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

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Abstract

Boran cattle provide livelihood to thousands of households in the arid and semi arid lands (ASAL) of Kenya. Due to their superior adaptive and productive traits in comparison to other breeds of cattle, they have also become a popular choice for breeders in Eastern and Southern Africa. Continued genetic improvement of the breed is important and therefore accurate performance and pedigree records are required. 178 Pedigree records and blood samples of four Boran stud herds were evaluated for accuracy of parentage allocation using 11 microsatellite markers recommended by ISAG for parentage verification. The panel of the 11 microsatellite markers were found to be highly polymorphic (PIC of 0.6901) with a combined probability of exclusion (CPE) of 0.9997. The dam misidentification was low ranging between 0 and 5% for the herds tested. The estimated rate of mispaternity however ranged between 4.3 and 80% among the four stud herds and more than 50% of the offspring of some herds were misidentified. The high rate of mispaternity will have a negative impact on the response to selection. The use of DNA markers for parentage assignment will improve the accuracy of the pedigree records of Boran stud cattle in Kenya and contribute to more accurate selection of superior animals.

Keywords: Boran cattle, microsatellite markers, pedigree records, sire misidentification, genetic improvement

Introduction

The livestock sector in Kenya contributes 10 - 12% to the gross domestic product (GDP) (Mwangi & Omore, 2004; Kabubo-Mariara, 2009), of which the Boran breed contributes approximately 2%. Improving the productivity of the livestock production systems is crucial for poverty alleviation (Rege & Gibson, 2003; Scarpa *et al.,* 2003; Rewe *et al*., 2010) and the harsh conditions of the ASAL dictates the need for the use and improvement of the local cattle breeds

such as the Boran. The Boran is a suitable breed for grass-fed beef production as they are well adapted to the Kenyan habitat and show a lower susceptibility to ticks and diseases (Hansen, 2004; Zander & Drucker, 2008). Boran bulls are used in crossbreeding systems and crossbred Boran-sired calves have been shown to outperform *Bos taurus* sired contemporaries with regard to birth and weaning weights (Lunstra & Cundiff, 2003). Boran embryos have been exported to Zambia, Zimbabwe, Australia, America, Brazil and South Africa (Cherogony & Kios, 2008; Kios, 2008; KBCBS, 2010), confirming the importance and the growing popularity of the breed.

Boran cattle are kept on large commercial ranches where a lack of internal paddocks and fencing limit sound breeding and management practices. Especially the stud breeders experience problems in keeping their mating groups apart and depend on the herders to accurately record both mating and calving activities. Due to large farming units, breeders often make use of multiple sire mating strategies that further aggravates the problem of accurate sire identification. Although pedigree records are kept for stud Boran cattle by the breeders, most Boran cattle herds in Kenya have developed without the use of herd books (Zander *et al.*, 2008) or an official scheme (Kahi *et al*., 2006). The unavailability of information from relatives in many Boran cattle populations has hindered the development of optimum breeding strategies.

Inaccurate pedigree information is a common problem in the livestock industry and paternity pedigree errors have a substantial negative impact on national genetic evaluation and estimates of inbreeding (Ron *et al*., 1996; Banos *et al*., 2001 Pollak, 2005; Dodds *et al*., 2007). The proportion of misidentified progeny varies between 2.9 and 23% in cattle worldwide (Geldermann *et al*., 1986; Weller *et al*., 2004; Van Eenennaam *et al.,* 2007). Parentage verification is crucial as it forms the basis for accurate selection and improved rate of genetic progress (Dodds *et al*., 2005; Visscher *et al.,* 2002) and becomes more critical with the wide spread use of artificial insemination, embryo transfer and multiple sire breeding schemes (Senneke *et al.,* 2004; Sherman

et al., 2004; Van Eenennaam *et al*., 2007). Beef producers should be able to determine the sires of all progeny in order to estimate their relative performance (Van Eenennaam *et al*, 2007), and this is possible with the aid of DNA based markers (Van Marle-Köster & Nel, 2003; Gomez-Raya *et al*., 2008).

The accuracy of pedigree records of Boran cattle has not previously been determined and the level of parentage misidentification is unknown. The objective of the study was to quantify parentage recording errors and evaluate DNA-based parentage verification as a possible solution in Kenyan Boran stud herds.

