genetic evaluation methods that allow breeding

value estimation with fractional assignment of parents is likely to increase the use of DNA parentage technology. Marker based pedigree assignment is now a feasible option for commercial beef producers due to the declining cost of genotyping and the possibility of generating accurate EBV's (Dodds *et al*., 2005b; Van Eenennaam *et al.*, 2007).

The main objective of the study was to quantify and validate, using molecular markers, the degree to which errors exist in paternity allocations as presented by pedigree records from four Boran herds in Kenya. The secondary objectives included the selection of the most suitable panel of markers for parentage determination, assessing the level to which the sample population is inbred and the population structure within the Boran cattle, based on the four sampled populations.

The objective was achieved through:

1. Quantification of the errors in the pedigree records of Boran stud herds through the genotyping of four sire families from four different herds using microsatellite markers recommended by the International Society for Animal Genetics (ISAG) (http://www.isag.org.uk) for parentage verification.
2. Identification of the causes contributing to the observed errors.
3. Evaluation of possible mitigation factors to eradicate/minimize the errors.
4. Selection of the most effective combination of microsatellite marker set for use in Boran cattle parentage verification based on allelic frequencies and polymorphism.
5. Determination and evaluation of the population structure and inbreeding trends based on pedigree records of the four Boran stud herds at the Kenya stud book.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

There are approximately 454 beef cattle ranches in Kenya which includes 321 group ranches, 84 private company ranches, 30 government ranches, 17 cooperative ranches and two public ranches (Rewe *et al*., 2008; KBCBS, 2010; MOLD, 2010). 29 of these ranches are members of the Kenya Boran Cattle Breeders' Society (KBCBS). Only 15 of the 29 ranches affiliated to KBCBS register their stud herds with Kenya stud book (KSB). The Boran cattle breeders depend on the relatively small number of registered pedigree Boran stud herds to provide superior sires for their stud and commercial breeding herds. The closed nucleus breeding scheme has been used for many years in stud herds which may lead to an increase in inbreeding with resultant reduction of performance. Both pedigree information and records of performance are important for genetic evaluation and estimation of population characteristics and inbreeding. Though indigenous cattle are not as productive, the harsh environmental conditions of ASAL may not support exotic breeds and improvement of the indigenous types through selection and performance testing remains a practical choice**.** The aim of the review was to present the historical background of Boran cattle and their importance and to give an overview of parentage recording and inbreeding and the consequences thereof.

2.2 Boran cattle

Boran cattle were historically found in Northern Kenya, Southern Ethiopia and Somalia and are now in many other countries. It is believed their ancestors arrived in the horn of Africa about 1,300 to 1,500 years ago from southwest Asia (ILRI, 2006; DAGRIS, 2010; KBCBS, 2010). Large numbers of these animals migrated from the Liben plateau of southern Ethiopia to Somalia and Kenya. In the 1920s, European ranchers in Kenya purchased Boran cattle and through selection developed the improved Boran or Kenyan Boran (ILRI, 2006; KBCBS, 2010). The Kenyan Boran has proved popular in Tanzania, Uganda and the Democratic Republic of Congo (DAGRIS, 2010; KBCBS, 2010).

There are approximately nine million beef cattle in Kenya, 99% of them are local East African Short horned Zebu (EASZ) with Boran cattle being the dominant breed as shown

in Table 2.1. Boran has four ecotypes, namely the Kenyan Boran, the Orma Boran, the Somali Boran and the Ethiopian Boran (Rege &Tawah, 1999; KBCBS, 2010). Lack of genetic characterization hampers the clear classification of the Boran breed and the degree of pureness of the subtypes cannot be defined (Zander & Drucker, 2008). The estimated population of Boran sub types are shown in Table 2.1. Statistical data for the Somali Boran is not available (Rege & Tawah, 1999; DADIS, 2010). The livestock are kept on ranches and communal grazing systems with multiple sires mating schemes and diverse breeding goals (Rege & Tawah, 1999; Scarpa *et al.,* 2003). There is a lack of consistent supply of quality breeding stock for Boran cattle breeders (Rewe *et al*., 2008).

Table 2.1: Breeds / Ecotypes of beef cattle in Kenya (DADIS, 2010; MOLD, 2010)

Breed		**Estimated number**	**Classification**
Aberdeen Angus		1,000	Bos Taurus
Belgium blue		Unknown and few	Bos Taurus
Boran -	Kenyan	580,570	EASZ
	Orma	547,000	EASZ
	Ethiopian	1,896,000	EASZ
	Somali	Unknown	EASZ
Charolais		1,500	Bos Taurus
Galloway		Unknown and few	Bos Taurus
Hereford		1,000	Bos Taurus
Kamasia		346,000	EASZ
Kamba		1,040,000	EASZ
Karapokot		Unknown	EASZ
Masai		1,500,000	EASZ
Nandi		389,000	EASZ
Redpoll		Unknown and few	Bos Taurus
Santa Gerdrudis		Unknown and few	Exotic
Simmental		Unknown and few	Bos Taurus
Turkana		198,000	EASZ
Winam		2,280,000	EASZ

The Kenyan Boran subtype is associated with higher total economic values, e.g. carcass traits, growth traits and reproductive traits compared to the other sub types (Zander e*t al.*, 2008). Boran cows hardly experience calving difficulties, even when carrying crossbred calves sired by larger breeds, such as Charolais (KBCBS, 2010). Their crossbred offspring produce high quality carcasses hence they are preferred by commercial ranchers in Kenya, and increasingly in other parts of the world (DAGRIS, 2010; KBCBS, 2010).

The breed is already popular in Australia, North America, and South Africa where Boran breed societies exist (KBCBS, 2010; SABCBS, 2010). They are docile, highly fertile, and mature earlier compared to the other Bos Indicus breeds (Zander e*t al.*, 2008). It has been shown that Boran cattle exhibit compensatory growth, which enables them to gain weight after losing it during the peak dry season, are very versatile, and adapt well to various environments (ILRI, 2006). The cows are efficient converters of pasture forage into body fat deposits, which are later mobilized during periods of feed scarcity and lactation (Zander & Drucker, 2008). The cows hardly lose condition during lactation and less severe droughts as shown in Figure 2.1.

Figure 2.1 Suckling Boran cows during the dry period in the ranch

2.2.1 Breed standards of Boran

Boran cattle are medium framed. The bulls have short and wide heads with a broad muzzle, prominent eyebrows and small ears (Figure 2.2). They have a short strong neck which is deep and muscular with a well developed dewlap with plenty of loose skin. The

shoulders are deep with a broad brisket and a prominent well developed hump. The back is strong and straight, the hindquarters are broad and full with wide, well fleshed hips. The legs are of medium length, strong and square. The testes mature early and are well developed. The prepuce sheath has firm and good muscle control which protects the prepuce from shrubs, thorns and rough pasture (KBCBS, 2010).

Figure 2.2: Young Boran bulls

The Boran has a loose, thick and extremely pliable skin which acts as an insect repellent. They have dark pigment and fine short hair which is important for heat tolerance (KBCBS, 2010). The height of a mature bull is 117 to 147 cm while that of the cow is 114 to 127 cm measured at the withers. The mature weight of a bull is 500 to 850 Kg and that of the cow is 380 to 550 Kg. The mature weight of steers at 3 to 3.5 years old fed on grass is 380 to 460 Kg, the carcass yield is 194 to 266 Kg and dressing percent is 52 to54% (KBCBS, 2010).

2.2.2 Performance recording of Boran cattle

There are no routine performance evaluation of Boran and other beef cattle in Kenya due to the low number of registered beef cattle and inadequate performance record keeping by the breeders (Rege & Tawah, 1999; Wasike *et al*., 2006). The Livestock recording centre (LRC) is a governmental department tasked with maintenance of databases of animal records and genetic evaluation (Kahi *et al*., 2006; Rewe *et al*., 2008). Political interference over the years led to poor performance and underutilization of the institution. The low human resource output and lack of government priority on animal breeding has a negative impact on genetic evaluation (Kahi *et al*., 2006; Rewe *et al*., 2008). Appropriate breeding policies should be developed to activate the LRC (Rewe *et al*., 2008). The breeding policy is at the parliamentary stage in Kenya and will soon be ready to guide livestock breeding programmes according to Muchemi (2010, K. Muchemi, Pers. Comm., MOLD, Nairobi, Kenya, email muchemikariuki@yahoo.com). The average performance traits of the unimproved Boran subtypes (Orma, Ethiopian and Somali ecotypes) are shown in Table 2.2.

Table 2.2 Average performance measurements of unimproved Boran subtypes (Orma, Ethiopia and Somali ecotypes) (DAD IS, 2010).

Trait	Male	Female
Birth weight (Kg)	25	25
Mature weight (Kg)	400	250
Withers height (cm)	130	121
Milk yield per lactation (Kg)		1200
Lactation length (Days)		170

The performance parameters of the Kenyan Boran (improved Boran in the Ranches) are listed in Table 2.3. Birth weight and weaning weights are traits regularly measured in Kenyan ranches (Rewe *et al*., 2006; Wasike *et al*., 2009).

Table 2.3 Average performance measurements of the Kenyan Boran cattle (improved ecotype) (DAGRIS, 2010; KBCBS, 2010).

Trait	Male	Female	Steers
Age at first heat (months)		15.6	
Age at first service (months)		21.7	
Age at first calving (months)		32.2	
Calving interval (months)		13.8	
Calving rate (%)		89	
Birth weight (Kg)	25	25	
Weaning weight (252 days, Kg)	185	168	
Average Milk production per lactation (Kg)		1130	
A DG (Pasture, g/day)			385-700
A DG (Pasture & Concentrates, g/day)			546-1000
A DG (Hay & Concentrates, g/day)			696-1300
Mature weight (Kg)	500-850	380-550	380-460
Carcass weight (Kg)			194-266
Dressing percent (%)			52-54
Height	117-147	114-127	

ADG: average daily gain, Kg: Kilograms, g: grams

The heritability estimates for weights recorded at different ages of the Kenyan Boran cattle are shown in Table 2.4. These heritability estimates were very low compared with previously reported values for other beef cattle in the literature (Table 2.5). This was probably due to inadequate performance records for the traits reported. The records used to estimate birth weight (1147), 12 month weight (888), 18 month weight (761) and 24 month weight (761) were limited with a high residual error variance estimates (Wasike *et al*., 2009).

Table 2.4 Heritability estimates for weights at different ages, AFC and CI of the Kenyan Boran cattle (Wasike *et al*., 2009)

Trait	BWT	WWT	YWT	18MWT	24MWT	AFC	CI
Direct heritability	0.34	0.12	0.19	0.08	0.14	0.04	0
Maternal heritability		0.14	0.34	0.04	0.11		
Total heritability	0.34	0.14	0.14	0.08	0.11	0.04	0

BWT=birth weight, WWT=weaning weight, YWT=Yearling weight, 18MWT=18 month weight, 24MWT=24 month weight, AFC=age at first calving and CI=calving interval

The heritability estimates for Korean beef cattle were also very low and was probably due to sire misidentification (Lee *et al*., 2000). The lack of adequate pedigree and performance records hinders the genetic improvement of Boran cattle in Kenya.

Table 2.5 Heritability estimates for weights at different ages in beef cattle

Trait	h^2	m^2	h^2t	Breed	Reference
BWT	0.45	0.1		Hereford	Dodenhoff *et al*., 1998
	0.46	0.11	0.54	Hereford	Koch *et al*., 2004
	0.52	0.08		Gelbvieh	Iwaisaki *et al*., 2005
WWT	0.18	0.18		Hereford	Dodenhoff *et al*., 1998
	0.17	0.18	0.19	Hereford	Koch *et al*., 2004
	0.36	0.13		Gelbvieh	Iwaisaki *et al*., 2005
	0.33	0.08		Nellore	Boligon *et al*., 2010
YWT	0.47	0.09		Hereford	Dodenhoff *et al*., 1998
	0.14	0.04		Korea native cattle	Lee *et al*., 2000
	0.42	0.08	0.48	Hereford	Koch *et al*., 2004
	0.59	0.13		Gelbvieh	Iwaisaki *et al*., 2005
	0.37	0		Nellore	Boligon *et al*., 2010
18MWT	0.11	0		Korea native cattle	Lee *et al*., 2000
24MWT	0.27	0		Korea native cattle	Lee *et al*., 2000
	0.36	0.14	0.46	Hereford	Koch *et al*., 2004

BWT: Birth weight, WWT: Weaning weight, YWT: Yearling weight, 18MWT: 18 Month weight, 24MWT: 24 Month weight, h^2: Direct heritability, m^2: Maternal heritability, h^2t: Total heritability

Recording in the beef cattle industry is still in its infancy stage in Kenya with only a few beef cattle breeders participating. On-farm records are kept by a few breeders of *Bos taurus* and Kenyan Boran cattle breeds. Though the benefits of animal recording are well known, the low level of participation can be attributed to the lack of understanding of their importance by the breeders and the low input / low output beef production systems (Rege, 1994). The Boran stud herd breeders only register animals with KSB that have passed breed inspection and this will bias population parameter estimates.

A physical comparison of the body condition of a pure bred Boran and a relatively pure Belgian Blue steers of the same age, raised on the same farm and pasture in the stressful tropical rangeland are shown in Figure 2.3 and 2.4. The Boran steer has a much better body condition compared to the Belgian Blue. The steers were raised in a semi arid land with fluctuating quality of the pastures.

Figure 2.3: Boran steer on pasture (ADC, 2007)

Figure 2.4: Belgian Blue steer on the same pasture as Boran steer on Figure 2.3 (ADC, 2007)

2.2.3 Selection criteria of Boran cattle

Breeding objectives for Boran cattle have evolved over many years to meet the market demands. The development of breeding objectives and selection indices is often generalized for national beef improvement and may not produce similar rates of genetic improvement compared to breeding objectives tailor-made for specific production and marketing sectors (Enns & Nicoll, 2008). The different breeders have different groups of traits that influence the breeding objective with their own emphasis on each trait. Progeny testing, animal recording and use of genetic markers can provide individuality to many breeders (Garrick & Golden, 2009). In most Boran cattle herds, animals that are highly superior in productive and reproductive performance are few and achieving high selection intensity is a difficult task without accurate pedigree and performance records.

Boran producers use scarce feed resources to produce cattle that are in demand, with appropriate levels of return on investment. The traits of economic importance to the Boran breeders include adaptive, reproductive, growth and carcass traits. The adaptive, growth and carcass traits have moderate to high heritability estimates. The reproductive traits have low heritability estimates and this may limit the rate of genetic progress (Burrow, 2001; Nephawe *et al*., 2004; Wasike *et al*., 2009). Poor nutrition, diseases, poor management, climate change and inappropriate policies and methods of evaluation are some non-genetic factors that hamper the improvement of Boran production (Rewe *et al*., 2006; Wasike *et al*., 2006; Kabubo-Mariara, 2009). Selection of Boran cattle is based on visual appraisal, relative growth rate and reproductive success (KBCBS, 2010). Currently no selection is based on genetic evaluations and economic indices but this may soon become a reality with the current efforts of various researchers and breeders (Wasike *et al*., 2009: Rewe *et al*., 2010).

The expected increase in mean performance of a population per generation through genetic selection is proportional to the accuracy with which the breeding value of selection candidates can be estimated (Dekkers & Hospital, 2002). Accurate prediction of breeding value is obtained from large amounts of high quality data with appropriate statistical techniques used in making the genetic predictions (Bourdon, 2000). The beef

industry has overemphasised the evaluation of productive traits with inadequate regard for other economically important traits, such as adaptive, reproduction, animal health, and feed efficiency (Garrick & Golden, 2009).

In the tropics, productivity of beef cattle enterprises depends on the ability of the animals to withstand the environmental stress (Burrow, 2001). The effects of high temperature and humidity, ecto-and endo-parasites, disease incidence and seasonal fluctuations in nutrition in the tropics leads to more sources of (co)variation in growth than in temperate environments (Burrow & Prayaga, 2004). Breeding programmes in beef cattle have not paid much attention to reproductive traits (Yagüe *et al.,* 2009). Lifetime production is an important measure of efficiency of beef production and is influenced by fertility, maternal ability, and survival of the cow and her offspring (Martinez *et al*., 2004a, b). Fertility evaluations are difficult and expensive. Females without offspring may result due to failure of the owner to record the offspring or reproductive failure (Rust & Groeneveld, 2002).

The research has shown that selection for high growth rate in young animals raised in the tropics is effective in increasing birth, weaning, yearling and 18 month weights. It also increased the period weight gains from birth to 18 months but mature cow size were restricted by environmental factors (Burrow & Prayaga, 2004). The maternal component of growth contributes to responses in growth at all ages between birth and 18 months in Boran and other beef cattle reared at pasture in the tropics (Burrow & Prayaga, 2004; Wasike *et al*., 2009). The feed intake and efficiency are economically important traits (Clarke *et al*., 2009) and more so in the ASAL with poor quality pasture during the dry period on the Kenyan ranches. These traits have not been recorded by Boran breeders.

The genetic evaluation for carcass traits relies on progeny testing which is difficult as parentage is often not recorded in the herds, different animal identification systems may be used, the owners of the animals may not be members of the breed association, and animals may change ownership and identification between weaning and slaughter ages

(Pollak, 2005). Though carcass traits are not important currently on the Kenyan market, the changing lifestyles and demand for export may soon influence the perception.

Despite the importance of adaptive traits to Boran breeders, there are no routine recording of the traits. The routine genetic evaluations of reproductive, growth and carcass traits are seldom carried out due to lack of adequate records. To improve meat production in Kenyan ranches, the calving rate must be increased and calving interval reduced. The growth rate and off-take must be improved without much interference with adaptive traits. The productive and reproductive potential of Boran cattle are relatively low compared to *Bos taurus* breeds and worthwhile gains may be achieved by accurate pedigree and performance recording of the relevant traits and selection over many generations (Wasike *et al*., 2009: Rewe *et al*., 2010).

2.3 Parentage verification

Parentage verification is crucial as it forms the basis for accurate pedigrees and genetic evaluations (Israel & Weller, 2000; Banos *et al.,* 2001; Visscher *et al.,* 2002) and becomes more critical with the wide spread use of artificial insemination, embryo transfer and multiple pasture or rotational sire breeding schemes (Senneke *et al.,* 2004; Pollak, 2005; Van Eenennaam *et al*., 2007; Riojas-Valdes *et al.*, 2009). Accurate cattle pedigrees are essential for optimal development of the breed and selection programmes improving productivity in the beef industry (Pollak, 2005; Cervini *et al.*, 2006; Van Eenennaam *et al*., 2007). It's important to use correct pedigree records in order to obtain accurate estimates of heritability and genetic correlations (Geldermann *et al*., 1986; Israel & Weller, 2000; Senneke *et al.,* 2004) which provide the basis for designing optimum breeding strategies (Van Vleck *et al*., 1987; Falconer & Mackay, 1996). Paternity pedigree errors have substantial impact on national genetic evaluations, estimates of inbreeding, genetic trends, sire variance and correlations (Banos *et al*., 2001; Visscher, *et al*., 2002).

Misidentification of parentage can lead to breeding inaccuracy with great financial loss to the beef industry (Pollak, 2005; Cervini *et al.*, 2006; Van Eenennaam *et al*., 2007). The

proportion of misidentified progeny varies between 12.2% in the USA's commercial beef population, 5 and 15% in Denmark, 4 and 23% in Germany, 8 and 20% in Ireland, 12% in Netherlands, 2.9 and 11.7% in Israeli Holstein, 10% in United Kingdom dairy herds and 10.73% in Czech Holstein cattle (Geldermann *et al*., 1986; Israel & Weller, 2000; Banos *et al*., 2001; Visscher *et al*., 2002; Weller *et al*., 2004; Rehout *et al.,* 2006; Van Eenennaam *et al.,* 2007). Pedigree errors are considered to be a common problem in the livestock enterprise and Parentage testing has become a feasible option due to the declining cost of DNA collection and genotyping (Dodds *et al.,* 2007).

2.3.1 Causes of misidentification

The documented causes of parentage misidentification include errors when the bull's herd book number or name is entered into the insemination record. The neighbouring bulls jumping the fence, precocious bull calves or the interchange of calves on the farm. Mistakes may occur in the paternity confirmation laboratory that result in the rejection of paternity caused by genotyping errors, null alleles, mutation, sample switching and omission of sire from sample collection. Other causes include the insemination of cows already pregnant from a previous insemination or the use of natural service bulls leading to pregnancies of previously inseminated cows which were assumed to be pregnant from the A.I service. Mistakes in sire identification may occur when a cow enters the milking herd schemes where pedigree information is obtained through the milk recording. Also mistakes may occur at the Artificial Insemination (A.I) institution in semen labelling or the A.I technicians incorrectly identifying semen straws during insemination (Weller, *et al*., 2004; Van Eenennaam *et al.,* 2007).

