

Research Application Summary

**Understanding the genetic resources and adaptive traits of locally adapted cattle breeds:
the case of Afrikaner and Bonsmara cattle in Namibia**

Haikukutu, L.¹, Amushendje, T.O.¹ & Lyaku, J.R.²

¹Department of Animal Science, Faculty of Agriculture and Natural Resources, University of Namibia,
P/Bag 13301, Windhoek, Namibia

²School of Veterinary Medicine, Faculty of Agriculture and Natural Resources, University of Namibia,
P/Bag 13301, Windhoek, Namibia

***Corresponding author:** tamushendje@unam.na

Abstract

There is rising interest in locally adapted livestock breeds as reservoirs of genetic diversity and adaptive fitness traits. In Namibia locally adapted cattle breeds are mainly reared in commercial farms south of the veterinary cordon fence. This study focused on the Afrikaner and Bonsmara cattle which are locally adapted breeds and have for many years been reared under low management levels in Namibia and subjected to strong environmental pressures and diverse disease challenges. The objective of this study was to explore the adaptation potential of these two breeds by investigating its genetic diversity and resistance to ticks. The genotypic data obtained revealed that Afrikaner cattle exhibit high levels of genetic diversity with an expected heterozygosity (H_e) of 0.660 and 5.50 mean number of alleles. Bonsmara cattle also exhibited high genetic diversity with an expected heterozygosity (H_e) of 0.637 and 7.00 mean number of alleles. A clear deficit of heterozygotes was observed as evidenced by the high FIS value of 0.247 suggesting high levels of inbreeding. Contrarily, an excess of heterozygotes was observed in Bonsmara cattle suggesting outbreeding. A low tick burden of 11-30 ticks on average in summer season was observed in both cattle breeds indicating high resistance to ticks. Results suggest that Afrikaner and Bonsmara cattle possess valuable traits such as tick resistance and therefore hold potential for production in harsh and unpredictable Namibian environmental conditions which are exacerbated by climate change. The Afrikaner and Bonsmara breeds like many other indicine-taurus cattle breeds have proved to be productive in harsh environments and selecting and breeding from their high producing phenotypes and ecotypes would go a long way towards concentrating desired genes for production and tolerance to diseases, thus mitigating climate change.

Key words: Afrikaner breed, Bonsmara cattle, genetic diversity, Namibia, tick resistance

Résumé

Il existe un intérêt croissant pour les races de bétail adaptées localement en tant que réservoirs de diversité génétique et de caractères adaptatifs de morphologie. En Namibie, les races bovines adaptées localement sont principalement élevées dans des fermes commerciales au sud de la ceinture vétérinaire. Cette étude s'est concentrée sur les bovins Afrikaner et Bonsmara qui sont des races adaptées localement et ont été élevés pendant des nombreuses années sous des faibles niveaux de gestion en Namibie et soumis à de fortes pressions environnementales et à divers problèmes de maladies. L'objectif de cette étude était d'explorer le potentiel d'adaptation de ces deux races en étudiant leurs diversités génétiques et leur résistance aux tiques. Les données génotypiques obtenues ont révélé que les bovins Afrikaner présentent des niveaux élevés de diversité génétique avec une

hétérozygotie (H_e) escomptée de 0,660 et un nombre moyen d'allèles de 5,50. Les bovins Bonsmara présentaient également une grande diversité génétique avec une hétérozygotie (H_e) attendue de 0,637 et un nombre moyen d'allèles de 7,00. Un déficit clair d'hétérozygotes a été observé comme en témoigne la valeur FIS élevée de 0,247 suggérant des niveaux élevés de consanguinité. Au contraire, un excès d'hétérozygotes a été observé chez les bovins Bonsmara suggérant une hybridation. Une faible charge de tiques de 11 à 30 tiques en moyenne était observée pendant la saison estivale chez les deux races bovines, indiquant une résistance élevée aux tiques. Les résultats suggèrent que les bovins Afrikaner et Bonsmara possèdent des caractéristiques précieuses telles que la résistance aux tiques et ont donc un potentiel de production dans des conditions environnementales Namibiennes difficiles et imprévisibles qui sont exacerbées par le changement climatique. Les races Afrikaner et Bonsmara, comme des nombreuses autres races bovines Zebu indicine, se sont avérées productives dans des environnements difficiles. Par conséquent, la sélection et l'amélioration à partir de leurs phénotypes et écotypes à haute production contribueraient grandement à concentrer les gènes souhaités pour la production et la tolérance aux maladies, atténuant ainsi l'effet de changement climatique.