Materials and Methods

Materials

Four Boran stud herds that are members of the Kenya Boran Cattle Breeders Society (KBCBS) and Kenya Stud Book (KSB) provided samples and pedigree records for this study. Three of the herds were kept in large ranches of over 50,000 acres of land with at least 4,000 head of cattle each with limited fencing and herd management. The breeding cow herds are reared in groups of 150 – 200 heads which are exposed to a bull for ten weeks and allowed to rest for two weeks before introducing the next bull. No specific mating season was followed and mating took place throughout the year. The fourth herd was kept on a smaller farm of 300 head of cattle with both external and internal paddocks. Mating and calving was closely monitored due to the effective separation of the livestock.

The cattle were handled in their normal environment in accordance with the protocols. Samples of 5ml blood was collected from each of the 178 head of cattle from the four sire families (Table 1) in vacutainer tubes containing EDTA and kept at -20˚ C for further analysis. Permission to use Boran cattle DNA was obtained from the Ethics committee on Biohazards in the Faculty of

Natural and Agricultural Sciences of the University of Pretoria (Ref. No: EC091005-54), the Director of Animal Health of the Republic of South Africa, the Director of Veterinary services in Kenya and the KBCBS.

Table 1 The distribution of samples and herd size / ranch size of the four stud herds

Stud	No Sampled	Herd size	Dams	Progeny	Sire	Ranch size (acres)
I	48	>4000	23	24	1	>50,000
II	41	>4000	20	20	1	>50,000
III	41	>4000	20	20	1	>50,000
IV	48	300	23	24	1	<10,000

Methods

DNA extraction was performed using GFX Genomic Blood DNA Purification kits from Amersham Biosciences (www.amersham.com) and the DNeasy Blood and Tissue kit for DNA purification from Qiagen (www.qiagen.com) according to the manufacturers' protocol. DNA quantification was performed using agarose gel electrophoresis (Amersham Pharmacia Biotech Inc) and nanodrop spectrophotometer (NanoDrop Technologies, Inc., *http*://www.nanodrop.com). The 178 DNA samples had concentrations of 50 - 100 ng/µl, adequate for the research.

Nine of the 12 microsatellite markers (BM1824, BM2113, SPS115, TGLA122, TGLA126, TGLA227, INRA023, ETH10 and ETH225) used in this study were selected based on the recommendation of the International Society for Animal Genetics (ISAG) (www.isag.org.uk) for routine use in cattle parentage verification. Three additional microsatellite markers, TGLA53, BM1818 and SPS113 were selected based on their high heterozygosity and polymorphic information content, their ease of amplification and fragment size relative to the other nine, as reported in the literature. SPS 113 was excluded from this study due to repeated failure to amplify.

DNA amplification was done 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) and 4 µl DNA (50 – 100 ng/µl). Deionised water was added to obtain a final solution of 15 µl.

Genotyping was performed with ABIPRISM® 3100 Genetic Analyzer (Applied Biosystems, Foster City, USA) for DNA sequencing and fragment analysis. The electropherogram was analysed using Genemarker software version 1.8 (Softgenetics) (http://www.softgenetics.com) and the genotypes were verified for consistency, peak sizes and fragment size. Allelic frequencies were estimated for the genotype dataset using Microsatellite toolkit (Park, 2001) and Cervus version 3.0 (Marshall *et al*., 1998) software. Parentage assignment and exclusion statistics were performed using Cervus 3.0 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 (Marshall *et al*., 1998; Kalinowski *et al*., 2007).

Results

The results of the individual microsatellite marker evaluation are shown in Table 2. The markers were highly informative and complied with the prerequisites for parentage verification which requires expected heterozygosity (H_{exp}) and polymorphic information content (PIC) values of above 0.5 (Marshall *et al.,* 1998). The combined probability of exclusion for the first (CPE-1) and second parent (CPE-2) of the 11 microsatellite markers panel were 0.990 and 0.9997 respectively. The average amplification success over all samples was 95.3%, with six markers showing a

success rate in excess of 97%. Three samples failed to amplify repeatedly and were excluded from the study. The microsatellite markers were non significant for Hardy – Weinberg equilibrium test, and are assumed to be in linkage equilibrium. Very low null-allele frequencies were reported for all markers, with no marker approaching the 0.05 limit proposed by Marshall *et al.* (1998) for exclusion from a parentage verification panel.