2.3.2 Consequences of misidentification

Pedigree errors have a large impact on the efficiency of progeny testing and the accuracy of the predicted breeding values. The errors will reduce the EBV of elite bulls assumed to be sires of inferior daughters and increase the EBV of low ranking bulls (Israel & Weller, 2000). Misidentifications can bias estimates of parameters needed for genetic evaluations of beef cattle such as direct and maternal heritability, direct – maternal correlations leading to decreased genetic gain from selection with a corresponding potential loss of

income (Senneke *et al*., 2004). In a study using commercial ranch records and 625 calves from a multiple sire breeding pasture, Van Eenennaam *et al.* (2007) found a large variability in calf out-put with a large number of young bulls that did not sire any offspring. Five of the 27 herd sires produced over 50% of the calves, 10 sires produced no progeny of which nine were yearlings. Senneke *et al*. (2004) showed in a simulation study that as the fraction of misidentification of progeny increased, the average estimates of direct and maternal heritability decreased almost linearly though the decrease of maternal heritability was not as great as the decrease in direct heritability. Estimates of both additive and maternal variance decreased dramatically.

The impact of paternity errors on genetic gain includes a reduction on the reliability of the bull EBV's because of a lower correlation between the EBV and true breeding value. For milk production the genetic trend for cows and bulls is reduced by 11% and 14% respectively and variance in EBV is reduced by 9% with a sire misidentification rate of 10% (Visscher *et al*., 2002). In a simulation study of a large dairy cattle population undergoing selection, the effect of a 10% paternity misidentification was a reduction of annual rate of response by 3.5 – 4.3% (Israel & Weller, 2000). Visscher *et al.* (2002) predicted a loss in response of 2 – 3% for one round of sire selection using heritability of 0.25 and progeny group size of 100 as a result of reduced reliability. Banos *et al.* (2001) found that a postulated error rate of 11% for sires used in international genetic evaluations decreased genetic trends by 11 – 15%. The 5% misidentification in the Israel Holstein contributed to the loss in annual genetic progress on milk production. The loss of 5 kg per cow per year due to sire misidentification for 100,000 cows is equal to 500,000 kg and this will cost 50,000 US dollars (Ron *et al.,* 1996).

2.3.3 Tools for parentage verification

Blood typing and protein polymorphism have traditionally been used in parentage verification (Geldermann *et al*., 1986; Cunningham, 1999; Weller *et al*., 2004). As early as 1940, blood group antigens were used for parentage verification in cattle and their application for detection of incorrect parentage in the 1950's proved to be a powerful tool with significant implications to the stud breeders (Van Marle-Köster & Nel, 2003). The

number of loci used in blood groups and protein polymorphisms result in relatively low precision and cannot always exclude a putative sire even if it's not the real parent (Visscher *et al*., 2002) and cannot be done retrospectively after the death of the sire. Due to the low polymorphism of the blood groups and milk proteins, the estimates of parentage misidentification were underestimates of the true misidentification rate (Ron *et al*., 1996).

Molecular markers play an important role in genetic studies including breed characterization and parentage verification and are now the tools of choice for parentage verification (Beuzen *et al.*, 2000; Van Marle- Köster & Nel, 2003; Van Eenennaam *et al*., 2007). The initial high cost of DNA parentage verification restricted the use to individuals of high value and situations, where there was doubt about the recorded parentage, for quality assurance diagnostic for particular breeds, for MOET offspring or males chosen for breeding and situations were several males have been found in a supposedly single sire mating group (Dodds *et al*., 2007). The decrease in cost of genotyping has increased the attractiveness of using genetic markers for identification of parents in a genetic improvement programme (Dodds *et al*., 2005b).

Genotyping with microsatellite markers is done using an automated capillary electrophoresis system that can separate, detect and analyze fluorescently labelled DNA fragments. Molecules from the sample are electrophoretically injected into thin, fused-silica capillaries filled with polymer when voltage is applied across the capillaries. The DNA fragments migrate from the negative to positive end of the capillaries with the shorter fragments moving faster. The laser beam at the detection cell excites the dye on the fragments to fluoresce and is captured by charge-coupled device camera which converts the fluorescence information into electronic information (www.AppliedBiosystems.com).

Microsatellite markers have been used widely as a genetic marker of choice in bovine pedigree verification (Van Marle-Köster & Nel., 2003; Bolormaa *et al.*, 2008). Microsatellite markers are used to amplify a specific section of interest on DNA

molecule. Any sample from an individual containing DNA can be used and enables the use of non invasive sampling techniques and retrospective samples from stored tissues or semen (Weller, *et al.,* 2004). Commercial molecular kits are readily available and easy to use with good reproducible results (Verbaarschot, 2007). The International Society for Animal Genetics (ISAG) recommends the use of 12 – 14 microsatellite markers with nine compulsory international markers.

2.4 Evaluation and Control of inbreeding using pedigree information

The effects of inbreeding are well documented. Inbreeding unmasks undesirable lethal or semi-lethal genes which are recessive in nature (Van Vleck *et al.,* 1987) as it reduces heterozygosity and increases homozygosity. Inbreeding depression occurs after certain threshold levels are reached (Alvarez *et al.*, 2005) and leads to the decline in performance particularly of the fitness traits thus reducing farm profitability (Miglior *et al.,* 1992; Mc Parland *et al*., 2007). There are two hypothesis used to explain the genetic basis of inbreeding depression. The partial dominance postulates that inbreeding increases the frequency of homozygotes which leads to inbreeding depression through expression of deleterious recessive alleles. While that of over dominance is based on the fact that the heterozygotes are superior to both homozygotes and a reduction in heterozygotes due to inbreeding will reduce the frequency of expression of the over dominance (Croquet *et al*., 2007).

The use of inbreeding in animal production has been associated with selection to obtain more uniform characteristics in cattle breeds or lines to be used in crossing and for scientific research (Fioretti *et al*., 2002). Reproductive technologies currently available allow for high selection intensity in most livestock species. This in combination with selection methods, such as best linear unbiased prediction (BLUP) has enhanced the response to selection. The rates of inbreeding have also increased and represent a serious concern for several breeding programmes due to the possible consequences of inbreeding depression and reduced genetic variability (Carolino & Gama, 2008b). Inbreeding affects estimates of the accuracy of genetic evaluations and leads to increased overestimation of

reliability under the animal model. This results from accounting for the several contributions that an inbred animal has from the same ancestor (Wiggans *et al.,* 1995).

2.4.1 Consequences of inbreeding

Performance and viability traits decrease at the rate of 1% of the mean for every 1% increase in the inbreeding coefficient. Inbreeding increases the frequency of embryonic death and stillbirths (Nicholas, 2003) and as it progresses there is a decline in fertility, a decrease in progeny survival and a lowering of growth rate and milk production (Malcom *et al.*, 1998; Alvarez, *et al*., 2005; Carolino & Gama, 2008a). There is an increase of between 0.146 - 0.623 days in age at first insemination and between 0.209 - 0.763 days in age at first calving for every 1% increase in inbreeding (Fioretti *et al*., 2002).

Dam and calf inbreeding leads to increased perinatal mortality in beef cattle with incidence ranging from 3.4 – 6.3 % in first parity animals (Adamec *et al*., 2006; Mc Parland *et al*., 2008). The effects of inbreeding are strongly related to dam parity and sex of the calf with the largest effects of 0.417% and 0.252% for dystocia and stillbirths per 1% increase of inbreeding in first parity cows giving birth to male calves (Adamec *et al*., 2006). Losses in reproductive traits due to inbreeding are of economical concern because low fertility and slow growth could lead to increased age at first calving and prolonged generation interval. Genetic homogeneity compromises the quality of embryos in super ovulated cows. There is a decrease in the number of transferable embryos in cows with more than 9% inbreeding coefficient. Some of the undesirable effects of inbreeding are manifested in embryo quality (Alvarez, *et al.*, 2005).

The ease with which a few elite bulls can be used repeatedly in global breeding programs to develop elite breeding stock has led to a rapid increase in inbreeding levels. The negative influence of inbreeding depression may be a contributing factor to the decline in fertility (Funk, 2006). The mating among relatives affects phenotypic performance particularly in young and growing animals. Inbreeding leads to reduction in live weights at birth through to mature weight in tropical beef cattle with increased meat toughness (Burrow, 1998). The estimated decrease in production traits is between 0.04 - 2.07 kg in

live weight of beef cattle at different ages for every 1% increase in inbreeding (Burrow, 1998; Fioretti *et al*., 2002). Inbred animals have decreased carcass weight, less carcass fat and are smaller with poorly developed muscle. The decrease in carcass weight is between 0.87 - 1.90 kg per 1% increase of inbreeding (Mc Parland *et al*., 2008). Research has shown that beef breeds kept in extensive systems, under serious climatic and feed constraints show more pronounced impact of inbreeding depression (Carolino & Gama, 2008a).

The consequences of inbreeding depression in dairy production is a reduction of between 9 to 29.6 kg of milk, 0.55 to 1.09 kg of fat and 0.69 to 0.97 kg of protein per lactation for each 1% increase in inbreeding (Miglior *et al*., 1992; Wiggans *et al*., 1995; Croquet *et al*., 2007). The summit milk yield declines by between 0.06 to 0.12 kg/day per 1% increase in inbreeding, with the average estimates of inbreeding of 3.50% and 4.16% (Cassell *et al*., 2003). There is no documented evidence of inbreeding in Boran cattle, though breeders have observed some the listed effects (KBCBS, 2010).

2.4.2 Evaluation and control of inbreeding

The inbreeding coefficient of an individual is sensitive to the accuracy and completeness of the available pedigree information and absolute inbreeding levels provide less winformation than the average rate of increase per generation (Mc Parland *et al*., 2007). True inbreeding would be affected by sire misidentification (Banos *et al.,* 2001), data structure, base year, estimation procedure and the statistical model (Cassell *et al*., 2003; Adamec *et al*., 2006). The inclusion of unknown parent groups increases the number of animals with low inbreeding as they are assumed to be unrelated (Wiggans *et al*., 1995). The amount of pedigree information from which inbreeding is estimated affects the estimates of inbreeding depression. Partial pedigrees reduce the average inbreeding estimate and the variance of such estimates (Cassell *et al*., 2003). Missing pedigree information of the parent will lead to the inbreeding coefficient to be estimated at zero, though the missing ancestors may not be less related than the general population. The genetic merit of the offspring with missing pedigree records will be overestimated because inbreeding depression in progeny is estimated as zero (Cassell *et al*., 2003).

More complete pedigree information will produce more accurate estimates of inbreeding coefficients and depression, though many other factors i.e. the accuracy of the pedigree and phenotypes, the size of datasets, statistical procedures, and models used will impact such a conclusion. Missing records of the maternal grand dam of an individual in an otherwise complete pedigree would eliminate 39.6% of information available to detect inbreeding, leaving a pedigree 60.4% complete (Cassell *et al*., 2003). Accuracy of selection would improve if evaluations were adjusted to include the inbreeding depression expected. Accounting for inbreeding depression is expected to increase estimates of genetic trends by the amount of inbreeding depression multiplied by the trend in inbreeding (Wiggans *et al*., 1995). Selection and mating programs that directly account for inbreeding will automatically choose and pair less related parents. To maintain fitness in a breed, the Food and Agricultural Organisation has recommended the avoidance of an increase in the rate of inbreeding of more than 1% per generation (Mc Parland *et al*., 2007). It is critical to maintain accurate, complete pedigree records of several generations for ease of estimation and control of inbreeding.

2.5 Conclusion

Breed differences in traits of economic importance serve as a genetic resource for selection and improving beef production efficiency, meat composition and quality as no single breed excels in all traits (Wheeler *et al*., 2004; Bidner *et al*., 2009). These breed differences should be considered in breeding programmes and should provide producers with more information when deciding on sire breeds that will maximize the profit potential in their production system (Wheeler *et al*., 2004; Wheeler *et al*., 2005). Boran sired F1 crosses weigh more at birth through to weaning compared to *Bos taurus* sired calves (Lunstra & Cundiff, 2003; Amen *et al*., 2007). The use of parentage verification may have an important role in enhancing selection especially in multi-sire systems. Accurate pedigrees are an essential component of breeding value estimation. It has been shown in literature that the consequence of parentage misidentification and inbreeding depression justifies the verification of pedigrees.

CHAPTER 3: MATERIALS AND METHODS

3.1 Materials

There are 29 ranches affiliated to Kenya Boran Cattle Breeders' Society (KBCBS). Olpejeta ranch (80,000 acres) and Mutara ranch (60,000 acres) are the largest with 8,000 and 6,000 head of cattle respectively. Other large ranches include Soysambu and Segera ranches (50,000 acres each) with 6,000 and 4,500 head of cattle respectively. Kisima and Lolldaiga Hills have more than 40,000 acres of land with 2,700 and 4,500 head of cattle respectively. The smaller ranches generally are between 3,000 to 40,000 acres with 300 to 4000 head of cattle. Only 15 of the 29 ranches affiliated to KBCBS register their stud herds with Kenya stud book (KSB). KBCBS was established in 1951 with the aim of promoting and standardizing the Boran breed (KBCBS, 2010). The organization has well trained breed inspectors to verify every Boran animal before registration. Only cattle that meet the set Boran standards are approved for registration with the KSB.

KSB is a livestock breeders' organization established in 1920 with the mandate of livestock registration and maintenance of pedigree registers. KSB was initially managed by the Agricultural society of Kenya (ASK), but is now under the management of the Kenya livestock breeders' organization (KLBO), a consortium of all livestock breed societies in Kenya. KLBO was formed in 1994 under the auspices of ASK to manage and guide the activities of KSB and Dairy recording services of Kenya (DRSK).

3.1.1 Blood samples

The four stud herds used in the study are members of the KBCBS and KSB. Three of the herds are kept in the large ranches with more than 50,000 acres of land and at least 4,000 head of cattle. The fourth herd is kept in a smaller farm (less than 10,000 acres) and 300 head of cattle (Table 3.1). The three large ranches are located in the Laikipia district in Rift valley province, central part of Kenya while the smaller farm is in South Nyanza district (near Lake Victoria) in Nyanza province in the western part of Kenya and marked as X and shown by the arrows (Figure 3.1).

Table 3.1 The group, number sampled, herd/ranch size and its location

Group	No Sampled	Herd size	Ranch size	Location
Stud herd I	48	>4000 cattle	>50,000 acres	Laikipia
Stud herd II	41	>4000 cattle	>50,000 acres	Laikipia
Stud herd III	41	>4000 cattle	>50,000 acres	Laikipia
Stud herd IV	48	300 cattle	10,000 acres	Kisumu

The three ranches in Laikipia district have no internal paddocks and depend on the herders to record mating and calving events on pasture. Most of the ranches have no external perimeter fence and cattle may stray from one herd to the next or from one ranch to the next especially under the scare of predators common within the ranches or during

Figure 3.1 The Kenyan map showing districts

watering at common watering points. The cow herds are reared in groups of 150 – 200 heads and are exposed to a bull for ten weeks and allowed to rest for two weeks before introduction of the next bull. This is meant to aid in the identification of the sire of the offspring. The smaller farm in South Nyanza district has both external and internal paddocks. Mating and calving is closely monitored due to the effective separation of the livestock. There are no predators in this part of the country.

The cattle were handled in their normal environment in accordance with the protocols of Veterinary regulation on blood collections in Kenya (Figure 3.2). Permission to use Boran DNA was sought from the University of Pretoria and the KBCBS (Authority letter attached as appendix A). The protocol for using DNA from Kenya was submitted to the Ethics committee on Biohazards in the Faculty of Natural and Agricultural Sciences (Ref. No: EC091005-54).

Figure 3.2 Blood collection at one of the ranches

The authority to import DNA to the Republic of South Africa from Kenya was sought from the Director of Animal Health of the Republic of South Africa and the Director of Veterinary services in Kenya (Authority letters attached as appendix B). 5mls of blood was collected from each of the 178 head of cattle from four sire families with pedigree records, in vacutainer tubes containing EDTA and kept at -20˚ C for further analysis.

3.1.2 Pedigree records

KSB has 34,382 pedigree records of registered Boran cattle from 15 ranches covering the period 1951 to 2007. There are three categories of breeding records: foundation records with minimal information (if any) on both parents and may have no information on the grand parents. This are mainly records of individuals resulting from unrecorded mating and the sire may be missing from the records. Also individuals with registered parents but don't meet the stringent breed standards are registered as foundation. The pedigree and pure bred records have complete details of parentage but differ on the depth of the pedigree information over generations with pedigree records being more elaborate. Pure bred cattle have records of their parents and may have records of the grand parents and the great grand parents. A pedigree cow mated with a pure bred bull will produce a pure bred offspring but a pure bred cow mated to a pedigree bull will result in a pedigree offspring. Pedigree cattle have ancestral records for three or more generations. The progeny of the pedigree and pure bred cattle are subjected to stringent scrutiny by breed inspectors and only those that meet the minimum breed standards are registered and the remainder down-graded to foundation or sent to commercial herds. The culled males are castrated for fattening and all culled animals (bulls and heifers) are not registered or recorded with KSB. Over 50% of bulls are culled annually during inspection and most of the animals registered in KSB are females.

Despite recording from 1951, most of the Boran records are foundation with just a few pedigree records. The records include 1,841 pedigrees, 13,993 pure bred and 18,548 foundation records. 5000 records of the registered Boran cattle in the four research stud herds were collected for estimation of population structure and inbreeding. Boran pedigree records are processed and kept manually by the KSB. The records were for

cattle born between 1972 and 2007. The pedigree records were accessed with the permission of KSB, KBCBS and KLBO. The records were picked from the breeding forms manually, a difficult task but surmountable with patience.

3.2 Methods

3.2.1 DNA Extraction

DNA extraction was performed at the Central Veterinary Laboratories (CVL) in Kabete, Kenya. CVL is a specialised veterinary referral laboratory under the management of the Director of Veterinary services (DVS) whose authority was obtained (see letter in Appendix C). DNA was extracted from 178 blood samples using GFX Genomic Blood DNA Purification kits from Amersham Biosciences (www.amersham.com) according to the manufacturer's protocol for direct method of DNA extraction. The Qiagen, DNeasy Blood and Tissue kit for purification of total DNA from animal blood was also used (www.qiagen.com) according to the manufacturer's protocol. The DNA was kept at -20 ˚C until exported to the University of Pretoria in South Africa according to the conditions specified by the Director of Animal Health of the Republic of South Africa (see letter in appendix B & C).

3.2.2 DNA Quantification

DNA quantification was performed on arrival at the Animal Breeding and Genetics Laboratory of the Department of Animal and Wildlife Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria. The quantification was done using both agarose gel and nanodrop spectrophotometer according to the manufacturer's protocol (NanoDrop Technologies, Inc., *http*://www.nanodrop.com). The 178 DNA samples had concentrations of 50 - 100 ng/µl, adequate for the research.

The agarose gel electrophoresis was done using Hoefer HE 33 Mini Horizontal Submarine Unit© (Amersham Pharmacia Biotech Inc). The technique is used to separate DNA fragments based on the principle that DNA is negatively charged at neutral pH due to the phosphate backbone. When an electrical current is applied on the DNA, it will

move towards the positive pole. A 1% gel was prepared by mixing 0.35 grams of agarose powder with 35ml of 1X TAE buffer. 3µl of Ethidium bromide was added to the mixture ready for use. TAE buffer maintains the pH and salt concentration while agarose forms a porous lattice which the DNA molecule must weave through on its movement towards the positive pole. Larger DNA molecules will be slowed down by the lattice and move slower than small molecules (Sambrook & Russel, 2001). Ethidium bromide binds strongly to DNA by intercalating between the bases and fluoresces when exposed to ultra violet light transmitting energy visible as orange light. The strength of the orange light gives an indication of the concentration of the DNA. The stronger the orange light, the more concentrated the DNA.

The NanoDrop spectrophotometer uses a sample retention system with fibre optic technology. This allows path length to change in real time for a given sample. During each measurement cycle, the sample is assessed at both 1-mm and 0.2-mm path, providing an extensive dynamic range (2 ng/µl to 3,700 ng/µl) (NanoDrop Technologies, Inc., *http*://www.nanodrop.com). 1 µl of DNA was used for each sample measured.

3.2.3 Selection of microsatellite markers

The 12 microsatellite markers used in this research were selected based on the recommendation of the International Society for Animal Genetics (ISAG) (www.isag.org.uk) on microsatellite markers for parentage verification analysis. Nine microsatellite markers i.e. BM1824, BM2113, SPS115, TGLA122, TGLA126, TGLA227, INRA023, ETH10 and ETH225 were recommended by ISAG for routine use in cattle. The ISAG microsatellite markers were selected based on their high heterozygosity and polymorphism, factors important for calculation of exclusion probabilities (Visscher *et al*., 2002; Sherman *et al*., 2004; Riojas-Valdes *et al.*, 2009). They are easy to amplify, score, with appropriate fragment size.

The additional three microsatellite markers, TGLA53, BM1818 and SPS113 were selected based on their high heterozygosity and polymorphic information content, their ease in amplification and fragment size relative to the other nine, as reported in literature.

SPS 113 was excluded from this study due to repeated failure to amplify. In Table 3.2 the sequence, chromosomal location, annealing temperature, fragment length and fluorescent dye of the microsatellite markers are presented. The microsatellite markers were manufactured by Applied Biosystems (www.AppliedBiosystems.com) and labelled with appropriate fluorescent dye as shown in Table 3.2.

The microsatellite markers were optimized for genotyping based on the guidelines of the manufacturer and Sambrook & Russel (2001). Optimization was achieved after testing at various annealing temperatures and magnesium chloride concentration as shown in Table 3.3.

Table 3.2 Primers used for parentage verification of four Boran stud herds

Locus	Primer	Primer sequence 5'-3'	Chrom	TA ˚C	Size	Dye
BM1824	Forward	GAG CAA GGT GTT TTT CCA ATC	1	58	181-195	PET
	Reverse	CAT TCT CCA ACT GCT TCC TTG				
BM2113	Forward	GCT GCC TTC TAC CAA ATA CCC	2	58	125-145	PET
	Reverse	CTT CCT GAG AGA AGC AAC ACC				
TGLA122	Forward	CCC TCC TCC AGG TAA ATC AGC	21	58	134-164	6-FAM
	Reverse	AAT CAC ATG GCA AAT AAG TAC ATA C				
TGLA126	Forward	CTA ATT TAG AAT GAG AGA GGC TTC T	20	60	115-129	VIC
	Reverse	TTG GTC TCT ATT CTC TGA ATA TTC C				
TGLA227	Forward	CGA ATT CCA AAT CTG TTA ATT TGC T	18	60	76-98	6-FAM
	Reverse	ACA GAC AGA AAC TCA ATG AAA GCA				
ETH10	Forward	GTT CAG GAC TGG CCC TGC TAA CA	5	58	206-220	6-FAM
	Reverse	CCT CCA GCC CAC TTT CTC TTC TC				
ETH225	Forward	GAT CAC CTT GCC ACT ATT TCC T	9	54	135-155	VIC
	Reverse	ACA TGA CAG CCA GCT GCT ACT				
TGLA53	Forward	GCT TTC AGA AAT AGT TTG CAT TCA	16	58	150-180	NED
	Reverse	ATC TTC ACA TGA TAT TAC AGC AGA				
SPS115	Forward	AAA GTG ACA CAA CAG CTT CTC CAG	10	56	243-255	VIC
	Reverse	AAC GCG TGT CCT AGT TTG GCT GTG				
INRA023	Forward	GAG TAG AGC TAC AAG ATA AAC TTC	3	58	193-213	NED
	Reverse	TAA CTA CAG GGT GTT AGA TGA ACT C				
BM1818	Forward	AGC TGG GAA TAT AAC CAA AGG	23	56	256-270	NED
	Reverse	AGT GCT TTC AAG GTC CAT GC				

Chrom: Chromosome, TA: Annealing temperature, Size: Size range across breeds

Most microsatellite markers worked well with 0.8 µl of magnesium chloride (25mM) concentration, except TGLA 126 and TGLA227, which required 1.0 µl of magnesium chloride (25mM).

Table 3.3 Optimization of the microsatellite markers for Boran parentage verification

	Annealing temperature (TA) ˚C				
Primer	**52**	**54**	**56**	**58**	**60**
BM1818	0	0	Annealed	0	0
BM1824	0	0	0	Annealed	0
BM2113	0	0	0	Annealed	0
ETH10	0	0	0	Annealed	0
ETH225	0	Annealed	0	0	0
INRA023	0	0	0	Annealed	0
SPS115	0	Smeared	Smeared	Annealed	0
TGLA53	0	0	0	Annealed	0
TGLA122	0	0	0	Annealed	0
TGLA126	Faint bands	Faint bands	0	Faint bands	Annealed
TGLA227	0	Faint bands	0	Faint bands	Annealed

3.2.4 DNA Amplification

DNA amplification was performed using the polymerase chain reaction (GeneAMP® PCR system 9700 thermocycler, Applied Biosystems, Foster City, USA) with the following PCR program: An initial denaturation for 5 minutes at 94˚C, followed by 30 cycles of; denaturation at 94˚C for 30 seconds, annealing temperature for 1 minute and extension at 72˚C for 1 minute, and a final extension at 72˚C for 7 minutes. The reaction solution for PCR was: 2.0 µl PCR buffer (5x GoTaq buffer), 0.8 – 1.0 µl MgCl (25mM), 0.5 µl dNTPs (10nM), 0.4 µl forward primer (10pmol/µl), 0.4 µl reverse primer (10pmol/µl), 0.5 µl GoTaq polymerase (5 U/µl), 4 µl DNA (50 – 100 ng/µl). Deionised water was added to obtain a final solution of 15 µl.

The PCR products were quantified using gel electrophoresis on a 3% agarose gel to ascertain the success of amplification. The same procedure as in quantification was followed and PCR products that amplified poorly were repeated. The repeats in this research were minimal due to the high amplification success rate. The PCR products were then prepared for genotyping by mixing 1 µl of each of the 11 PCR products for each individual. 1 µl of the mixture was mixed with 9 µl of Liz/Formamide (14:1000) solution. 1 µl of this mixture per sample was sent to the Sequencing laboratory, Forestry and Agricultural Biotechnology Institute (FABI), University of Pretoria, for genotyping.

3.2.5 Genotyping

Genotyping was performed with ABIPRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) for DNA sequencing and fragment analysis. The electronic information was transferred to the computer work station for processing using the 3100 Data Collection Software (Applied Biosystems, Foster City, USA) and stored as an electropherogram.

3.2.6 Genotype analysis

The electropherogram created by the Genetic Analyser was analysed with Genemarker software version 1.8 (Softgenetics) (http://www.softgenetics.com). The data of the 178 animals from the Genetic Analyzer was extracted and transferred into the Genemarker. The data was processed by the Genemarker using a microsatellite marker panel designed by the researcher, the Boran microsatellite panel. The panel had 11 microsatellite markers as shown in Table 3.2 and bins were automatically created for each detected peak. The genotypes were verified for consistency, peak sizes and fragment size and stored for further use.

3.2.7 Allelic frequency analysis

Allelic frequencies were estimated for the genotype dataset using Microsatellite toolkit (Park, 2001) and Cervus version 3.0 (Kalinowski *et al*., 2007) software. Microsatellite toolkit contains Microsoft excel visual basic macros and was used to check for errors, duplicates, genetically identical samples, calculate allele frequency and diversity statistics. Cervus analyses genetic data from co-dominant markers such as microsatellites (Marshall *et al*., 1998; Kalinowski *et al*., 2007). The following parameters were calculated namely: the expected and observed heterozygosity, polymorphic information content, average exclusion probabilities, Hardy-Weinberg equilibrium Chi-square statistics and null allele frequency. Deviations from Hardy-Weinberg equilibrium were assessed using the Chi-square goodness-of-fit test and compared the observed genotype frequency with expected genotype frequency. The Chi-square value and the number of degrees of freedom were used to calculate the significance of any deviation from Hardy-Weinberg equilibrium (Marshall *et al*., 1998; Kalinowski *et al*., 2007).

3.2.8 Parentage verification analysis

Parentage assignment and exclusion statistics were performed using Cervus based on likelihood equations that accommodate genotyping errors and increase the number of paternities that can be assigned at 80% (relaxed) and 95% (strict) confidence level (Kalinowski *et al*., 2007). The identification of the most likely father was achieved through the calculation of the natural logarithm of the likelihood-odds (LOD) ratio given the genotypes of the offspring, known mother and candidate fathers (Marshall *et al*., 1998). A positive LOD indicates that a male was more likely to be the father than a male randomly drawn from the population. A negative LOD indicates the male was less likely to be the father than a male randomly drawn from the population (Marshall *et al*., 1998). Once LOD scores were calculated for all males, the male with the highest score was the most likely father. The statistical confidence of this estimate was measured by the difference between LOD scores of the male with the highest score and the male with the second highest score. If the difference was large, the male with the highest LOD score was the actual father (Marshall *et al*., 1998; Kalinowski *et al*., 2007). The LOD score values of three and above were conclusive to confirm paternity, LOD scores of between 3 and -3 were considered inconclusive and further analysis of other potential sires should be considered. LOD score values below -3 indicate rejected paternity (Slate *et al.,* 2000).

3.2.9 Population structure analysis

The 5000 pedigree records were checked for completeness, errors and duplication with dBase plus software (dataBased Intelligence, 2004). dBase plus checks whether sires and dams have been included as individuals and for any error in the entries of pedigree and other records. It was used to check and delete any duplicates, count the number of sires and dams in the dataset and save the out-put files for use in other programs. The pedigree information was ordered and individuals defined before they were used as parents.

The records were analysed to determine the number of breeding males and females, the age structure of parents by birth year of offspring, the distribution of parity of dams at birth of their offspring, the generation interval and family sizes using PopRep 1.0

software (Groeneveld *et al.,* 2009). Poprep is a web based resource for the scientific community maintained by the Institute of Farm Animal Genetics (IFL) and can be accessed through the website http://www.poprep.tzv.fal.de/.

3.2.10 Inbreeding coefficients analysis

The inbreeding coefficient (F) was calculated using Proc Inbreed (SAS 9.2, 2007, SAS Institute Inc, Cary NC, USA) and Animal Breeders Toolkit (ABTK) (Golden *et al*., 1995). The ABTK used data stored as text files (ASCII) under UNIX user utilities through an object oriented approach to solve a variety of equation systems (Golden *et al*., 1995). The INBREED procedure carried out analysis either on the assumption that all the individuals belong to the same generation or by use of non overlapping generations and analyses each generation separately with the assumption that the parents of individuals in the current generation were defined in the previous generation. It also computes the averages of the inbreeding coefficients within and over sex categories. The rate of inbreeding per generation was estimated by use of the INBREED procedure for non overlapping generations within the period 1972 to 2007.

Proc INBREED used the VAR variable statement that defines the individuals name, parent 1 (sire) and parent 2 (dam) and recognizes names up to 12 characters. The CLASS variable identified the generations in a class statement and the GENDER statement specified the variable that indicates the sex of the individuals. The GENDER statement was needed only when the AVERAGE option was specified to average the inbreeding coefficients within sex categories (SAS 9.2, 2007).

CHAPTER 4: RESULTS

4.1. Introduction

178 animals grouped within four Boran sire families were genotyped using 11 microsatellite markers to test the accuracy of pedigree records kept by the breeders. The 11 microsatellite markers were then tested in panels of 11, 10, 9, 8, 7 and six markers based on allelic frequency performance on the four sire families for their suitability in parentage assignment. 5000 pedigree records were analysed for population structure and inbreeding coefficients were calculated. The results of allele frequency and parentage verification analyses, microsatellite marker panel comparisons, population characteristics and inbreeding coefficients are presented.

4.2 Individual marker evaluation

The results of the observed and expected heterozygosity, polymorphic information content, null allele frequency and exclusion probabilities are presented in Tables 4.1 and 4.2 for 11 microsatellite markers tested for their suitability in parentage verification analysis.

Table 4.1 Parameters of microsatellite markers used for parentage verification

Locus	k	n	Hobs	Hexp	PIC	PE-1	PE-2	Null
TGLA122	9	174	0.770	0.758	0.727	0.376	0.557	-0.0061
ETH10	7	174	0.690	0.635	0.609	0.247	0.435	-0.0561
BM1818	7	174	0.707	0.723	0.688	0.328	0.511	0.0057
TGLA126	7	173	0.815	0.793	0.761	0.414	0.593	-0.0196
ETH225	7	173	0.613	0.618	0.593	0.233	0.420	0.0030
TGLA53	13	173	0.867	0.824	0.800	0.481	0.655	-0.0261
BM1824	6	172	0.703	0.730	0.681	0.311	0.485	0.0224
TGLA227	10	172	0.651	0.664	0.613	0.253	0.421	0.0136
BM2113	10	171	0.830	0.812	0.782	0.445	0.622	-0.0134
INRA023	10	165	0.727	0.754	0.715	0.361	0.538	0.0157
SPS115	7	161	0.671	0.658	0.623	0.258	0.440	0.0229
CPE						**0.9901**	**0.9997**	
MEAN	**8.45**			**0.7246**	**0.6901**			

K: Number of alleles, n: Number of samples, Hobs: Observed heterozygosity, Hexp: Expected heterozygosity, PIC: Polymorphic information content, PE-1: Probability of exclusion first parent, PE-2: Probability of exclusion second parent, CPE: Combined probability exclusion and Null: Null allele frequency

The 11 microsatellite markers were highly informative and sufficient for parentage verification analyses which require microsatellite markers with Hexp and PIC of above 0.5 (Marshall *et al.,* 1998; Visscher *et al.,* 2002). Hexp is a measure of variation of each locus and is a useful measure of the informativeness of the locus. The marker with the lowest Hexp, ETH225 (0.618) was above the minimum recommended value for parentage verification. The PIC is a measure of informativeness related to heterozygosity and the higher the value the more informative and suitable for parentage verification. The 11 markers had PIC values of above 0.5 which is adequate for parentage verification analysis of the Boran stud herds. ETH225 had the lowest PIC value of 0.593 while TGLA53 (0.800) had the highest. The CPE-2 of the 11 markers combined as a panel was 0.9997, adequate for parentage assignment (Dodds *et al*., 2005b; Van Eennenaam *et al*., 2007, Bolormaa *et al*., 2008). The results of the comparative amplification success of the 11 microsatellite markers used in the study are given in Table 4.2. Only three of the total of 178 samples used in this study failed to amplify (98.3% success).

Table 4.2 Amplification success of the microsatellite markers used for parentage verification

Locus	Animals genotyped	%	PE-PP	PE-I	PE-SI
TGLA122	174	97.6	0.753	0.911	0.606
ETH10	174	97.6	0.645	0.841	0.527
BM1818	174	97.6	0.709	0.889	0.583
TGLA126	173	97.2	0.777	0.926	0.627
ETH225	173	97.2	0.630	0.829	0.515
TGLA53	173	97.2	0.835	0.947	0.648
BM1824	172	96.6	0.664	0.879	0.584
TGLA227	172	96.6	0.606	0.837	0.540
BM2113	171	96.1	0.800	0.936	0.639
INRA023	165	92.7	0.729	0.902	0.601
SPS115	161	90.4	0.639	0.848	0.540
CPE			0.99999		0.99993

PE-PP: Probability of exclusion parent pair, PE-I: Probability of exclusion identity and PE-SI: Probability of exclusion sib identity

The average amplification success of the 11 microsatellite markers on 178 samples was 95.3%. Six markers had over 97% success rate while nine markers had above 96% success rate. SPS115 (90.4%) and INRA023 (92.7%) had the lowest amplification success rates.

The results for Hardy – Weinberg equilibrium test are presented in Table 4.3. The 11 microsatellite markers used in the study were non significant for the Hardy – Weinberg equilibrium test.

Table 4.3 Hardy - Weinberg equilibrium statistics for the microsatellite markers tested

Locus	Chi-Square	Degrees of freedom	P-Value	Significance
TGLA122	0.3901	3	0.9423	NS
ETH10	4.6771	1	0.0306	NS
BM1818	0.2345	3	0.9719	NS
TGLA126	7.5354	6	0.2742	NS
ETH225	0.0016	1	0.9685	NS
TGLA53	0.0109	1	0.9169	NS
BM1824	11.9627	3	0.0075	NS
TGLA227	1.2242	3	0.7472	NS
BM2113	13.8801	6	0.0310	NS
INRA023	3.9586	3	0.2660	NS
SPS115	1.5878	1	0.2076	NS

NS: Non significant

The test was necessary for parentage verification and a deviation from Hardy – Weinberg equilibrium in a single or two loci may indicate natural or indirect selection acting on a nearby gene or segregating null allele. This will have an impact on the effectiveness of the markers on parentage verification analysis (Marshall *et al*., 1998). Deviation at many loci is an indication of diverse population sub structure i.e. inbred family groups or hybridization and may also be indicative of failure to specify the column number with the first allele in the genotype file during parentage verification analyses (Marshall *et al*., 1998). The 11 markers tested are suitable for parentage verification analysis of the Boran breed as they were in Hardy – Weinberg equilibrium. No heterozygote deficiency was indicated.

4.3 Parentage verification analysis

The results of parentage verification analyses of the four sire families are presented in Tables 4.4 to 4.7. The results of the dam parentage verification analysis for stud herd 1 (Table 4.4) indicate a perfect match for the offspring/dam pair loci and only one pair loci had four mismatches with a negative LOD score.

Table 4.4 Parentage verification analysis results of Boran Stud Herd 1

Offs	L1	Dam	L2	C	M1	LOD score	Sire	L3	C	M1	LOD score	Delta	PC	TL	M2	Trio LOD	Delta	TC
SH2	11	SH42	11	11	0	6.20E+00	SH1	11	11	4	-1.39E+01	0.00E+00		11	5	-1.78E+01	0.00E+00	
SH3	11	SH49	10	10	0	6.36E+00	SH1	11	11	4	-1.59E+01	0.00E+00		11	6	-2.16E+01	0.00E+00	
SH4	11	SH32	11	11	0	7.74E+00	SH1	11	11	5	-1.89E+01	0.00E+00		11	8	-3.09E+01	0.00E+00	
SH5	11	SH39	11	11	0	6.76E+00	SH1	11	11	1	-1.87E+00	0.00E+00		11	4	-1.04E+01	0.00E+00	
SH6	11		0	0	0	0.00E+00	SH1	11	11	3	-5.40E+00	0.00E+00		0	0	0.00E+00	0.00E+00	
SH7	11	SH30	11	11	0	5.26E+00	SH1	11	11	0	**4.09E+00**	**4.09E+00**	*	11	0	**6.68E+00**	**6.68E+00**	*
SH8	11	SH36	11	11	0	3.76E+00	SH1	11	11	5	-1.72E+01	0.00E+00		11	6	-1.95E+01	0.00E+00	
SH9	11		0	0	0	0.00E+00	SH1	11	11	0	**6.09E+00**	**6.09E+00**	*	0	0	**0.00E+00**	**0.00E+00**	
SH10	11	SH48	11	11	0	5.64E+00	SH1	11	11	0	**2.06E+00**	**2.06E+00**	*	11	1	**2.74E+00**	**2.74E+00**	*
SH12	11	SH37	11	11	0	3.34E+00	SH1	11	11	4	-1.50E+01	0.00E+00		11	5	-1.58E+01	0.00E+00	
SH13	11	SH47	11	11	0	2.39E-01	SH1	11	11	3	-9.25E+00	0.00E+00		11	5	-1.50E+01	0.00E+00	
SH14	**10**	**SH46**	**10**	**9**	**4**	**-1.38E+01**	**SH1**	**11**	**10**	**4**	**-1.33E+01**	**0.00E+00**		**10**	**7**	**-1.08E+01**	**0.00E+00**	
SH15	11	SH27	11	11	0	4.63E+00	SH1	11	11	4	-1.51E+01	0.00E+00		11	7	-2.69E+01	0.00E+00	
SH16	11	SH41	9	9	0	4.62E+00	SH1	11	11	6	-2.53E+01	0.00E+00		11	9	-3.49E+01	0.00E+00	
SH17	11	SH40	11	11	0	7.15E+00	SH1	11	11	3	-7.86E+00	0.00E+00		11	3	-5.66E+00	0.00E+00	
SH18	11	SH31	11	11	0	3.21E+00	SH1	11	11	1	**4.27E+00**	**4.27E+00**	*	11	1	**8.33E+00**	**8.33E+00**	*
SH19	11	SH38	11	11	0	7.02E+00	SH1	11	11	1	**2.18E+00**	**2.18E+00**	*	11	4	-1.06E+01	0.00E+00	
SH20	11	SH33	11	11	0	5.36E+00	SH1	11	11	0	**7.91E+00**	**7.91E+00**	*	11	1	**6.60E+00**	**6.60E+00**	*
SH21	11	SH45	10	10	0	8.34E+00	SH1	11	11	4	-1.24E+01	0.00E+00		11	6	-2.01E+01	0.00E+00	
SH22	11	SH34	11	11	0	1.04E+01	SH1	11	11	2	-1.63E+00	0.00E+00		11	2	-5.84E-02	0.00E+00	
SH23	10	SH29	11	10	0	8.71E+00	SH1	11	10	0	**5.98E+00**	**5.98E+00**	*	10	0	**9.99E+00**	**9.99E+00**	*
SH24	11	SH44	11	11	0	4.09E+00	SH1	11	11	3	-9.11E+00	0.00E+00		11	6	-2.08E+01	0.00E+00	
SH25	11	SH28	11	11	0	7.25E+00	SH1	11	11	6	-2.61E+01	0.00E+00		11	8	-3.17E+01	0.00E+00	
SH26	11	SH43	11	11	0	6.59E+00	SH1	11	11	4	-1.56E+01	0.00E+00		11	7	-2.56E+01	0.00E+00	

Offs: Offspring, L1: Offspring loci, L2: Dam loci, C: Loci compared, PC: Pair confidence, TC: Trio confidence, M1: Pair loci mismatching, L3 = Sire loci, TL = Trio loci compared, M2 = Trio loci mismatching, * : 95% confidence

The sire/offspring pair loci comparison results for stud herd 1 indicate five pairs matched while seven had positive LOD scores. Seven and five offspring were assigned paternity with 95% confidence based on pair and trio loci respectively.