Table 2 Parameters of microsatellite markers used for parentage verification

Locus	**k**	**n**	**% Suc**	**Hobs**	**Hexp**	**PIC**	**PE-1**	**PE-2**	**Null**	**HW**
TGLA122	9	174	97.6	0.77	0.758	0.727	0.376	0.557	-0.0061	NS
ETH10	7	174	97.6	0.69	0.635	0.609	0.247	0.435	-0.0561	NS
BM1818	7	174	97.6	0.707	0.723	0.688	0.328	0.511	0.0057	NS
TGLA126	7	173	97.2	0.815	0.793	0.761	0.414	0.593	-0.0196	NS
ETH225	7	173	97.2	0.613	0.618	0.593	0.233	0.42	0.003	NS
TGLA53	13	173	97.2	0.867	0.824	0.8	0.481	0.655	-0.0261	NS
BM1824	6	172	96.6	0.703	0.73	0.681	0.311	0.485	0.0224	NS
TGLA227	10	172	96.6	0.651	0.664	0.613	0.253	0.421	0.0136	NS
BM2113	10	171	96.1	0.83	0.812	0.782	0.445	0.622	-0.0134	NS
INRA023	10	165	92.7	0.727	0.754	0.715	0.361	0.538	0.0157	NS
SPS115	7	161	90.4	0.671	0.658	0.623	0.258	0.44	0.0229	NS
CPE							**0.9901**	**0.9997**		
MEAN	**8.45**		**95.3**		**0.7246**	**0.6901**				

K: Number of alleles, n: Number of samples, % Suc: comparative amplification success as percentage, 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 (first and second parent), Null: Null allele frequency, HW: Hardy – Weinberg equilibrium

The results of parentage verification analyses of the four sire families are presented in Table 3. Of the 87 offspring analysed, the genotypes of 85 matched with that of their dams and only two mismatches were reported. There were four loci mismatches between the dam and her offspring in stud herd I with a resulting -1.33 LOD score. The dam and her offspring in stud herd III had three loci mismatches with a -1.25 LOD score. All maternity records of stud herds II and IV were

confirmed by the molecular analyses, with no mismatches. One offspring each in stud herds I and III mismatched with their reported dams. This was in sharp contrast with the paternity records. The genotypes of only 39 offspring matched with that of their recorded sires, while 48 offspring were misallocated. The sire misallocation percentage varied greatly between herds (4.3% in herd IV vs. 80% in herd III). The average dam misidentification across all herds was 2.3%, while the sire misidentification was very high (55.2%) over the four stud herds.

Table 3 Parentage verification results of the four Boran stud herds showing number of animals compared, number of matched and mismatched offspring and parents, mismatched loci and percentage mismatches

Stud	N	Offsp	MD	MM	ML	%MM	MP	Mispat	PL	%MP
Herd I	47	24	23	1	4	4.2	7	17	1-6	71
Herd II	41	20	20	0	0	0	6	14	2-6	70
Herd III	41	20	19	1	3	5	4	16	1-6	80
Herd IV	46	23	23	0	0	0	22	1	3	4.3
Total	**175**	**87**	**85**	**2**		**2.3**	**39**	**48**		**55.2**

N: number of animals (sire, dam, offspring), Offsp: offspring, MD: number of matched offspring and dams, MM: number of mismatched offspring and dams, ML: number of loci mismatch between dam and offspring, % MM: % offspring and dam mismatch, MP: number of matched offspring and sires, Mispat: number of mismatched offspring and sires, PL: range of loci mismatch between sires and offspring and % MP: % offspring and sire mismatch

Discussion

In this study DNA based parentage verification was used to evaluate parentage recording in Kenyan Boran cattle. The microsatellite markers selected were first analysed for individual parameters influencing their suitability for inclusion in a parentage verification panel. The 11 microsatellite markers were polymorphic with an average number of alleles of 8.45 per locus. It should be noted that the animals tested were related and a higher number of alleles will be

expected if larger samples of Boran cattle is to be tested. Other studies in beef cattle have reported mean number of alleles of 9 (Sherman *et al*., 2004; Van Eennenaam *et al.*, 2007). The H_{exp} range (0.618 to 0.824) and H_{obs} (0.613 to 0.867) were in agreement to those reported by Sherman *et al*., (2004) in a similar study (0.588 to 0.843). The mean polymorphic information content (PIC) was 0.6901 and corresponded to those determined previously, which ranged from 0.626 to 0.640 in beef cattle (Cervini *et al.*, 2006; Van Eennenaam *et al.*, 2007).