The offspring/dam pair loci comparison for stud herd 2 (Table 4.5) matched and only one pair had one mismatch with positive LOD score. One LOD score value was negative although the offspring/dam pair loci had no mismatch. The offspring/sire loci analyses reported six pair LOD scores and five trio LOD scores with positive values, five of which indicated one pair mismatch. One offspring with LOD score of 7.07 and zero Delta value was assigned paternity without confidence while five offspring were assigned paternity at 95% confidence level based on both pair and trio loci.

The maternity test results for stud herd 3 (Table 4.6) indicate most of the offspring/dam pair loci matched and only two had one and three loci mismatch respectively. The offspring/dam pair loci with three mismatches also had a negative LOD score. Most of the offspring/sire pair loci mismatched in more than one locus and only four pairs matched with positive LOD scores. The trio offspring/dam/sire loci mismatched in most trios and only three resulted in positive LOD scores. Four and three offspring were each assigned paternity at 95% confidence level based on pair and trio loci respectively.

The maternity test results for stud herd 4 (Table 4.7) shows all offspring/dam loci matched with positive LOD scores. Most offspring/sire loci matched with positive LOD scores and Delta values and only one pair loci had three mismatches. The pair loci with three mismatches had also a negative LOD score and zero Delta value. Most offspring were assigned paternity at 95% confidence level based on pair and trio loci and only one offspring was not assigned paternity.

Table 4.5 Parentage verification analysis results of the Boran Stud Herd 2

Offs	L1	Dam	L2	C	M1	LOD score	Sire	L3	C	M1	LOD score	Delta	PC	TL	M2	Trio LOD	Trio Delta	TC
SH53	11	SH73	11	11	0	9.85E+00	SH52	11	11	0	**7.80E+00**	**7.80E+00**	*	11	1	**3.59E+00**	**3.59E+00**	*
SH54	10	SH74	11	10	0	5.20E+00	SH52	11	10	3	-8.29E+00	0.00E+00		10	4	-1.29E+01	0.00E+00	
SH55	11	SH75	11	11	0	6.19E+00	SH52	11	11	1	**1.91E-01**	**1.91E-01**	*	11	5	-1.64E+01	0.00E+00	
SH56	11	SH76	11	11	0	5.90E+00	SH52	11	11	4	-1.20E+01	0.00E+00		11	5	-1.58E+01	0.00E+00	
SH57	11	SH77	11	11	0	3.22E+00	SH52	11	11	1	**5.67E+00**	**5.67E+00**	*	11	1	**8.94E+00**	**8.94E+00**	*
SH58	11	SH78	10	10	0	5.97E+00	SH52	11	11	6	-2.12E+01	0.00E+00		11	6	-2.33E+01	0.00E+00	
SH59	11	SH79	10	10	0	9.34E+00	SH52	11	11	1	**1.91E-02**	**1.91E-02**	*	11	1	**3.65E+00**	**3.65E+00**	*
SH60	11	SH80	11	11	0	6.43E+00	SH52	11	11	3	-7.37E+00	0.00E+00		11	4	-1.10E+01	0.00E+00	
SH61	10	SH81	10	9	0	**-4.16E-01**	SH52	11	10	2	-7.82E+00	0.00E+00		10	5	-1.39E+01	0.00E+00	
SH62	11	SH82	11	11	0	6.04E+00	SH52	11	11	1	**7.07E-01**	**0.00E+00**		11	1	**3.87E+00**	**3.87E+00**	*
SH63	11	SH83	11	11	1	2.26E+00	SH52	11	11	1	**2.68E+00**	**2.68E+00**	*	11	3	**6.81E-01**	**6.81E-01**	*
SH64	11	SH84	8	8	0	4.75E+00	SH52	11	11	3	-8.03E+00	0.00E+00		11	3	-8.17E+00	0.00E+00	
SH65	11	SH85	11	11	0	7.55E+00	SH52	11	11	3	-9.60E+00	0.00E+00		11	6	-2.09E+01	0.00E+00	
SH66	11	SH86	11	11	0	6.59E+00	SH52	11	11	4	-1.10E+01	0.00E+00		11	5	-1.55E+01	0.00E+00	
SH67	11	SH87	11	11	0	1.22E+01	SH52	11	11	4	-1.51E+01	0.00E+00		11	5	-1.72E+01	0.00E+00	
SH68	11	SH88	11	11	0	5.34E+00	SH52	11	11	3	-1.49E+01	0.00E+00		11	6	-2.15E+01	0.00E+00	
SH69	11	SH89	10	10	0	9.15E+00	SH52	11	11	2	-5.24E+00	0.00E+00		11	4	-1.09E+01	0.00E+00	
SH70	11	SH90	11	11	0	9.57E+00	SH52	11	11	4	-1.17E+01	0.00E+00		11	5	-1.50E+01	0.00E+00	
SH71	9	SH91	10	8	0	2.00E+00	SH52	11	9	5	-1.97E+01	0.00E+00		9	6	-2.22E+01	0.00E+00	
SH72	11	SH92	10	10	0	4.24E+00	SH52	11	11	2	-7.89E+00	0.00E+00		11	8	-2.80E+01	0.00E+00	

Offs: Offspring, L1: Offspring loci, L2: Dam loci, M1: Pair loci mismatching, C: Loci compared, L3: Sire loci, TL: Trio loci,
M2: Trio loci mismatching, PC: Pair confidence, TC: Trio confidence, * : 95% confidence

Table 4.6 Parentage verification analysis results of the Boran Stud Herd 3

Offs	L1	Dam	L2	C	M1	LOD score	Sire	L3	C	M1	LOD score	Delta	PC	TL	M2	Trio LOD	Delta	TC
SH94	11	SH114	11	11	0	7.17E+00	SH93	11	11	3	-1.26E+01	0.00E+00		11	5	-1.82E+01	0.00E+00	
SH95	11	SH115	11	11	0	4.61E+00	SH93	11	11	2	-6.82E+00	0.00E+00		11	6	-1.97E+01	0.00E+00	
SH96	11	SH116	10	10	0	6.59E+00	SH93	11	11	4	-1.65E+01	0.00E+00		11	7	-2.63E+01	0.00E+00	
SH97	11	SH117	11	11	0	8.28E+00	SH93	11	11	5	-1.95E+01	0.00E+00		11	6	-2.32E+01	0.00E+00	
SH98	11	SH118	11	11	0	7.56E+00	SH93	11	11	3	-1.24E+01	0.00E+00		11	6	-2.18E+01	0.00E+00	
SH99	**11**	**SH119**	**8**	**8**	**3**	**-1.25E+01**	**SH93**	**11**	**11**	**6**	**-2.45E+01**	**0.00E+00**		**11**	**8**	**-1.99E+01**	**0.00E+00**	
SH100	11	SH120	11	11	0	8.88E+00	SH93	11	11	3	-1.14E+01	0.00E+00		11	7	-2.54E+01	0.00E+00	
SH101	11	SH121	10	10	0	5.67E+00	SH93	11	11	3	-1.18E+01	0.00E+00		11	7	-2.55E+01	0.00E+00	
SH102	11	SH122	11	11	0	5.13E+00	SH93	11	11	0	**4.94E+00**	**4.94E+00**	*	11	0	**9.30E+00**	**9.30E+00**	*
SH103	11	SH123	11	11	0	3.01E+00	SH93	11	11	1	-6.12E+00	0.00E+00		11	6	-1.96E+01	0.00E+00	
SH104	11	SH124	11	11	0	6.55E+00	SH93	11	11	2	-7.41E+00	0.00E+00		11	5	-1.72E+01	0.00E+00	
SH105	11	SH125	11	11	0	7.60E+00	SH93	11	11	4	-1.55E+01	0.00E+00		11	6	-2.23E+01	0.00E+00	
SH106	11	SH126	11	11	0	8.56E+00	SH93	11	11	4	-1.60E+01	0.00E+00		11	7	-2.55E+01	0.00E+00	
SH107	11	SH127	10	10	0	5.03E+00	SH93	11	11	0	**1.90E+00**	**1.90E+00**	*	11	5	-1.60E+01	0.00E+00	
SH108	11	SH128	10	10	0	6.59E+00	SH93	11	11	0	**4.05E+00**	**4.05E+00**	*	11	0	**7.76E+00**	**7.76E+00**	*
SH109	11	SH129	10	10	0	6.66E+00	SH93	11	11	3	-1.18E+01	0.00E+00		11	6	-2.06E+01	0.00E+00	
SH110	11	SH130	10	10	0	8.76E+00	SH93	11	11	4	-1.69E+01	0.00E+00		11	7	-2.59E+01	0.00E+00	
SH111	11	SH131	11	11	0	1.11E+01	SH93	11	11	4	-1.25E+01	0.00E+00		11	6	-2.14E+01	0.00E+00	
SH112	11	SH132	11	11	0	1.02E+01	SH93	11	11	0	**3.55E+00**	**2.87E+00**	*	11	0	**7.15E+00**	**7.15E+00**	*
SH113	10	SH133	11	10	1	7.11E-01	SH93	11	10	3	-9.66E+00	0.00E+00		10	4	-1.09E+01	0.00E+00	

Offs: Offspring, L1: Offspring loci, L2: Dam loci, C: Loci compared, PC: Pair confidence, TC: Trio confidence, M1: Pair loci mismatching, L3: Sire loci, M2: Trio loci mismatching, TL: Trio loci, * : 95% confidence

Table 4.7 Parentage verification analysis results of the Boran Stud Herd 4

Offs	L1	Dam	L2	C	M1	LOD score	Sire	L3	C	M1	LOD score	Delta	PC	TL	M2	Trio LOD	Delta	TC
SH158	10	SH183	9	8	0	3.43E+00	SH157	11	10	0	4.00E+00	4.00E+00	*	10	1	3.34E+00	3.34E+00	*
SH159	11	SH184	10	10	0	4.19E+00	SH157	11	11	0	6.67E+00	6.67E+00	*	11	0	1.11E+01	1.11E+01	*
SH160	11	SH185	10	10	0	6.72E+00	SH157	11	11	0	4.02E+00	4.02E+00	*	11	0	7.36E+00	7.36E+00	*
SH161	11	SH186	9	9	0	1.36E+00	SH157	11	11	0	4.35E+00	4.35E+00	*	11	1	3.12E+00	3.12E+00	*
SH162	10	SH187	10	9	0	4.11E+00	SH157	11	10	0	3.40E+00	3.40E+00	*	10	0	6.43E+00	6.43E+00	*
SH164	11	SH189	10	10	0	9.14E+00	SH157	11	11	0	5.66E+00	5.66E+00	*	11	0	8.80E+00	8.80E+00	*
SH166	11	SH191	10	10	0	9.03E+00	SH157	11	11	0	7.88E+00	7.88E+00	*	11	0	8.19E+00	8.19E+00	*
SH167	11	SH192	10	10	0	8.23E+00	SH157	11	11	0	7.27E+00	7.27E+00	*	11	0	1.03E+01	1.03E+01	*
SH168	11	SH193	11	11	0	5.06E+00	SH157	11	11	0	5.66E+00	5.66E+00	*	11	0	9.97E+00	9.97E+00	*
SH169	11	SH194	11	11	0	4.82E+00	SH157	11	11	0	5.83E+00	5.83E+00	*	11	0	9.95E+00	9.95E+00	*
SH170	11	SH195	11	11	0	5.41E+00	SH157	11	11	0	5.56E+00	5.56E+00	*	11	0	9.14E+00	9.14E+00	*
SH171	11	SH196	11	11	0	4.60E+00	SH157	11	11	0	4.52E+00	4.52E+00	*	11	0	6.98E+00	6.98E+00	*
SH172	11	SH197	11	11	0	6.21E+00	SH157	11	11	0	5.41E+00	5.41E+00	*	11	0	9.24E+00	9.24E+00	*
SH173	11	SH198	11	11	0	2.76E+00	SH157	11	11	0	6.38E+00	6.38E+00	*	11	1	5.50E+00	5.50E+00	*
SH174	11	SH199	11	11	0	5.84E+00	SH157	11	11	0	6.99E+00	6.99E+00	*	11	0	7.59E+00	7.59E+00	*
SH175	11	SH200	11	11	0	3.68E+00	SH157	11	11	0	6.08E+00	6.08E+00	*	11	0	8.76E+00	8.76E+00	*
SH176	11	SH201	11	11	0	8.67E+00	SH157	11	11	0	5.77E+00	5.77E+00	*	11	0	7.64E+00	7.64E+00	*
SH177	10	SH202	11	10	0	4.60E+00	SH157	11	10	0	3.16E+00	3.16E+00	*	10	0	6.62E+00	6.62E+00	*
SH178	11	SH203	11	11	0	9.39E+00	SH157	11	11	0	5.15E+00	5.15E+00	*	11	0	6.29E+00	6.29E+00	*
SH179	11	SH204	11	11	0	9.18E+00	SH157	11	11	3	**-9.52E+00**	**0.00E+00**		11	4	**-1.32E+01**	**0.00E+00**	
SH180	11		0	0	0	0.00E+00	SH157	11	11	0	7.05E+00	7.05E+00	*	0	0	**0.00E+00**	**0.00E+00**	
SH181	11	SH206	11	11	0	5.09E+00	SH157	11	11	0	1.69E+00	1.69E+00	*	11	0	5.88E+00	5.88E+00	*
SH182	11	SH207	11	11	0	6.35E+00	SH157	11	11	0	5.74E+00	5.74E+00	*	11	1	4.13E+00	4.13E+00	*

Offs: Offspring, L1: Offspring loci, L2: Dam loci, C: Loci compared, PC: Pair confidence, TC: Trio confidence, M1: Pair loci mismatching, L3: Sire loci, TL: Trio loci, M2: Trio loci mismatching, * : 95% confidence

4.4 Microsatellite marker panel comparison

Six panels of microsatellite markers were selected based on their Hobs, Hexp and PIC as shown in Table 4.8. The panels were compiled by sequentially excluding the worst performing marker based on Hobs, Hexp and PIC until a 6-marker panel was obtained. ETH225 had the lowest Hobs, Hexp and PIC values and TGLA53 had the highest.

Table 4.8 Composition of the six microsatellite marker panels

Markers	Panel					
	1	2	3	4	5	6
TGLA122	√	√	√	√	√	√
BM2113	√	√	√	√	√	√
INRA023	√	√	√	√	√	√
TGLA53	√	√	√	√	√	√
BM1818	√	√	√	√	√	√
TGLA126	√	√	√	√	√	√
BM1824	√	√	√	√	√	
SPS115	√	√	√	√		
TGLA227	√	√	√			
ETH10	√	√				
ETH225	√					

The parameters of the six microsatellite marker panels that may be used for routine parentage verification in Boran cattle are presented in Table 4.9 showing the mean number of alleles, expected heterozygosity and polymorphic information content. The results also show the combined probability of exclusion of first parent (CPE-1) and both parents (CPE -2). CPE-1 and CPE-2 values declined with every reduction in the number of markers and this was more pronounced after the third panel.

Table 4.9 Comparative results of six microsatellite marker panels

Panel	k	Hexp	PIC	CPE-1	CPE-2
1 (11 microsatellite markers)	8.45	0.724	0.690	0.990	0.9997
2 (10 microsatellite markers)	8.60	0.735	0.699	0.987	0.9995
3 (9 microsatellite markers)	8.78	0.746	0.709	0.982	0.9992
4 (8 microsatellite markers)	8.63	0.756	0.722	0.976	0.9985
5 (7 microsatellite markers)	8.86	0.770	0.736	0.968	0.9973
6 (6 microsatellite markers)	9.33	0.777	0.745	0.954	0.9947

Hexp: Expected heterozygosity, PIC: Polymorphic information content, CPE -1: Combined probability exclusion of first parent, CPE-2: Combined probability exclusion of second parent, k: Number of alleles

The comparison of CPE-1 and CPE-2 for the six panels is presented in Figure 4.1 and shows a downward trend as the number of markers in the panels decrease.

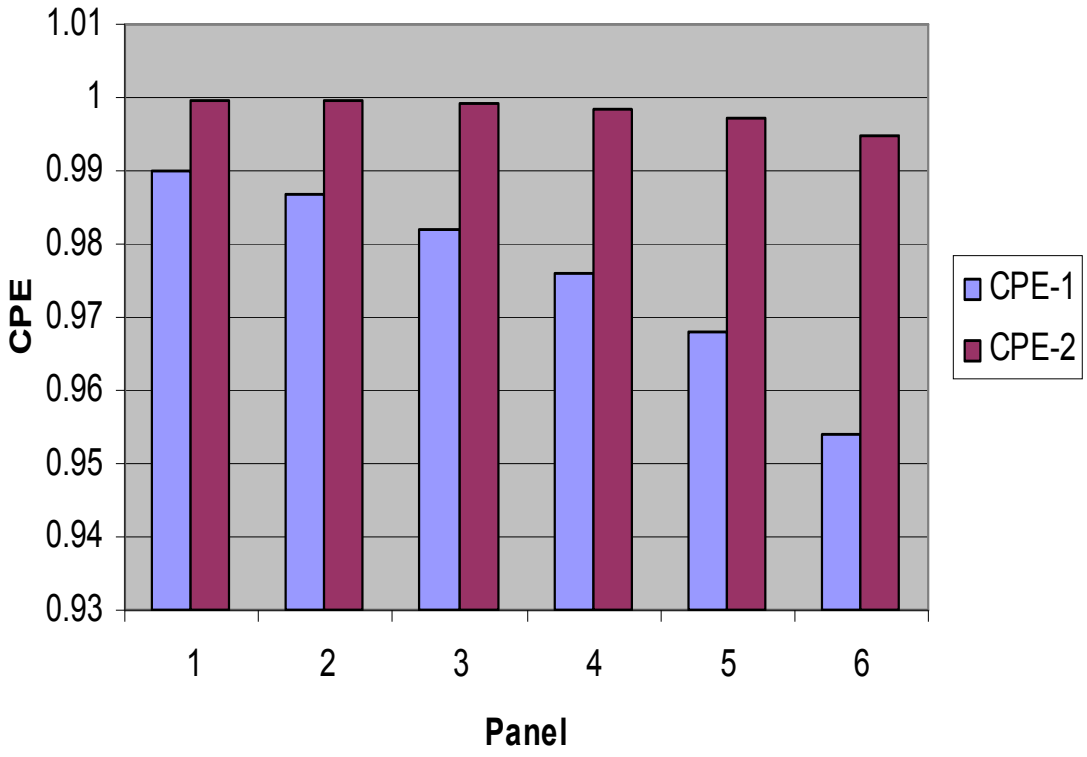

Figure 4.1 Comparison of the CPE-1 and CPE-2 for the six panels.

Panels 1 to 3 had CPE-2 of above 0.999 while panels 4 to 6 tend to be slightly lower with a CPE-2 of less than 0.999. The allelic frequency analysis and exclusion probabilities of the six panels indicate that panels 1 to 3 may be more effective for application in parentage verification compared to panels 4 to 6.

The results of the six panels tested in the four stud herds (Table 4.10) indicate that panel 1 and 2 with 11 and 10 microsatellite markers respectively had similar success rate though panel 2 had slightly lower LOD score values (see appendix D). Panels 1 to 3 assigned paternity only at 95% (strict) confidence level while Panels 4 to 6 assigned paternities at both 95% (strict) and 80% (relaxed) confidence levels.

Table 4.10 Comparison of sire assignment using six microsatellite marker panels

	Assigned at 95%				Assigned at 80%				Not Assigned				Total offspring			
Panel	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**	**S1**	**S2**	**S3**	**S4**
1	7	5	4	22	0	0	0	0	17	15	16	1	24	20	20	23
2	7	5	4	22	0	0	0	0	17	15	16	1	24	20	20	23
3	8	4	4	22	0	0	0	0	16	16	16	1	24	20	20	23
4	8	4	4	22	0	1	0	0	16	15	16	1	24	20	20	23
5	7	4	5	21	1	0	2	1	16	16	13	1	24	20	20	23
6	6	4	3	18	2	2	4	4	16	14	13	1	24	20	20	23

S1: Stud herd 1, S2: Stud herd 2, S3: Stud herd 3, S4: Stud herd 4, Assignment at 95% and 80% confidence level.

Detailed parentage assignment using the six microsatellite marker panels in the four sire Boran studs herds are attached in appendix D.

4.5 Boran population structure and inbreeding

4,456 animals from the four stud herds used in the study were analysed after exclusion of 544 animals due to duplication, inconsistency in birth dates and sex. The results of the population structure analysis showing the number of males and females in reproduction by the year of birth of their offspring are presented in Figure 4.2 for the period 1981 - 2007.

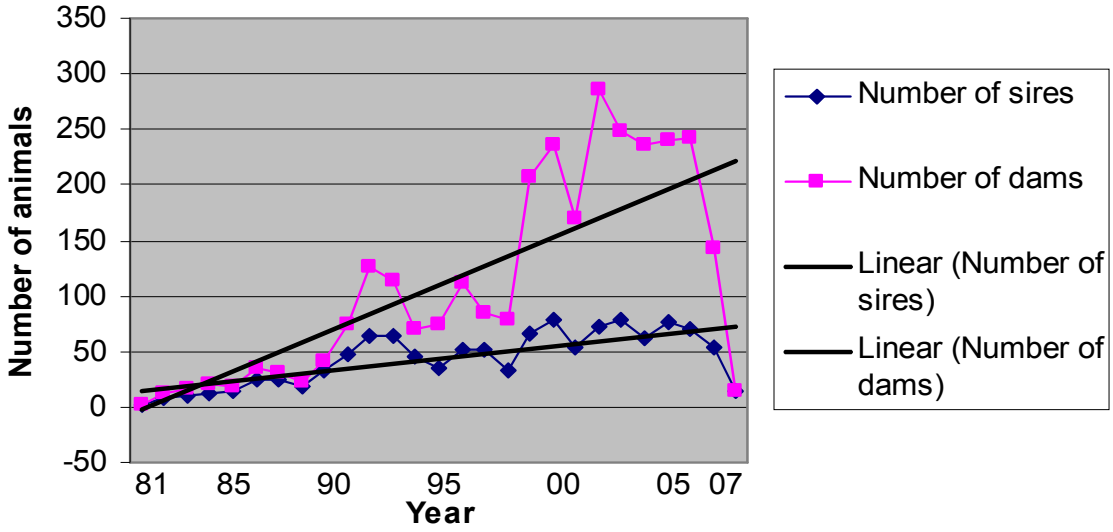

Figure 4.2 Number of males and females in reproduction by the year of birth of their offspring.

The trend shows an increase in the total number of animals in the four herds. The sires had a slight increase in numbers over the years and the females had a high increase between 1995 and 2000 and remained constant up to 2006 before a decrease in 2007. The sires also decreased after 2006. The results of age distribution analysis are shown in Figure 4.3 showing the age of the sires and dams at birth of their offspring. Most of the sires had their offspring between the ages of 5 to 8 years while the dams had more of their offspring between the ages of 4 to 8 years.

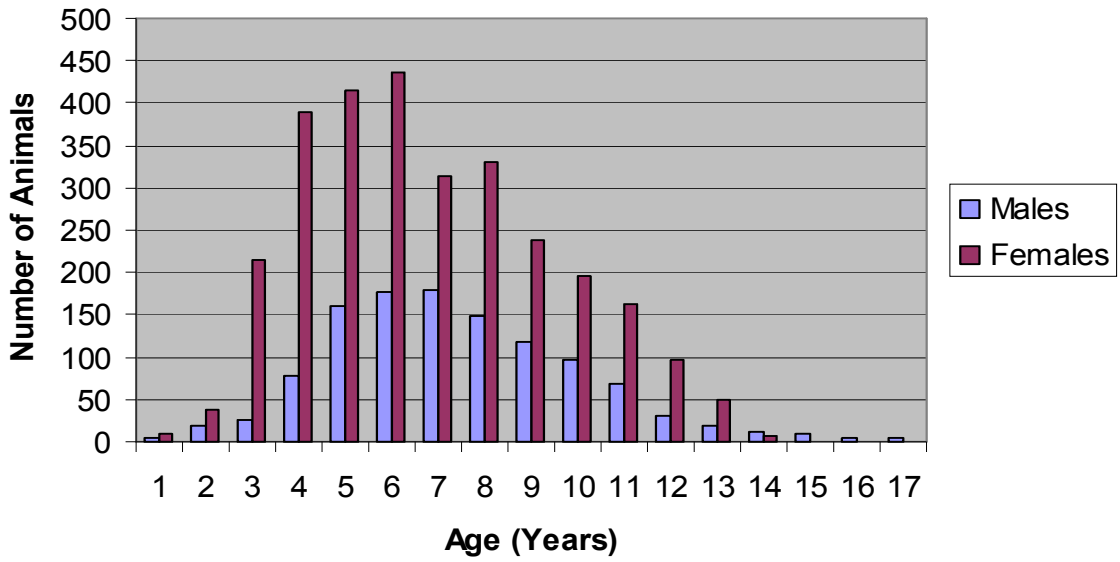

Figure 4.3 Age distribution of males and females in reproduction by birth year of their offspring

The results of the generation interval (1981 to 2007) analysis are shown in Figures 4.4 – 4.6. Generation intervals show minimal difference between the four selection paths in the four stud herds: sire to sons (ss), sire to daughters (sd), dam to sons (ds) and dam to daughters (ds).

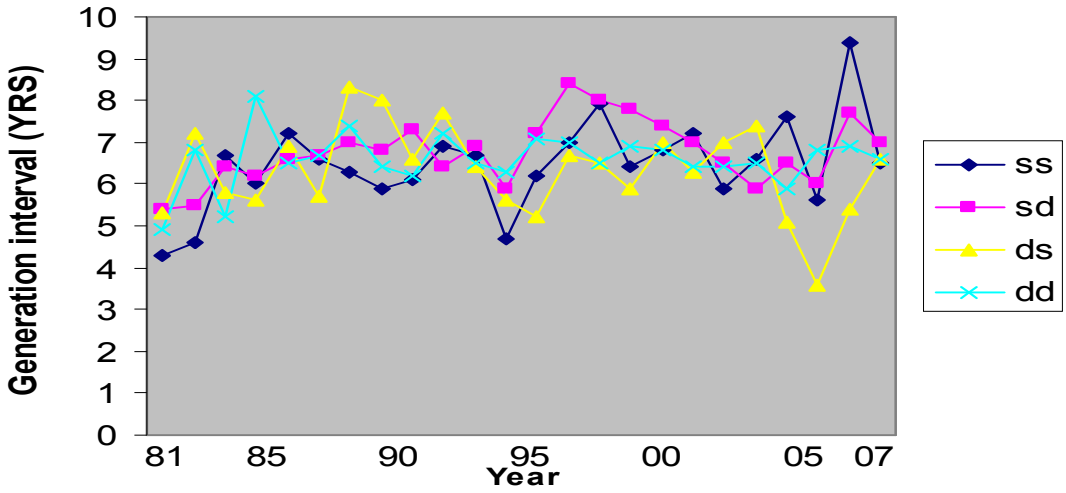

Figure 4.4 Generation interval of the four different selection paths (ss: sire to son, sd: sire to daughter, ds: dam to son and dd: dam to daughter).

The sire to offspring selection pathways indicates a slightly increasing trend though the increase is gradual (Figure 4.5).

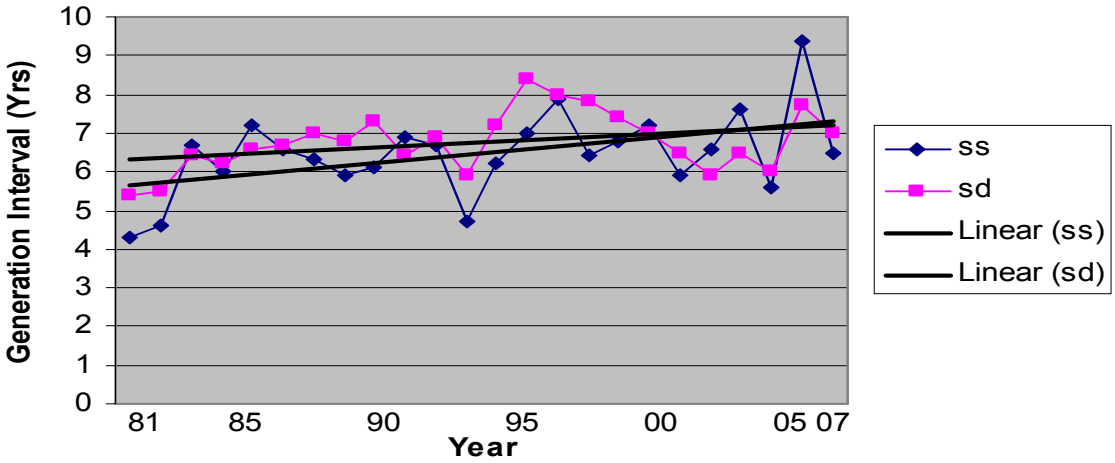

Figure 4.5 Generation interval of the sire to offspring pathways.

The dam to sons' selection pathway indicates a gradual downward trend while that of the dam to daughters' was constant over generations in the four stud herds (Figure 4.6).

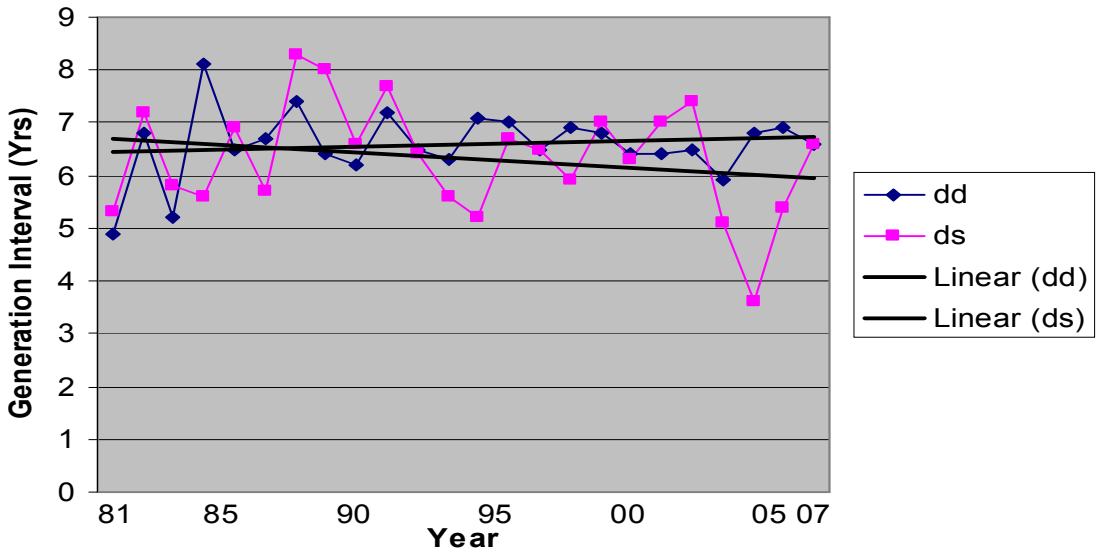

Figure 4.6 Generation interval of the dam to offspring pathways.

The results of the distribution of parity of the dams for the period 1980 to 2007 are presented in Figure 4.7. Most cows had only a single parity though the age structure indicates a high incidence of cows older than five years (Figure 4.3).

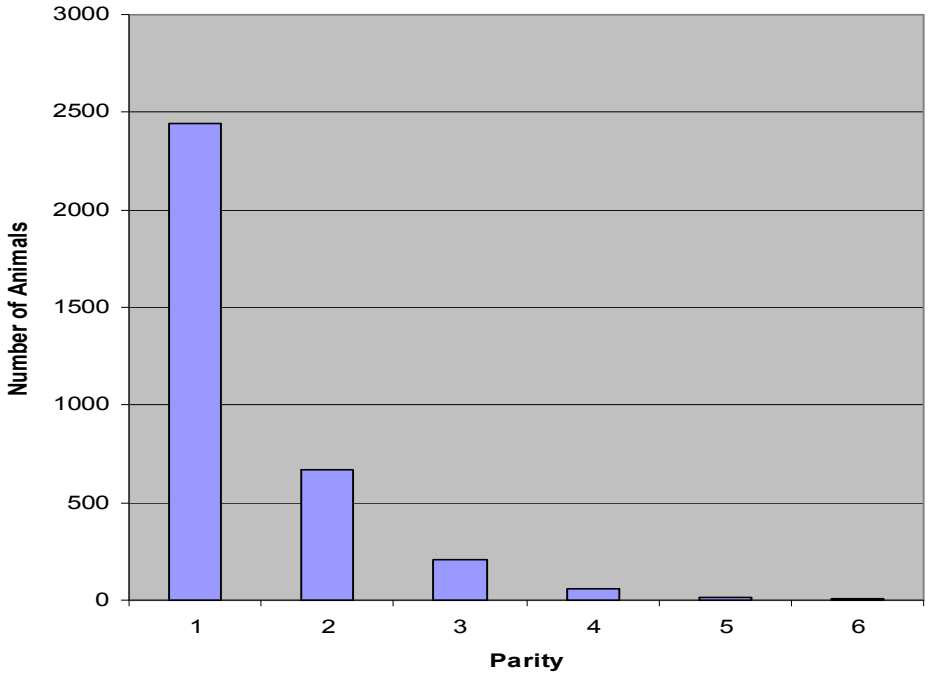

Figure 4.7 Distribution of females by parity number for the period 1980 to 2007

The results of the family sizes are presented in Figure 4.8 for the four stud herds.

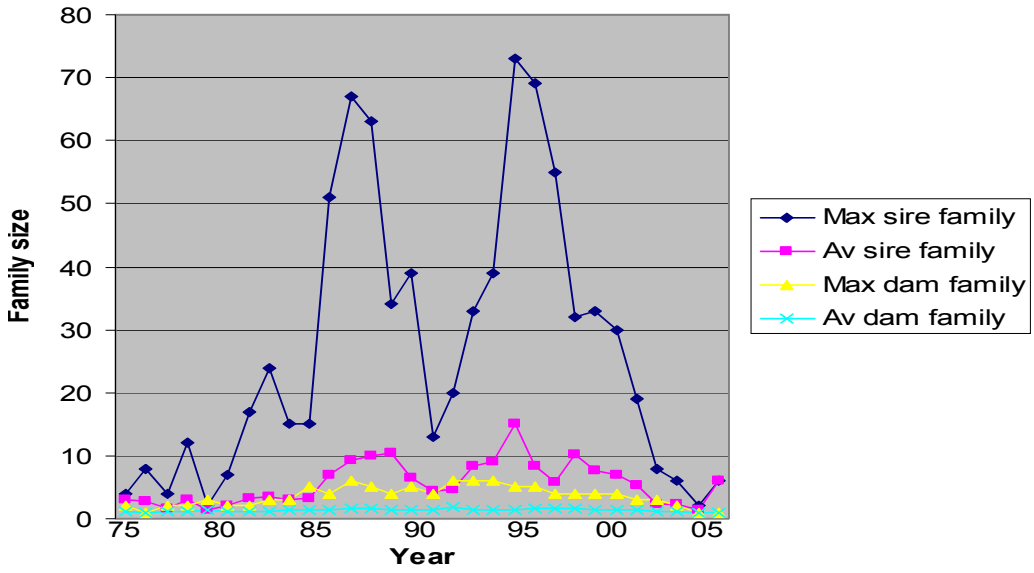

Figure 4.8 Maximum and average family sizes

The average dam family size (1.3) was constant over the years while the average sire family size showed a slight rise from 1975 to 1995, followed by a downward trend. Sire families had a maximum of 74 animals and dam families had a maximum of six animals.

The results of inbreeding coefficients analysis are presented in Tables 4.11 and 4.12, showing the level of inbreeding, co-ancestry, distribution of inbreeding and the number of animals at each level of inbreeding.

Table 4.11 Average of inbreeding coefficients of the four stud herds

	Male x Male	Male x Female	Female x Female	Over Sex
Inbreeding	0.0019(0.19%)	0	0.0022(0.22%)	0.0022(0.22%)
Co-ancestry	0.0027(0.27%)	0.002(0.2%)	0.0016(0.16%)	0.0018(0.18%)

The inbreeding coefficients of the study herds were very low with minimal difference between males and females. The average inbreeding of 0.22% was below the anticipated in a closed nucleus breeding scheme that has been in existence for over five generations.

Table 4.12 Distribution of inbreeding coefficients of the four stud herds

Inbreeding coefficients %	0	<5	5 to 10	10 to 15	>15
Number of Animals (SAS)	4283	76	44	24	12
%	96.4	1.8	1	0.5	0.3
Number of Animals (ABTK)	4697	75	44	25	10
%	96.8	1.6	0.9	0.5	0.2

SAS: Statistical Analysis Software, ABTK: Animal Breeders Toolkit Software

Between 10 and 12 animals had inbreeding coefficients of more than 15% and only 3.2 to 3.6% of the animals in the study herds were considered inbred. The results based on SAS (SAS 9.2, 2007) and ABTK (Golden *et al*., 1995) were similar, only the total number of animals was slightly more with ABTK due to the additions of sires and dams that had not been included as individuals on the dataset.

The results of inbreeding over generations for the four stud herds are presented in Table 4.13 showing inbreeding coefficients per generation for each sex and over sex. Animals in generations one and two were not inbred and inbreeding of females was detected from generation three with a slight increase in trend over the generations. Inbreeding in males was only detected at generation five.

Table 4.13 Inbreeding coefficients of the four stud herds over five generations

Generation	**1**	**2**	**3**	**4**	**5**
Male x Male	0	0	0	0	0.0011
Male x Female	0	0	0	0	0
Female x Female	0	0	0.0004	0.0003	0.001
Over sex	0	0	0.0003	0.0002	0.001

CHAPTER 5: DISCUSSION

5.1 Introduction

Parentage was verified in four Boran families using microsatellite markers. Parentage verification is critical with the use of multiple pasture or rotational sire breeding schemes (Senneke *et al.,* 2004; Van Eenennaam *et al*., 2007) as practised by Boran cattle breeders in Kenya. Accurate cattle pedigrees are essential for optimal development of the breed and selection programmes improving productivity in the beef industry (Sherman *et al*., 2004; Pollak, 2005; Bolormaa *et al.*, 2008) and provides the basis for designing optimum breeding strategies. Paternity pedigree errors have substantial impact on national genetic evaluations, estimates of inbreeding, genetic trend, sire variance and correlations (Banos *et al*., 2001; Visscher, *et al*., 2002). The sire misidentification in this study was 4.3 - 80% while dam misidentification was only 0 - 5% across the four stud herds. The average generation interval was 6.8 years and inbreeding coefficient was 0.0022. Despite the low inbreeding coefficient, the actual rate of inbreeding may be higher due to the high sire misidentification rate and lack of complete pedigree records. To improve production and productivity of the Boran cattle, it is crucial that parentage verification is included in breeding strategies. This will improve the accuracy of the pedigrees, response to selection and monitoring of inbreeding.

5.2 Parentage verification

5.2.1 Individual marker evaluation

For this study, 12 microsatellite markers were selected from the recommended ISAG (www.isag.org.uk) panels for parentage verification. In the final analysis, one marker was excluded due to repeated poor amplification. The genotypic dataset was first subjected to analyses for the different parameters that have an influence on the suitability of markers in a panel for parentage verification (Marshall *et al.,* 1998; Slate *et al*., 2000; Sherman *et al*., 2004).

The 11 microsatellite markers in this study were found to be polymorphic with an average number of alleles of 8.45 per locus. It should be noted that the animals tested are related and higher number of alleles will be expected if larger samples of Boran is to be tested. The allele range (6 to 13) corresponded to those determined previously, which ranged from 4 to 16 (Peelman *et al.,* 1998; Maudet *et al.,* 2002; Visscher *et al.,* 2002; Van Eennenaam *et al.*, 2007). The Hexp range (0.618 to 0.824) and Hobs (0.613 to 0.867) corresponded to those reported previously, which ranged from 0.21 to 0.91 for Hexp and 0.22 to 0.92 for Hobs (Peelman *et al.,* 1998; Maudet *et al.,* 2002; Visscher *et al.,* 2002; Van Eennenaam *et al.*, 2007).

The polymorphic information content (PIC) range (0.593 to 0.800) corresponded to those determined previously, which ranged from 0.18 to 0.90 (Peelman *et al.,* 1998; Van Eennenaam *et al.*, 2007). The mean PIC was 0.690, close to 0.626 reported by Van Eenennaam, *et al.* (2007). The 11 microsatellite markers were highly informative and sufficient for parentage verification analyses of the four stud herds which require microsatellite markers with Hexp and PIC of above 0.5 (Marshall *et al.,* 1998; Visscher *et al.,* 2002). Parameters for ETH 225 were lower than for the other markers in this study and may have resulted from the fact that it had a high frequency of allele 155. Most of the individuals studied had this allele and it's almost fixed in the population under study and could have resulted from selection.