The H_{exp} and PIC values of above 0.5 and null frequency values of below 0.05 were adequate for inclusion in a parentage verification panel (Marshall *et al.,* 1998). When combined into a panel, the microsatellite markers had a high exclusion power with a 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 similar markers have reported CPE-1 values of 0.9684 – 0.9936 and CPE-2 values of 0.9989 to 0.9999 (Sherman *et al*., 2004; Cervini *et al.*, 2006; Van Eenennaam *et al.,* 2007). The CPE values were sufficient to exclude non parents in the parentage analysis of the four Boran stud herds.

Two of the four herds managed 100 % correct recording of dams to offspring, while the other two herds had one recording error each. The two instances of incorrect dam records 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 low level of dam misidentification in this study indicates this as a minor problem.

The rate of sire misidentification in the four herds ranged between 4.3 and 80%. Previously reported rates of misidentification or missing sires in beef cattle include 12% reported by Van Eenennaam *et al*. (2007), 14% reported by Sherman *et al*. (2004) and 0-33.6% reported by Holroyd *et al*. (2002). The high rate of sire misidentification in this study is cause for concern as

paternal identification is critical for improvement of the rate of genetic progress (Pollak, 2005) and effective monitoring of inbreeding (Cassell *et al*., 2003).

The mating strategy of all year joining practiced by the Boran cattle breeders makes it difficult to accurately record most of the mating events. 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 was meant to aid in identification of the sire of the offspring but the two week rest period is too short to adequately identify the offspring of the sires if a supposedly pregnant cow is served by the new bull in the field. The lack of internal and external paddocks to separate breeding herds during the mating period may also lead to other sires straying from commercial herds and the neighboring farms to the stud herds. Similar observations have been reported on other beef ranches (Van Eenennaam *et al*., 2007).

The extensive system of beef production in most ranches may lead to ineffective monitoring of mating events (Gomez-Raya *et al*., 2008; Yagüe *et al*., 2009) and limit the use of the reproductive information for selection. 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. Late identification and registration of the offspring may also contribute to sire misidentification. The offspring in the three ranches with high sire misidentification rate were branded at weaning and sire records may have been incorrectly entered into the herd book. Similar observation was reported for sheep with late pedigree recording (Bolormaa *et al.*, 2008).

Some of these causes can 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 the solution. The breeder of stud herd IV had the most accurate pedigree records for both dams and sires. Cattle recording require accurate identification

of the sire, dam, and the progeny with proper maintenance of the records, a difficult task in the multiple sire breeding systems in use. Stud herd IV is kept in a relatively smaller fully paddocked farm and the recording and identification of calves is done immediately after birth. These could be the reasons for the low sire misidentification rate in this herd.

The overall sire misidentification rate in this study was 55.2% and with such a high rate, genetic progress of the breed will be slow and may even be negative (Banos *et al*., 2001). It renders the use of pedigree records inappropriate to monitor genetic progress and inbreeding trends (Wiggans *et al*., 1995). The stud herds rely on performance and progeny testing 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 (Gomez-Raya *et al*., 2008). Incorrect identification of sires can bias estimates of heritability, breeding values and genetic progress (Israel & Weller, 2000). The use of DNA-based parentage verification can mitigate several of the management challenges in Kenya and result in more accurate selection and a faster rate of genetic progress.

Conclusion

This study has shown that DNA technology can positively contribute to increased accuracy of pedigree information. The high rate of sire misidentification, the extensive production system practiced by Boran cattle breeders and infrastructural challenges render the genetic improvement of this breed difficult without the use of molecular techniques. This was the first attempt to verify pedigree accuracy in Kenyan ranches using DNA based parentage verification.

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