The 11 microsatellite markers had high exclusion power with the combined probability of exclusion for first parent (CPE-1) of 0.9901 and second parent (CPE-2) of 0.9997. Studies performed in beef cattle using the same markers or part of them have reported CPE-2 of 0.9924 to 0.9999 (Cervini *et al.*, 2006; Rehout *et al.*, 2006; Riojas-Valdes *et al.,* 2009) and 0.999 (Van Eenennaam *et al.,* 2007). The CPE was sufficient to exclude non parents in parentage verification analysis of the four stud herds. These microsatellite markers had excellent amplification success rates and were easy to score. The markers were in Hardy-Weinberg equilibrium, a prerequisite for effective assignment of parentage. The null allele frequencies were very low (less than 0.05) and were considered

of little effect on the assignment of parentage. A marker with null allele frequency of above 0.05 is not suitable for parentage verification analysis (Marshall *et al.,* 1998).

The results of the parameter analysis for the 11 microsatellite markers indicate a highly polymorphic informative panel that could be used for parentage verification. This panel was used to analyse and assign parentage to 87 offspring of four sire families.

5.2.2 Parentage assignment

The parentage assignment in the four stud herds was based on the magnitude and sign of the LOD and Delta scores (Marshall *et al.,* 1998; Slate *et al*., 2000; Kalinowski *et al.,* 2007) and loci match. Results with positive LOD scores of above 3 indicated confirmed parentages, LOD scores of between 3 and -3 indicated inconclusive parentage while LOD score values below -3 indicated misidentified parentages. Positive LOD score results of a residential sire confirmed parentage. Parent/offspring pair loci that matched were indicative of confirmed parentage.

The overall rate of dam misidentification was only 2.3% and two stud herds had 100% correct maternal identification. The maternal misidentification rate was 4.2% in stud herd 1 and 5% in herd 3. Dam 133 of stud herd 3 was not excluded as a parent due to the one loci mismatch probably caused by null allele. The dam genotype (245/245) and offspring genotype (243/243) were homozygous at locus SPS115. The dam misidentification in the two herds could have been caused by switching of the calves at birth, incorrectly identifying the dams and offspring during blood sampling or incorrect recording of the pedigree information in the herd book (Weller, *et al*., 2004). The dam misidentification was low and the records were considered reliable for identification.

The rate of sire misidentification in the four herds ranged from 4.3 to 80%. The high rate of sire misidentification is cause for concern as paternal identification is critical for improvement of genetic potential (Banos *et al*., 2001; Pollak, 2005; Van Eenennaam *et al*., 2007) and effective monitoring of inbreeding (Cassell *et al*., 2003) of the Boran stud

herds. The identification of the correct sire and offspring pairs was not as clear as for the dams.

Sire SH1 of stud herd 1 was the parent of five offspring with zero mismatches and two offspring with one mismatch each and positive LOD scores. The mismatch could have resulted probably from null alleles as the sire genotype (86/86) and both offspring genotypes (76/76) were homozygous at locus TGLA227. Allelic drop out has been shown to be a complicating factor in parentage verification analysis using microsatellite markers (Dakin & Avise, 2004; Weller *et al*., 2004; Van Eenennaam *et al*., 2007). The sire was not the parent of 17 offspring with negative LOD scores and more than two pair loci mismatches. Paternity misidentification rate was 71% in this stud herd.

In stud herd 2, the Sire misidentification rate (70%) was also high. Sire SH52 was the parent of the six offspring with positive LOD scores and was not the sire of 14 offspring with negative LOD scores and more than two mismatches. Five offspring/sire pairs had one mismatch each at locus ETH225 with positive LOD scores. The five offspring were homozygous for allele 155, though the sire was heterozygous. This locus had a higher rate of mismatch in this herd than could be accounted by chance and was not considered during paternity assignment.

Sire misidentification rate in stud herd 3 was 80%. Sire SH93 was the parent of the four offspring with positive LOD scores and was not the parent of the 16 offspring with negative LOD scores and more than two mismatches. The sire was not considered the parent of offspring SH103 with one locus mismatch due to the large negative LOD score.

The high rate of sire misidentification could be due to the true sire not been sampled and may be amongst the sires that were used in the herd during the mating season and not recorded as parents of the offspring, neighbouring sires straying to the herd due to lack of paddocks or precocious bull calves (Holroyd *et al*., 2002; Van Eenennaam *et al*., 2007). To conclude the parentage assignment, all sires used during the mating season in the three stud herds and neighbouring stud/commercial herds should be sampled and

genotyped. This was beyond the scope of this study which sought to assess the accuracy of pedigree records, possible causes of the observed misidentification and evaluate mitigation factors.

Sire misidentification rate in stud herd 4 was only 4.3%. The stud bull, SH157 was the parent of most of the offspring in stud herd 4. Only one offspring, SH179 had three pair loci mismatch and negative LOD score. The three mismatches in an otherwise perfectly matched offspring/sire stud herd were sufficient to confirm a misidentification.

The overall sire misidentification rate across the herds was 55.2% and with such a high rate of misidentification, genetic progress will be slow and may even be negative (Israel & Weller, 2000; Banos *et al*., 2001; Pollak, 2005). Boran stud breeders' practice closed nucleus breeding system and may suffer from the effects of inbreeding depression if the causes of sire misidentification are not corrected. The high rate of sire misidentification leads to large pedigree errors and renders the use of pedigree records inappropriate to monitor genetic progress and trends in inbreeding (Wiggans *et al*., 1995; Cassell *et al*., 2003; Adamec *et al*., 2006). The stud herds rely on performance and progeny testing for selection of sires and heifers for replacement and match mating for continued improvement of the breed. Superior sires with misidentified progeny may be culled due to poor performance of the purported progeny leading to loss of superior genetics (Visscher *et al*., 2002; Senneke *et al.,* 2004; Gomez-Raya *et al*., 2008).

The rate of misidentification or missing sires recorded in the literature in beef cattle include: 12% reported by Van Eenennaam *et al*. (2007) using 23 microsatellite markers, 14% reported by Sherman *et al*. (2004), using 12 microsatellite markers and 0-33.6% reported by Holroyd *et al*. (2002) using 11 microsatellite markers. The three studies were on parentage assignment of sires on multiple mating strategies unlike in the current study which analysed the accuracy of the pedigree records. The researchers sampled all the sires used during the mating season in those herds. In the current study, only sires that were recorded as parents in the herd book were sampled to test the accuracy of the recorded entries. The breeder of stud herd 4 had the most accurate pedigree records while

the breeders of stud herds 1, 2 and 3 had relatively accurate records of dams but highly inaccurate records of sires.

Cattle recording require accurate identification of the sire, dam, and the progeny with proper maintenance of the records. It is not an easy task in multiple or rotational sire breeding systems as with Boran stud herds. This requires the ability to record both sire and dam during mating season and at birth or shortly thereafter (Dodds, *et al*., 2005b). Inaccuracies that hinder genetic progress are in the recording systems and the integrity of the pedigrees. It's important to use correct pedigree records in order to obtain accurate estimates of heritability and genetic correlations which are used as tools for developing selection programmes in livestock (Bolormaa *et al.*, 2008).

5.2.3 Causes of parentage misidentification in Boran herds

The high rate of sire misidentification is a problem in most of the large scale ranches in Kenya due to:

1. The extensive system of beef production in most ranches may lead to ineffective monitoring of mating events (Dodds, *et al*., 2005b; Van Eenennaam *et al*., 2007; Gomez-Raya *et al*., 2008). Recording of successful mating in the field may not be effective as most of the herders in Kenyan ranches have no formal education and these records are prone to human error of omission. Similar observations have also been reported in sheep production (Bolormaa *et al.*, 2008).
2. The mating strategy of all year joining practiced by the Boran cattle breeders makes it difficult to accurately record most of the breeding events (Dodds, *et al*., 2005b). The sires in Kenyan ranches are used for 10 weeks, then rested and a new bull introduced after a two week rest of the breeding cows. This may not be effective for positive identification of paternity if not coupled with other strategies. The rest period of two weeks is too short to adequately differentiate the offspring of the sires if a supposedly pregnant cow is served by the new bull in the field.
3. The lack of internal paddocks and in some ranches, external paddocks to separate breeding herds during the mating period. Though the herds graze separately, the

possibility of meeting at some point during grazing or one herd sire straying to another herd is high. This may also lead to other sires straying from commercial herds and the neighbourhood to the stud herds. Similar observations have been reported in other ranches (Van Eenennaam *et al*., 2007).

4. Late identification and registration of the offspring. The offspring in the three ranches with high sire misidentification rate are given individual identity (branding) at weaning (7 to 9 months of age) and sire records may be incorrectly entered into the herd book. Similar observation was reported for sheep with late pedigree recording (Bolormaa *et al.*, 2008).
5. The switching of calves at birth or shortly thereafter (Weller *et al*., 2004). This could be the reason for the stud herds with dam misidentification but the low level of dam misidentification in this study indicates this as a minor problem.
6. Some ranches have experienced high personnel turn over in the past years. This may be a minor contributing factor and is unlikely to contribute much as seen by the accurate recording of dams and their offspring.
7. Genotyping errors that may arise due to dropped alleles and false alleles may contribute to exclusion of a true parent. This result from alleles at microsatellite loci that consistently fail to amplify during the PCR process (Dakin & Avise, 2004; Weller *et al*., 2004; Van Eenennaam *et al*., 2007). In allelic drop out, the heterozygote is genotyped as a homozygote due to the failure of one allele to amplify during the PCR process. This could be caused by poor primer annealing or poor DNA samples being used.

Stud herd 4 is kept in a relatively smaller fully paddocked farm with both external and internal paddocks. The recording and identification of calves is done immediately after birth or shortly thereafter unlike in the other three stud herds. This could be the reasons for the low sire misidentification. The dams in all the four stud herds have a low rate of misidentification and indicate that the calving process is well recorded and effective.

Though multiple sires are used in the four stud herds studied, they are used at different periods and the rate of sire misidentification should not be high if recording was done

well. The first four of the listed causes above are the main contributors to sire misidentification in the four herds. These causes can easily be controlled by improved recording but the extensive system of production under harsh climatic conditions and infrastructural challenges are limiting factors and DNA based parentage assignment may be a better option. The current hierarchy in beef cattle selection in most countries is such that genetic progress is achieved in the stud herds and passed on through the bulls to the commercial herds were no further assessment or selection of these bulls occur (Pollak, 2005; Van Eenennaam, *et al*., 2007). DNA paternity verification holds potential for free range ranch operations with no consistency of identification of bulls that sire poor performing calves (Gomez-Raya *et al*., 2008).

Parentage testing can be effective and the sires and dams only need to be genotyped once and data stored. The selected progeny for breeding are also genotyped once in a lifetime after selection. A number of countries have adopted molecular markers in their livestock improvement schemes including parentage testing (Beuzen *et al.*, 2000; Van Marle-Köster & Nel, 2003). Most of the performance records in suckling cow production systems have no data on successful mating. The unsupervised paddock mating is the most frequent and limits the use of the reproductive information for selection in beef cattle (Yagüe *et al*., 2009). The importance of correct pedigree information increased with the introduction of the animal model for national genetic evaluation (Visscher *et al.,* 2002). If the sire is missing from the mating group records and is not considered as a potential parent, it will have a greater impact on estimated breeding values than a dam as the sire has more progeny (Dodds *et al.,* 2005a). The animal model assumes that all relationships in the national herd book are correct. This is not the case in the current situation for Boran cattle in Kenya. Incorrect identification of sires can bias estimates of heritability, breeding values and genetic progress (Israel & Weller, 2000; Banos *et al*., 2001).

5.3 Microsatellite marker panel comparison

Panels of six to eleven microsatellite markers were tested for their suitability in parentage verification analyses of the Boran cattle in Kenya (Table 4.8 to 4.10 and Figure 4.1). The rationale of selecting the range of marker panels' was based on various studies in the

literature. Most of the researchers have recommended the use of 6 to 20 microsatellite markers for parentage assignment (Ron *et al*., 1996; Marshall *et al*., 1998; Visscher *et al*., 2002; Sherman *et al*., 2004; Cervini *et al*., 2006; Rehout *et al*., 2006; Bolormaa *et al.*, 2008; Gomez-Raya *et al*., 2008). The International Society for Animal Genetics (ISAG: http://www.isag.org.uk) have proposed the use of 12 microsatellite markers.

The microsatellite markers for parentage verification were selected based on their ease of scoring, high mean heterozygosity, high polymorphism, ease of amplification with Polymerase Chain Reaction and appropriate fragment length relative to the other microsatellite markers (Visscher *et al*., 2002; Weller, *et al*., 2004; Riojas-Valdes *et al.*, 2009). The higher the heterozygozity of the microsatellite markers above 0.5 the better they are for identification validation (Visscher *et al*., 2002).

Panels 1 to 3 had CPE-2 above 0.999 and only differed slightly as shown in Table 4.9 and Figure 4.1 while Panels 4 to 6 had CPE-2 below 0.999. Panels 1 to 6 differed on CPE-1 with panels 2 to 6 having CPE-1 below 0.99. CPE-1 is useful were parents of the offspring are unknown or when the recorded identification of the known parent is doubtful. To achieve high exclusionary power of CPE-1, more microsatellite markers are required. CPE-2 is useful were one parent is known with certainty and requires relatively few markers to achieve a high exclusionary power. In the four Boran stud herds, the dam is known with certainty and CPE-2 will be appropriate for paternity assignment were both parents and the offspring are genotyped. Panels 1 to 4 will be more appropriate based on their exclusionary power. For routine analysis of the Boran cattle in Kenya were only sires and offspring are genotyped, panels 1 and 2 with CPE-1 of 0.990 and 0.987 (approx 0.99) may be more suitable.

Parentage assignment was performed using the six microsatellite marker panels and the four stud herds (Table 4.10 and appendix D). Panel one and two had similar paternity assignments and only slightly differed in the magnitude of the LOD scores. Panel three assigned an extra calf to sire SH1 of stud herd 1 and led to a reduction of one assignment to sire SH52 of stud herd 2. Panels 1 to 3 only assigned offspring at 95% (strict)

confidence level while panels 4 to 6 assigned offspring at both 95% (strict) and 80% (relaxed) confidence level. This may be due to the loss of discriminatory power of the panels as the number of markers is reduced. This indicates that were dam records are more accurate, a smaller panel can be used but were the rate of misidentification is high a more powerful panel is needed.

Based on these observations, panel 2 with 10 microsatellite markers will be the most appropriate and cost effective for parentage verification of Boran cattle in Kenya. This panel is useful in cases were genotypes of both parents and offspring are sampled and also were only genotypes of one parent and offspring are available. The panel will be relatively cheaper than the 11 marker panel used in this study. The dam is mostly identified correctly in farm records and with a CPE-2 of more than 0.999 and 0.9985, panels 3 and 4 may be powerful enough for parentage assignment when both parents and offspring are sampled. The stud herds have between 70 to 300 breeding cows in most ranches and confirming paternity is possible. The gain in response to selection (Ron *et al*., 1996; Visscher *et al*., 2002; Senneke *et al.,* 2004; Pollak, 2005), reduction in involuntary culling of supposedly poor sires (Dodds *et al*., 2005b; Bolormaa *et al.*, 2008; Gomez-Raya *et al*., 2008) and reduction in potential inbreeding (Wiggans *et al*., 1995; Cassell *et al*., 2003; Adamec *et al*., 2006) could have a significant effect.

The use of non invasive DNA technology i.e. hair follicles or milk samples (Visscher *et al*., 2002; Weller *et al*., 2004) should be attractive to Boran cattle breeders and will reduce the cost of parentage verification analysis. The envisaged cost of the program in Kenya based on the use of hair follicles is presented in Table 5.1. The cost is exclusive of collection and transportation of samples to the laboratory. The figures are courtesy of Animal Breeding and Genetics laboratory, Department of Animal and Wildlife Sciences, University of Pretoria, Applied Biosystems, Foster City, USA, www.appliedbiosystems.com, Whitehead Scientific (Pty) Ltd, www.whitesci.co.za, and Inqaba Biotech, South Africa, http://www.inqababiotec.co.za/). Exchange rate used was Kenya shillings (Ksh) 10: 1 Rand and Ksh 75: 1 US Dollar.

Table 5.1 Approximate cost of parentage verification analysis in Kenya

ITEM	CONCENTRATION	UNIT PRICE (Ksh)	TOTAL(Ksh)
10 Primers	10 nm	9,000	90,000
50 pieces of Taq (Polymerase)	500 units	10,520	526,000
1 Liz standard		36,272	36,272
1 Agarose		10,000	10,000
1 set of dNTPs		23,490	23,490
20 packets of Tips (0.1-10µl)		1,600	32,000
16 packets of Tips (1-200µl)		2,150	34,400
2 pieces of Loading dye		5,474	10,948
2000 number of Genescan		200	400,000
Total			1,163,110
Contigency(20%)			232,622
Laboratory			56,000
Total			1,451,732
Labour (50%)			653,710
Total for 2000 samples			**2,105,442**
Cost per sample			**1,053**

Ksh: Kenya shilling

Boran cattle breeders could collect hair follicles in a clean envelope and send these NPP5DGVTU1D17E8Cthrough the postal system to a laboratory reducing the cost compared to the use of blood samples. The fact that systems are available for direct PCR procedures with hair follicles without conventional DNA extraction further reduces the costs. In South Africa, the cost of parentage verification based on use of hair follicles and direct PCR procedure is 140 Rand (Ksh 1,400) (Personal communication, Inqaba Biotech, South Africa, http://www.inqababiotec.co.za/). The rate is similar to that proposed for Boran cattle breeders in Kenya. Gomez-Raya *et al*. (2008), in a study in 8 beef ranches in Northern Nevada, USA, has demonstrated that benefits of $1.71 – 2.44 per dollar invested at bull culling rates of 0.2 – 0.3 are possible using a 12 microsatellite marker panel.

5.4 Boran population structure and Inbreeding

5000 pedigree records were collected for population structure and inbreeding analysis from four Boran stud herds in Kenya used for parentage verification study. 544 records were excluded from the final analysis due to duplication, incorrect date of birth or sex. These included parents born later than their offspring and records were sires were entered

as dams or dams as sires. The average generation interval was 6.8 years and inbreeding coefficient was 0.0022 (0.22%). Most of the animals in generation 1 to 3 had none or little information on their parents and ancestors and this may have contributed to the low inbreeding coefficients (Wiggans *et al*., 1995; Cassell *et al*., 2003). 50% of the records analysed were in the category of foundation, a common feature in Boran pedigree records. 18,548 of the total of 34,382 (54%) of Boran pedigree records at KSB are foundation records (KLBO, 2010).

The number of males and females in reproduction (Figure 4.3) indicate an increasing trend. The sharp decrease in the number of registered males and females between 2006 and 2007 was due to incomplete processing of records at KSB. During the collection of data for this study in September 2009, most application forms for registration of cattle born in 2007 had not been processed while some ranches had not submitted their forms. The manual system of recording and processing of registration at KSB for all breeds of cattle in Kenya is a difficult task and may slow progress in genetic evaluation and population studies. The computerisation of the records at KSB will allow faster processing of pedigrees with improved accuracy.

The age distribution of males and females in reproduction (Figure 4.4) follow a normal distribution curve with an average age of 5.9 years. The sires are normally used for longer periods and were expected to be older on average but this was not the case. The bulls were sold to other breeders when their heifers for replacement were ready for joining at three years old (Wasike *et al*., 2009: KBCBS, 2010). The 30 most influential sires produced over 30% of the progeny selected for breeding. Eight dams had five progeny each selected for breeding but the average was 1.3 progeny selected for breeding per dam in the total population studied.

Most of the females had single parity with only 30% having more parity. This may indicate either poor fertility or lack of registration of the offspring and may result from offspring not meeting the breeders' standards. The failure of the offspring to meet the breed standards may result from increased inbreeding that may not be detected early due

to the high sire misidentification. Culled males were castrated and raised as steers without registration with KSB (Wasike *et al*., 2009; KBCBS, 2010). Culled heifers were also not registered with KSB and this practice will bias genetic parameter estimates and monitoring of inbreeding. The recording system need to be reviewed to allow the registration of all births with KSB. Though all births are recorded by individual ranches, only animals that pass the breed standards are registered with the KSB.

Dams with culled offspring not registered will be categorised as failed reproduction denying researchers and breeders an opportunity to address the breeding constraints and is also a big problem if you want to select for improved reproduction. Approximately 80% of the 4,456 pedigree records used in this study were female records. This was due to unavailability of records for culled males at KSB. KBCBS need to consider the recording of culled males and females with KSB which will allow for accurate and meaningful interpretation of population characteristics and improved selection for reproductive traits.

The transfer of genes from parents to offspring occurs through the four selection paths, sires to sons (ss), sires to daughters (sd), dams to sons (ds) and dams to daughters (dd). The sire to sons and dam to sons' selection paths accounts for much of the genetic progress due to the greater intensity of selection. Fewer males are needed as parents for the next generation and allows for greater intensity of selection compared to that of females. The generation interval affects the rate of genetic progress and structure of the population (Groeneveld *et al*., 2009). Genetic gain per year is measured as a ratio of the product of the heritability and selection differential divided by generation interval in years. The shorter the generation interval, the more rapid is the genetic change in a population (Mc Parland *et al*., 2007).

Genetic gain/year = (heritability x section intensity)/generation interval in years

The generation interval for ss selection path was 6.5 years, similar to those reported by Mc Parland *et al*. (2007) (6.05 to 8.25) and Gutierrez *et al*. (2003) (2.86 to 7.75). The

generation interval for ss selection path was higher than reported by Carolino & Gama, (2008b) (6.0) and Groeneveld *et al*. (2009) on Bonsmara breed (5.4). The ss generation interval was lower than those reported for Nellore cattle (10.1) (Groeneveld *et al*., 2009). The generation interval for the sd selection path was 7.0 years, similar to those reported by Mc Parland *et al*. (2007) (6.23 to 8.14) and for Nellore cattle (7.2) (Groeneveld *et al*., 2009) and was higher than those reported by Gutierrez *et al*. (2003) (2.68 - 6.61) and for Bonsmara cattle (5.5) (Groeneveld *et al*., 2009). Though the trend indicates a slight increase of generation interval of the sire to offspring selection pathways in the Boran stud herds, the increase was very low.

The generation interval for the ds selection path was 6.6 years, higher than reported by Mc Parland *et al*. (2007) (3.99 to 6.03) and for Bonsmara cattle (5.7) (Groeneveld *et al*., 2009). This was similar to those reported by Gutierrez *et al*. (2003) (3.8 to 8.52), Carolino & Gama, (2008b) (6.8) and was lower than those for Nellore cattle (8.4) (Groeneveld *et al*., 2009). The generation interval for the dd selection path was 6.6 years, higher than those reported by Mc Parland *et al*. (2007) (3.97 to 5.83), Bonsmara cattle (5.7) and Nellore cattle (5.9) (Groeneveld *et al*., 2009), but similar to those reported by Gutierrez *et al.* (2003) (3.9 to 7.48). The dams were expected to have a longer generation interval over sires because of the longer period to produce a replacement. The sire can produce many offspring within a year but a dam can only produce one offspring which can be male or female. The dam to offspring selection pathways had a slight downward trend for ds path and was constant for dd selection path.

In this study, the generation interval of the four selection pathways had very small differences in sharp contrast to the observation of Mc Parland *et al*. (2007) and Nellore cattle (Groeneveld *et al*., 2009), were the dam to offspring pathways were shorter than sire to offspring pathways. Both the studies of Gutierrez *et al*. (2003) and Carolino & Gama, (2008b), had longer dam to offspring pathways compared to sire to offspring pathways. The results were similar to those reported for Bonsmara cattle which had similar generation intervals over the four pathways (Groeneveld *et al*., 2009).

The results of inbreeding analysis show most of the animals (96.4 – 96.8%) had zero inbreeding coefficients and only between 3.2 and 3.6% were inbred. Between 10 and 12 of a total of 156 inbred animals had inbreeding coefficients of 0.25 (25%). The overall inbreeding was extremely low at 0.0022 (0.22%), much lower compared to studies by Gutierrez *et al.* (2003) (0.25 to 3.13%) and Mc Parland *et al.* (2007) (0.54 to 2.19%). The inbreeding within the sexes was similar in the four Boran stud herds. The result in consideration of the high rate of sire misidentification and incomplete pedigree records may be grossly underestimated and may not reflect the actual inbreeding of these herds.

The inbreeding coefficients of male animals in generations 1 to 3 and females in generation 1 and 2 were zero. This was expected due to lack of complete pedigree information of animals in generations 1 to 3. Pedigrees records of Boran stud herds are processed and stored manually, a difficult task to retrieve and reconstruct complete pedigree information. The inbreeding is increasing though at a lower rate. The rate of inbreeding was lower than those reported for Bonsmara cattle (0.0011 to 0.0014) and higher than those of Nellore cattle (-0.0001 to -0.0002) (Groeneveld *et al*., 2009). The manual, incomplete pedigree recording in the four stud herds and high mispaternity could not allow for accurate estimation of inbreeding. The reported inbreeding is therefore most likely underestimated and may not be a useful indicator of the prevailing situation of the Boran stud herds.

CHAPTER 6: RECOMMENDATION AND CONCLUSION

The high rate of sire misidentification may lead to loss of response to selection, low genetic gain and increase in inbreeding. To minimize the sire misidentification, the following remedial measures are recommended:

1. The use of DNA based parentage assignment of sires. The availability of procedures that allow the use of hair follicles from the target individuals will reduce the cost of sampling and DNA extraction. This may be attractive to ranches due to the extensive nature of beef production in Kenya. In extensive production systems, practising year round joining, it is difficult to supervise mating and calving events. The availability of systems that use DNA testing to generate estimated breeding values (EBV) for sires is an option that Boran cattle breeders can explore to improve response to selection, parentage verification and reduce the threat of inbreeding without putting undue constraint on their production systems which are already under stress of unfavourable climatic conditions. There is lack of a clear selection strategy due to inadequate pedigree and performance records and ranking of the sires on their potential abilities cannot be done at this time. The few records available may not produce accurate estimates of population parameters and may lead to increased inbreeding.
2. Breeders may practice seasonal joining instead of the current all year joining. This may reduce the number of sires used in a herd per year and supervision of mating will be for a short period rather than for a whole year resulting in a reduced cost of manpower for supervision. Though it may decrease the rate of misidentification, it must be accompanied with proper paddocks to separate breeding herds.
3. Provision of paddocks may not be an option on most ranches due to the predators. The presence of elephants and lions calls for electric fences which are expensive to install and maintain.
4. Closer supervision of mating and calving to ensure correct dam and sire are entered into the mating records. The option is difficult to implement in the extensive production system.

5. Identification and recording of calves as soon as they are born to reduce misidentification through the switching of calves. This has been done successfully and positive identification of dams is reflected by the parentage analysis results.
6. Pregnancy diagnosis may be done immediately after the withdrawal of the bull and before introduction of the next sire. This will ensure all pregnant cows are identified and recorded and the records of those with a service but did not conceive are sorted.
7. Recording all offspring both for replacement and culled with KSB. This will lead to accurate estimation of breeding parameters especially reproductive traits which will be biased by the current practice. Sires and dams with culled offspring may seem to be inferior for reproductive traits and superior genetics may be lost due to failed recording and mating of closely related animals due to sire misidentification.

This was the first attempt to verify pedigree accuracy in Kenyan ranches using DNA based parentage verification in four stud herds. The Boran breed is increasingly gaining popularity in other countries and sustaining the supply of quality stock is possible with accurate and reliable pedigree records. To compete effectively with other meat sources, Boran breeders need technology that is robust, cost effective and relevant in the extensive production system in practice. The proposed panel of 10 microsatellite markers is effective in sire identification based on the combined probability of exclusion for the second parent of 0.9995. The dam of the offspring is known in most cases as shown by the parentage verification results across the four sire families. This leads to increased accuracy in paternity assignment at 95% (strict) confidence level.

The Boran cattle have an essential role in Kenyan beef production. The high rate of sire misidentification within the Boran herds will reduce the rate of response to selection leading to low genetic gain and this will hinder the envisioned genetic improvement of this breed. Population characteristics will be impossible to monitor accurately and the rate of inbreeding may rise with adverse consequences. The cost of paternity test (Ksh 1,053, exclusive of sampling costs) is reasonable. This study has shown that DNA

technology can positively contribute to increased accuracy of pedigree information. The availability of laboratory facilities in Kenya and systems for accurate estimation of breeding values (EBV) using DNA markers is an added advantage to the breeders who may wish to maintain the competitive edge of their stock. It is impossible to design an optimum breeding programme for Boran stud herds that will enhance the response to selection with the current rate of sire misidentification. Genetic evaluation is expensive and this will be wasted with a lack of positive response from targeted animals due to misidentification. The increase in production and productivity of the ranches will continue to reduce the threats for the livelihood of people who are supported directly or indirectly by these enterprises.

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APPENDIX A

Attached is the letter permitting the use of Boran cattle for research at the University of Pretoria.

Boran Cattle Breeders Society.

Tel: 020- 608194/5.
Fax: 020-607529.
E-mail: info-nbi@kisima.co.ke

c/o Kisima Farm,
P. O. Box 20139,
00200 City Square,
Nairobi, Kenya.

4th. May 2009,

To Whom It May Concern:

REF. DAVID KIOS:

I hereby confirm that the above student from University of Pretoria will be conducting research on Boran cattle in Kenya under the auspices of the Boran Cattle Breeders' Society, Kenya.

Please assist him to achieve this research which will be very important for the Boran Breed as a whole.

Sincerely,

Giles Prettejohn,
Chairman.

APPENDIX B

Attached are the Import and No Objection permits for the import of DNA from Kenya to South Africa.

agriculture
Department:
Agriculture
REPUBLIC OF SOUTH AFRICA

Directorate of Animal Health
Import-Export Policy Unit
Private Bag X138
Pretoria, 0001
Republic of South Africa

Tel: (27)-012-3197514
Fax: (27)-012-3298292

IMPORTER:
DR D. KIOS
DEPT OF ANIMAL AND WILDLIFE SCIENCES
UNIVERSITY OF PRETORIA
PRETORIA
0002

DEPARTEMENT VAN LANDBOU
DIREKTORAAT DIERESIEKTEBEHEER
PRIVAATSAK/PRIVATE BAG X138
2009 -10- 13
PRETORIA 0001
DIRECTORATE OF ANIMAL HEALTH
DEPARTMENT OF AGRICULTURE

PERMIT NO: 13/1/1/30/9/5-131
Valid from: 2009-10-09
Expiry date: 2010-01-09

VETERINARY IMPORT PERMIT FOR SPECIMENS
(Issued in terms of the Animal Diseases Act, 1984)

Authority is hereby granted for you to import 250 DNA EXTRACTS FROM BOVINE BORAN CATTLE into Republic of South Africa:

from: MOI UNIVERSITY, CHEPKOILEL CAMPUS, ELDORET, NAIROBI, KENYA
subject to the following conditions:

1. the consignment must be accompanied by this original permit;
2. the DNA EXTRACTS to be securely packed and transported in leakproof containers, sealed by an authorised official of the Veterinary Administration of the exporting country;
3. the consignment must be airfreighted through port of entry O. R. TAMBO INTERNATIONAL AIRPORT . **Samples may only be imported as manifest cargo under an airwaybill number and may not be imported as personal luggage.**
4. the DNA EXTRACTS must be kept and used for purposes of testing/research at the laboratories of DEPT OF ANIMAL AND WILDLIFE SCIENCES, UNIVERSITY OF PRETORIA, PRETORIA, under the personal supervision of DR. ESTE VANMARLE-KÖSTER
5. on completion of tests/research the DNA EXTRACTS must be destroyed by incineration;
6. The State Veterinarian: KEMPTON PARK Tel: 011 973 2827 must be advised timeously of the arrival of the consignment.
7. **This permit does not absolve the importer from compliance with the provisions of any other legislation relating to this import.**
8. This permit is subject to amendment or cancellation by the Director Animal Health at any time and without prior notice being given.
9. This permit is valid for three (3) months from date of issue and FOR ONE CONSIGNMENT ONLY.

DIRECTOR: ANIMAL HEALTH

NOTE:
From 1st January 2005 any consignment imported into South Africa packed with either wood packing material or dunnage, will require treatment t remove any pests present (by heat or methyl bromide fumigation). Treatment must be indicated on packing material. [Enquiries: Directorate Pla Health & Quality Fax: 012 319 6350 or www.nda.agric.za]
30: Lipath specimens

MINISTRY OF LIVESTOCK DEVELOPMENT

Telegrams: "VETLAB", Kabete
Telephone: Kabete 631390/4/5/7, 631287
and 631291
Fax: 631273
When replying please quote

Ref. No RES/POL/VOL.XXIV/4

All correspondences should be addressed to:
The Director of Veterinary Services
Parcels by rail: Nairobi Station

DEPARTMENT OF VETERINARY SERVICES
VETERINARY RESEARCH LABORATORY
P.O. KABETE, 00625 KANGEMI, NAIROBI

Date: 30th September, 2009

Dr. D.K. Kios
Moi University
Chepkoilel campus,
P.O. Box 1125
ELDORET

RE: LETTER OF NO OBJECTION

This is to certify that there is no objection for you to Export to University of Pretoria, South Africa, the following biological materials for research purposes only.

Material & Quantity:

207 doses of Deoxy ribonuncleic acid (DNA)

The materials should be securely packaged

Dr. S.K. Mbiria
For: DIRECTOR OF VETERINARY SERVICES

APPENDIX C

Attached are letters authorizing extraction of DNA in Kenya and outlining the terms and conditions of handling and importing/exporting the DNA.

MINISTRY OF LIVESTOCK DEVELOPMENT

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Telegrams: "VETLAB", Kabete
Telephone: Kabete 631390/4/5/7, 631287
and 631291
Fax: 631273
When replying please quote

DEPARTMENT OF VETERINARY SERVICES
VETERINARY RESEARCH LABORATORY
P.O. PRIVATE BAG
POST CODE 00625 KABETE
NAIROBI

Ref. No. **RES/9/VOL.I/25**

and date

Date **29th September 2009**

All correspondences should be addressed to:
The Director of Veterinary Services
Parcels by rail: Nairobi Station

Dr. David K. Kios
c/o Department of Animal Science
Moi University
P.O. Box 1125
Eldoret

RE: DNA EXTRACTION AT VIL KABETE

This is in reply to your letter dated 15th September 2009 requesting to carry out DNA extraction at Central Veterinary Laboratories Kabete from 200 bovine blood samples collected from three ranches in Laikipia District and a Boran stud herd in Koru. My understanding is that all this is in part fulfillment of your post graduate studies at the University of Pretoria.

The staff at the Central Veterinary Laboratories Kabete are hereby requested to give Dr. Kios the necessary assistance to expedite this extraction.

Please note, however, that your dissertation and all reports and publications arising from the use of these extracted DNA samples shall acknowledge the role played by the Director/Department of Veterinary Services.

Dr. P.M. Ithondeka (MBS)
Director of Veterinary Services.

UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

agriculture

Department:
Agriculture
REPUBLIC OF SOUTH AFRICA

Private Bag X138, Pretoria, 0001
Delpen Building, c/o Annie Botha & Union Street, Riviera.
0084

From: Directorate Animal Health
Tel: 012 319 7502
Fax: 012 319 7470
E-mail: ThaboMo@nda.agric.za
Enquiries: Mr. Thabo Motsisi
Our Ref: 12/11/1- University of Pretoria
Your Ref No : EC091005-054

Date: 06 October 2009

RE: Permission to do research in terms of Section 20 of the ANIMAL DISEASES ACT, 1984 (ACT NO. 35 of 1984)

Dear Dr Kios

Your fax / memo / letter dated 01 October 2009, requesting permission under Section 20 of the Animal Diseases Act, 1984 (Act No. 35 of 1984) to perform a research project or study refers.

I am pleased to inform you that permission is hereby granted to perform the following research/study, with the following conditions:

Conditions: As indicated in the revised and final research proposal, only DNA extracted at the Central Veterinary Laboratories Katebe Kenya, will be imported and used for this study.
Title of research/study: The role of parentage verification of Boran cattle in Kenya
Researcher (s): Dr David Kios (Researcher); Dr E. Van Marle-Koster (Promoter)
Institution: Department of Animal and Wild Life Sciences, Faculty of Natural and Agricultural Sciences, University of Pretoria
Your Ref. Number: EC091005-054
Our ref Number: 12/11/1-University of Pretoria

Kind regards

For DIRECTOR: ANIMAL HEALTH

APPENDIX D

Attached are parentage assignment results of the four Boran stud herds using six micro-satellite marker panels.

APPENDIX D

Tables 1D – 4D presents results of parentage assignment using six microsatellite marker panels in four Boran stud herds

Table 1D Microsatellite marker panel comparison on parentage verification of stud herd 1

		PANEL 1						PANEL 2						PANEL 3						PANEL 4						PANEL 5						PANEL 6					
Offsp	Sire	L1	L2	C	M	LOD	P T	L1	L2	C	M	LOD	P T	L1	L2	C	M	LOD	P T	L1	L2	C	M	LOD	P T	L1	L2	C	M	LOD	P T	L1	L2	C	M	LOD	P T
SH2	SH1	11	11	11	4	-1.39E+01		10	10	10	4	-1.49E+01		9	9	9	4	-1.54E+01		8	8	8	4	-1.60E+01		7	7	7	4	-1.59E+01		6	6	6	3	-1.20E+01	
SH3	SH1	11	11	11	4	-1.59E+01		10	10	10	4	-1.51E+01		9	9	9	4	-1.56E+01		8	8	8	3	-1.17E+01		7	7	7	3	-1.10E+01		6	6	6	2	-7.06E+00	
SH4	SH1	11	11	11	5	-1.89E+01		10	10	10	5	-1.87E+01		9	9	9	5	-1.86E+01		8	8	8	4	-1.46E+01		7	7	7	4	-1.52E+01		6	6	6	4	-1.53E+01	
SH5	SH1	11	11	11	1	-1.87E+00		10	10	10	1	-2.95E+00		9	9	9	1	-2.81E+00		8	8	8	1	-4.07E+00		7	7	7	1	-3.33E+00		6	6	6	1	-3.31E+00	
SH6	SH1	11	11	11	3	-5.40E+00		10	10	10	3	-6.47E+00		9	9	9	2	-2.55E+00		8	8	8	2	-3.13E+00		7	7	7	2	-3.72E+00		6	6	6	2	-3.77E+00	
SH7	SH1	11	11	11	0	4.09E+00	* *	10	10	10	0	4.26E+00	* *	9	9	9	0	4.40E+00	* *	8	8	8	0	3.13E+00	* *	7	7	7	0	3.20E+00	* *	6	6	6	0	2.54E+00	+ *
SH8	SH1	11	11	11	5	-1.72E+01		10	10	10	5	-1.88E+01		9	9	9	5	-1.87E+01		8	8	8	5	-1.92E+01		7	7	7	5	-1.92E+01		6	6	6	5	-1.99E+01	
SH9	SH1	11	11	11	0	6.09E+00	*	10	10	10	0	5.02E+00	*	9	9	9	0	4.48E+00	*	8	8	8	0	3.90E+00	*	7	7	7	0	3.97E+00	*	6	6	6	0	3.31E+00	*
SH10	SH1	11	11	11	0	2.06E+00	* *	10	10	10	0	2.89E+00	* *	9	9	9	0	3.03E+00	* *	8	8	8	0	2.46E+00	* *	7	7	7	0	3.20E+00	* *	6	6	6	0	2.50E+00	* *
SH12	SH1	11	11	11	4	-1.50E+01		10	10	10	4	-1.60E+01		9	9	9	4	-1.66E+01		8	8	8	4	-1.72E+01		7	7	7	4	-1.64E+01		6	6	6	4	-1.65E+01	
SH13	SH1	11	11	11	3	-9.25E+00		10	10	10	3	-9.08E+00		9	9	9	3	-9.63E+00		8	8	8	3	-1.02E+01		7	7	7	3	-1.01E+01		6	6	6	2	-6.22E+00	
SH14	SH1	10	11	10	4	-1.33E+01		9	10	9	4	-1.31E+01		8	9	8	4	-1.37E+01		7	8	7	3	-9.77E+00		7	7	7	3	-9.77E+00		6	6	6	3	-9.82E+00	
SH15	SH1	11	11	11	4	-1.51E+01		10	10	10	4	-1.49E+01		9	9	9	3	-1.10E+01		8	8	8	2	-7.11E+00		7	7	7	2	-7.04E+00		6	6	6	2	-7.02E+00	
SH16	SH1	11	11	11	6	-2.53E+01		10	10	10	6	-2.45E+01		9	9	9	6	-2.43E+01		8	8	8	5	-2.04E+01		7	7	7	4	-1.65E+01		6	6	6	3	-1.26E+01	
SH17	SH1	11	11	11	3	-7.86E+00		10	10	10	3	-7.02E+00		9	9	9	3	-7.57E+00		8	8	8	3	-8.14E+00		7	7	7	3	-8.07E+00		6	6	6	3	-8.77E+00	
SH18	SH1	11	11	11	1	4.27E+00	* *	10	10	10	1	4.43E+00	* *	9	9	9	1	3.89E+00	* *	8	8	8	0	7.80E+00	* *	7	7	7	0	7.21E+00	* *	6	6	6	0	6.51E+00	* *
SH19	SH1	11	11	11	1	2.18E+00	*	10	10	10	1	1.26E+00	*	9	9	9	1	7.14E-01	*	8	8	8	0	4.63E+00	*	7	7	7	0	5.37E+00	*	6	6	6	0	4.68E+00	*
SH20	SH1	11	11	11	0	7.91E+00	* *	10	10	10	0	8.07E+00	* *	9	9	9	0	7.53E+00	* *	8	8	8	0	6.95E+00	* *	7	7	7	0	7.02E+00	* *	6	6	6	0	6.33E+00	* *
SH21	SH1	11	11	11	4	-1.24E+01		10	10	10	4	-1.35E+01		9	9	9	4	-1.34E+01		8	8	8	3	-9.45E+00		7	7	7	3	-1.03E+01		6	6	6	3	-1.10E+01	
SH22	SH1	11	11	11	2	-1.63E+00		10	10	10	2	-1.47E+00	*	9	9	9	1	2.45E+00	* *	8	8	8	1	1.19E+00	* *	7	7	7	1	3.67E-01	+ *	6	6	6	1	3.21E-01	+ *
SH23	SH1	10	11	10	0	5.98E+00	* *	9	10	9	0	4.91E+00	* *	8	9	8	0	4.36E+00	* *	7	8	7	0	3.79E+00	* *	7	7	7	0	3.79E+00	* *	6	6	6	0	3.81E+00	* *
SH24	SH1	11	11	11	3	-9.11E+00		10	10	10	3	-1.02E+01		9	9	9	3	-1.07E+01		8	8	8	2	-6.81E+00		7	7	7	2	-6.07E+00		6	6	6	1	-2.15E+00	
SH25	SH1	11	11	11	6	-2.61E+01		10	10	10	6	-2.53E+01		9	9	9	6	-2.51E+01		8	8	8	5	-2.12E+01		7	7	7	5	-2.05E+01		6	6	6	4	-1.66E+01	
SH26	SH1	11	11	11	4	-1.56E+01		10	10	10	4	-1.48E+01		9	9	9	4	-1.46E+01		8	8	8	3	-1.07E+01		7	7	7	3	-9.97E+00		6	6	6	2	-6.05E+00	

L1: Offspring loci, L2: Sire loci, C: Pair loci compared, M: Pair loci mismatching, Offsp: Offspring, P: Pair confidence, T: Trio confidence, LOD: LOD score

Table 2D Microsatellite marker panel comparison on parentage verification of Boran stud herd 2

		PANEL 1							PANEL 2							PANEL 3							PANEL 4							PANEL 5							PANEL 6						
Offs	Sire	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T
SH53	SH52	11	11	11	0	7.80E+00	*	*	10	10	10	0	5.66E+00	*	*	9	9	9	0	4.23E+00	*	*	8	8	8	0	4.22E+00	*	*	7	7	7	0	4.29E+00	*	*	6	6	6	0	4.24E+00	*	*
SH54	SH52	10	11	10	3	-8.29E+00			9	10	9	2	-4.38E+00			8	9	8	2	-4.24E+00			7	8	7	2	-4.92E+00			6	7	6	2	-5.78E+00			6	6	6	2	-5.78E+00		
SH55	SH52	11	11	11	1	1.91E-01	*		10	10	10	1	-1.26E+00			9	9	9	1	-4.51E-01			8	8	8	1	-1.13E+00			7	7	7	1	-3.92E-01			6	6	6	0	3.53E+00	*	
SH56	SH52	11	11	11	4	-1.20E+01			10	10	10	4	-1.35E+01			9	9	9	4	-1.33E+01			8	8	8	3	-9.42E+00			7	7	7	3	-1.03E+01			6	6	6	2	-6.36E+00		
SH57	SH52	11	11	11	1	5.67E+00	*	*	10	10	10	0	9.59E+00	*	*	9	9	9	0	8.16E+00	*	*	8	8	8	0	7.25E+00	*	*	7	7	7	0	7.32E+00	*	*	6	6	6	0	3.99E+00	*	*
SH58	SH52	11	11	11	6	-2.12E+01			10	10	10	5	-1.73E+01			9	9	9	5	-1.87E+01			8	8	8	5	-1.96E+01			7	7	7	5	-1.89E+01			6	6	6	4	-1.49E+01		
SH59	SH52	11	11	11	1	1.91E-02	*	*	10	10	10	0	3.94E+00	*	*	9	9	9	0	2.62E+00	*	*	8	8	8	0	3.28E+00	*	*	7	7	7	0	3.35E+00	*	*	6	6	6	0	3.30E+00	*	*
SH60	SH52	11	11	11	3	-7.37E+00			10	10	10	3	-8.82E+00			9	9	9	2	-4.90E+00			8	8	8	2	-4.25E+00			7	7	7	2	-4.18E+00			6	6	6	2	-4.22E+00		
SH61	SH52	10	11	10	2	-7.82E+00			9	10	9	2	-8.80E+00			8	9	8	2	-7.99E+00			7	8	7	1	-4.07E+00			6	7	6	1	-3.33E+00			6	6	6	1	-3.33E+00		
SH62	SH52	11	11	11	1	7.07E-01		*	10	10	10	0	4.62E+00	*	*	9	9	9	0	3.20E+00		*	8	8	8	0	3.86E+00	+	*	7	7	7	0	3.00E+00		*	6	6	6	0	2.95E+00		*
SH63	SH52	11	11	11	1	2.68E+00	*	*	10	10	10	0	6.59E+00	*	*	9	9	9	0	5.17E+00	*	*	8	8	8	0	5.82E+00	*	*	7	7	7	0	4.97E+00	*	*	6	6	6	0	1.60E+00	+	
SH64	SH52	11	11	11	3	-8.03E+00			10	10	10	2	-4.11E+00			9	9	9	2	-3.97E+00			8	8	8	2	-3.99E+00			7	7	7	2	-3.25E+00			6	6	6	1	6.68E-01	+	*
SH65	SH52	11	11	11	3	-9.60E+00			10	10	10	2	-5.69E+00			9	9	9	2	-7.11E+00			8	8	8	2	-6.45E+00			7	7	7	2	-5.71E+00			6	6	6	2	-5.76E+00		
SH66	SH52	11	11	11	4	-1.10E+01			10	10	10	4	-1.25E+01			9	9	9	4	-1.24E+01			8	8	8	3	-8.45E+00			7	7	7	3	-8.38E+00			6	6	6	2	-4.46E+00		
SH67	SH52	11	11	11	4	-1.51E+01			10	10	10	4	-1.61E+01			9	9	9	4	-1.53E+01			8	8	8	4	-1.53E+01			7	7	7	4	-1.61E+01			6	6	6	3	-1.22E+01		
SH68	SH52	11	11	11	3	-1.49E+01			10	10	10	2	-1.09E+01			9	9	9	2	-1.01E+01			8	8	8	2	-9.48E+00			7	7	7	2	-8.73E+00			6	6	6	2	-8.78E+00		
SH69	SH52	11	11	11	2	-5.24E+00			10	10	10	2	-6.22E+00			9	9	9	2	-6.08E+00			8	8	8	2	-5.42E+00			7	7	7	2	-6.28E+00			6	6	6	1	-2.36E+00		
SH70	SH52	11	11	11	4	-1.17E+01			10	10	10	3	-7.80E+00			9	9	9	2	-3.88E+00			8	8	8	2	-3.90E+00			7	7	7	2	-4.76E+00			6	6	6	1	-8.39E-01		
SH71	SH52	9	11	9	5	-1.97E+01			8	10	8	4	-1.58E+01			7	9	7	4	-1.57E+01			6	8	6	4	-1.50E+01			6	7	6	4	-1.50E+01			5	6	5	3	-1.11E+01		
SH72	SH52	11	11	11	2	-7.89E+00			10	10	10	2	-8.87E+00			9	9	9	2	-8.06E+00			8	8	8	2	-7.41E+00			7	7	7	2	-8.05E+00			6	6	6	2	-8.09E+00		

L1: Offspring loci, L2: Sire loci, C: Pair loci compared, M: Pair loci mismatching, Offsp: Offspring, P: Pair confidence, T: Trio confidence, LOD: LOD score

Table 3D Microsatellite marker panel comparison on parentage verification of Boran stud herd 3

		PANEL 1							PANEL 2							PANEL 3							PANEL 4							PANEL 5							PANEL 6						
Offs	**Sire**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**	**L1**	**L2**	**C**	**M**	**LOD**	**P**	**T**
SH94	SH93	11	11	11	3	-1.26E+01			10	10	10	3	-1.17E+01			9	9	9	3	-1.09E+01			8	8	8	2	-7.02E+00			7	7	7	2	-6.95E+00			6	6	6	2	-6.56E+00		
SH95	SH93	11	11	11	2	-6.82E+00			10	10	10	2	-7.77E+00			9	9	9	2	-6.96E+00			8	8	8	1	-3.05E+00			7	7	7	1	-2.30E+00			6	6	6	1	-2.80E+00		
SH96	SH93	11	11	11	4	-1.65E+01			10	10	10	3	-1.26E+01			9	9	9	3	-1.18E+01			8	8	8	2	-7.86E+00			7	7	7	2	-7.11E+00			6	6	6	2	-7.61E+00		
SH97	SH93	11	11	11	5	-1.95E+01			10	10	10	4	-1.55E+01			9	9	9	4	-1.54E+01			8	8	8	3	-1.15E+01			7	7	7	3	-1.08E+01			6	6	6	3	-1.14E+01		
SH98	SH93	11	11	11	3	-1.24E+01			10	10	10	2	-8.45E+00			9	9	9	2	-8.31E+00			8	8	8	1	-4.40E+00			7	7	7	1	-3.65E+00			6	6	6	1	-4.32E+00		
SH99	SH93	11	11	11	6	-2.45E+01			10	10	10	5	-2.06E+01			9	9	9	5	-1.98E+01			8	8	8	4	-1.58E+01			7	7	7	4	-1.51E+01			6	6	6	4	-1.51E+01		
SH100	SH93	11	11	11	3	-1.14E+01			10	10	10	3	-1.24E+01			9	9	9	3	-1.16E+01			8	8	8	2	-7.64E+00			7	7	7	2	-6.90E+00			6	6	6	2	-6.51E+00		
SH101	SH93	11	11	11	3	-1.18E+01			10	10	10	2	-7.86E+00			9	9	9	2	-7.06E+00			8	8	8	1	-3.14E+00			7	7	7	0	7.77E-01	+		6	6	6	0	1.16E+00	+	
SH102	SH93	11	11	11	0	4.94E+00	*	*	10	10	10	0	3.99E+00	*	*	9	9	9	0	4.13E+00	*	*	8	8	8	0	3.55E+00	*	*	7	7	7	0	4.29E+00	*	*	6	6	6	0	4.32E+00	*	*
SH103	SH93	11	11	11	1	-6.12E+00			10	10	10	1	-5.29E+00			9	9	9	1	-4.48E+00			8	8	8	0	-5.65E-01			7	7	7	0	1.77E-01	+		6	6	6	0	5.66E-01	+	
SH104	SH93	11	11	11	2	-7.41E+00			10	10	10	2	-6.58E+00			9	9	9	2	-5.77E+00			8	8	8	1	-1.85E+00			7	7	7	1	-1.11E+00			6	6	6	1	-1.40E+00		
SH105	SH93	11	11	11	4	-1.55E+01			10	10	10	4	-1.47E+01			9	9	9	3	-1.08E+01			8	8	8	2	-6.84E+00			7	7	7	2	-6.10E+00			6	6	6	2	-5.71E+00		
SH106	SH93	11	11	11	4	-1.60E+01			10	10	10	3	-1.21E+01			9	9	9	3	-1.13E+01			8	8	8	2	-7.37E+00			7	7	7	2	-6.63E+00			6	6	6	2	-6.92E+00		
SH107	SH93	11	11	11	0	1.90E+00	*		10	10	10	0	9.50E-01	*		9	9	9	0	1.76E+00	*		8	8	8	0	1.18E+00	*		7	7	7	0	1.92E+00	*		6	6	6	0	1.63E+00	+	
SH108	SH93	11	11	11	0	4.05E+00	*	*	10	10	10	0	4.89E+00	*	*	9	9	9	0	3.84E+00	*	*	8	8	8	0	3.26E+00	*	*	7	7	7	0	2.44E+00	*	*	6	6	6	0	2.15E+00	*	*
SH109	SH93	11	11	11	3	-1.18E+01			10	10	10	3	-1.09E+01			9	9	9	2	-7.03E+00			8	8	8	1	-3.11E+00			7	7	7	1	-3.71E+00			6	6	6	0	2.11E-01	+	
SH110	SH93	11	11	11	4	-1.69E+01			10	10	10	3	-1.30E+01			9	9	9	3	-1.22E+01			8	8	8	3	-1.28E+01			7	7	7	3	-1.20E+01			6	6	6	2	-8.13E+00		
SH111	SH93	11	11	11	4	-1.25E+01			10	10	10	4	-1.35E+01			9	9	9	3	-9.55E+00			8	8	8	3	-1.01E+01			7	7	7	2	-6.20E+00			6	6	6	2	-6.49E+00		
SH112	SH93	11	11	11	0	3.55E+00	*	*	10	10	10	0	3.72E+00	*	*	9	9	9	0	2.53E+00	*	*	8	8	8	0	1.96E+00	*	*	7	7	7	0	2.03E+00	*	*	6	6	6	0	2.05E+00	*	*
SH113	SH93	10	11	10	3	-9.66E+00			9	10	9	2	-5.74E+00			8	9	8	2	-5.60E+00			7	8	7	2	-6.18E+00			6	7	6	1	-2.26E+00			5	6	5	1	-2.75E+00		

L1: Offspring loci, L2: Sire loci, C: Pair loci compared, M: Pair loci mismatching, Offsp: Offspring, P: Pair confidence, T: Trio confidence, LOD: LOD score

Table 4D Microsatellite marker panel comparison on parentage verification of Boran stud herd 4

		PANEL 1							PANEL 2							PANEL 3							PANEL 4							PANEL 5							PANEL 6						
Offs	Sire	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T	L1	L2	C	M	LOD	P	T
SH158	SH157	10	11	10	0	4.00E+00	*	*	9	10	9	0	3.48E+00	*	*	8	9	8	0	4.29E+00	*	*	7	8	7	0	3.96E+00	*	*	6	7	6	0	2.66E+00	*	*	5	6	5	0	1.69E+00	+	*
SH159	SH157	11	11	11	0	6.67E+00	*	*	10	10	10	0	6.15E+00	*	*	9	9	9	0	6.96E+00	*	*	8	8	8	0	6.94E+00	*	*	7	7	7	0	6.35E+00	*	*	6	6	6	0	5.37E+00	*	*
SH160	SH157	11	11	11	0	4.02E+00	*	*	10	10	10	0	3.50E+00	*	*	9	9	9	0	4.31E+00	*	*	8	8	8	0	3.97E+00	*	*	7	7	7	0	3.36E+00	*	*	6	6	6	0	3.07E+00	*	*
SH161	SH157	11	11	11	0	4.35E+00	*	*	10	10	10	0	4.51E+00	*	*	9	9	9	0	4.65E+00	*	*	8	8	8	0	4.31E+00	*	*	7	7	7	0	3.02E+00	*	*	6	6	6	0	2.73E+00	*	*
SH162	SH157	10	11	10	0	3.40E+00	*	*	9	10	9	0	2.88E+00	*	*	9	9	9	0	2.88E+00	*	*	8	8	8	0	2.55E+00	*	*	7	7	7	0	1.93E+00	*	*	6	6	6	0	9.52E-01	+	*
SH164	SH157	11	11	11	0	5.66E+00	*	*	10	10	10	0	5.82E+00	*	*	9	9	9	0	4.81E+00	*	*	8	8	8	0	5.47E+00	*	*	7	7	7	0	4.87E+00	*	*	6	6	6	0	3.90E+00	*	*
SH166	SH157	11	11	11	0	7.88E+00	*	*	10	10	10	0	7.36E+00	*	*	9	9	9	0	6.35E+00	*	*	8	8	8	0	5.78E+00	*	*	7	7	7	0	5.16E+00	*	*	6	6	6	0	4.18E+00	*	*
SH167	SH157	11	11	11	0	7.27E+00	*	*	10	10	10	0	7.43E+00	*	*	9	9	9	0	6.42E+00	*	*	8	8	8	0	6.09E+00	*	*	7	7	7	0	4.79E+00	*	*	6	6	6	0	4.50E+00	*	*
SH168	SH157	11	11	11	0	5.66E+00	*	*	10	10	10	0	5.14E+00	*	*	9	9	9	0	4.30E+00	*	*	8	8	8	0	4.28E+00	*	*	7	7	7	0	3.66E+00	*	*	6	6	6	0	3.37E+00	*	*
SH169	SH157	11	11	11	0	5.83E+00	*	*	10	10	10	0	5.31E+00	*	*	9	9	9	0	4.47E+00	*	*	8	8	8	0	4.14E+00	*	*	7	7	7	0	3.54E+00	*	*	6	6	6	0	3.25E+00	+	*
SH170	SH157	11	11	11	0	5.56E+00	*	*	10	10	10	0	5.73E+00	*	*	9	9	9	0	5.87E+00	*	*	8	8	8	0	5.85E+00	*	*	7	7	7	0	5.25E+00	*	*	6	6	6	0	4.96E+00	*	*
SH171	SH157	11	11	11	0	4.52E+00	*	*	10	10	10	0	4.00E+00	*	*	9	9	9	0	4.14E+00	*	*	8	8	8	0	4.12E+00	*	*	7	7	7	0	3.53E+00	*	*	6	6	6	0	3.23E+00	*	*
SH172	SH157	11	11	11	0	5.41E+00	*	*	10	10	10	0	4.90E+00	*	*	9	9	9	0	3.88E+00	*	*	8	8	8	0	3.87E+00	*	*	7	7	7	0	2.57E+00	*	*	6	6	6	0	2.28E+00	*	*
SH173	SH157	11	11	11	0	6.38E+00	*	*	10	10	10	0	6.54E+00	*	*	9	9	9	0	5.53E+00	*	*	8	8	8	0	5.51E+00	*	*	7	7	7	0	4.92E+00	*	*	6	6	6	0	4.63E+00	*	*
SH174	SH157	11	11	11	0	6.99E+00	*	*	10	10	10	0	6.48E+00	*	*	9	9	9	0	5.47E+00	*	*	8	8	8	0	5.13E+00	*	*	7	7	7	0	4.54E+00	*	*	6	6	6	0	3.56E+00	*	*
SH175	SH157	11	11	11	0	6.08E+00	*	*	10	10	10	0	5.56E+00	*	*	9	9	9	0	4.55E+00	*	*	8	8	8	0	4.22E+00	*	*	7	7	7	0	2.94E+00	*	*	6	6	6	0	2.64E+00	*	*
SH176	SH157	11	11	11	0	5.77E+00	*	*	10	10	10	0	5.25E+00	*	*	9	9	9	0	5.39E+00	*	*	8	8	8	0	5.37E+00	*	*	7	7	7	0	4.09E+00	*	*	6	6	6	0	3.11E+00	*	*
SH177	SH157	10	11	10	0	3.16E+00	*	*	9	10	9	0	2.64E+00	*	*	8	9	8	0	3.45E+00	*	*	7	8	7	0	2.88E+00	*	*	7	7	7	0	2.88E+00	*	*	6	6	6	0	2.59E+00	*	*
SH178	SH157	11	11	11	0	5.15E+00	*	*	10	10	10	0	5.32E+00	*	*	9	9	9	0	5.45E+00	*	*	8	8	8	0	5.12E+00	*	*	7	7	7	0	4.50E+00	*	*	6	6	6	0	3.53E+00	*	*
SH179	SH157	11	11	11	3	-9.52E+00			10	10	10	3	-1.00E+01			9	9	9	3	-9.23E+00			8	8	8	3	-9.24E+00			7	7	7	2	-5.33E+00			6	6	6	1	-1.41E+00		
SH180	SH157	11	11	11	0	7.05E+00	*		10	10	10	0	6.53E+00	*		9	9	9	0	7.34E+00	*		8	8	8	0	6.76E+00	*		7	7	7	0	5.48E+00	*		6	6	6	0	4.50E+00	*	
SH181	SH157	11	11	11	0	1.69E+00	*	*	10	10	10	0	1.17E+00	*	*	9	9	9	0	1.31E+00	*	*	8	8	8	0	9.73E-01	*	*	7	7	7	0	3.55E-01	+	*	6	6	6	0	6.46E-02	+	*
SH182	SH157	11	11	11	0	5.74E+00	*	*	10	10	10	0	5.91E+00	*	*	9	9	9	0	4.90E+00	*	*	8	8	8	0	4.56E+00	*	*	7	7	7	0	3.95E+00	*	*	6	6	6	0	2.97E+00	*	

L1: Offspring loci, L2: Sire loci, C: Pair loci compared, M: Pair loci mismatching, Offsp: Offspring, P: Pair confidence, T: Trio confidence, LOD: LOD score