Mots clés : race Afrikaner, bovins Bonsmara, diversité génétique, Namibie, résistance aux tiques

Background

Namibia has a rich diversity of livestock despite being the most arid country in sub-Saharan Africa. Popular cattle breeds in Namibia include the indigenous Nguni breed, and several locally adapted breeds such as Afrikaner – also known as Sanga, Bonsmara - also known as Sanga-exotic and Brahman or Zebu breed. The indigenous and locally adapted breeds are recognized for their hardiness, adaptability to extreme environmental conditions and most importantly, resistance to ecto-parasites and disease challenges (Muchenje *et al.*, 2008; Marufu *et al.*, 2011; Nyangiwe *et al.*, 2011). Namibia is vulnerable to climate change and experiences frequent droughts which affect agricultural production and food security through both crop and livestock losses. Lucrative export of beef to the European Union and other countries constitutes a significant portion of the agricultural contributions, to Namibia's GDP. However, ticks and diseases pose a serious threat to farmers, resulting in substantial economic losses to the beef cattle industry.

Locally adapted breeds of livestock are of considerable interest since they represent potential reservoirs of adaptive fitness traits that may contribute to the future of sustainable productivity in a changing climate. Therefore, it is crucial to determine the genetic diversity and adaptation potential of the locally adapted Bonsmara and Afrikaner cattle in Namibia to ensure viability of these breeds and for them to respond to selection pressures. Threats to genetic diversity of indigenous African cattle breeds include disease epidemics, unpredictable climate changes and inbreeding. Loss of genetic diversity in indigenous and locally adapted cattle reduces fitness components such as survival, reproductive output, growth rates and their ability to adapt to harsh climatic environments and to respond to ticks and disease challenges which animals are subjected to (Sommer, 2005). Genetically diverse livestock populations provide the population with a greater range of options by meeting the growing demand for animal products to feed the growing nation. Therefore, there is a need to explore the potential of locally adapted breeds by investigating their genetic diversity.

Materials and Methods

Sample collection and DNA extraction. A total of 100 animals including 58 Bonsmaras and 42 Afrikaners from Khomas and Omusati regions in Namibia were included in this study. In the Khomas region, samples were collected from the Neudamm experimental farm located 22°30'S, 17°22'E and Claratal Bonsmara stud farm located 22°48'S, 16°49'E. In the Omusati region, samples were collected from the University of Namibia Ogongo farm located 17.74°S, 15.30 °E. Ethical approval

(FANR/68/2015) for the study was obtained from the University of Namibia through the Centre for Research and Publications. Four millilitres of blood were collected from the coccygeal vein (tail) into EDTA vacutainer tubes and stored at 4°C until DNA extraction. Genomic DNA (gDNA) from fresh and frozen blood was extracted and purified using the ZR Zymo kit (Zymo Research, CA, USA). Blood samples and ticks were collected from the animals. Data on tick counts was collected from the animals naturally exposed to ticks, while tick scoring was performed according to Herrings (2014). Ticks were counted twice in summer (September- April) and winter (May-August) by lifting the tail of the animal and counting ticks in the perineal area.

PCR amplifications. Four microsatellite markers mapped in or in close proximity to the DRB3.2 region were used namely DRB3, DRBP1, RM185 and BM1815 (Untalan *et al.*, 2007). The microsatellites were fluorescently labelled, and PCR amplification was carried out using a GeneAmp® PCR System 9700 thermocycler (Applied Biosystems, Foster City, CA, USA). Successful amplicons were genotyped using an ABI PRISM® 3500XL DNA Genetic Analyser (Applied Biosystems). Raw genotyping results were processed with the Gene-Marker™ software (www.softgenetics.com/GeneMarker.html) to determine the fragment sizes for each locus. Excel Microsatellite Toolkit (Park, 2001) was used to estimate the basic population genetic descriptive statistics including heterozygosity values (H_O) and (H_E) and mean number of alleles (MNA). Arlequin version 3.1 (Excoffier *et al.*, 2004) was used to perform the analyses of molecular variance (AMOVA) and FSTAT v2.9.3 to compute the inbreeding coefficients (FIS) for each cattle breed.

Results

Within breed genetic variation was observed, with expected heterozygosity ranging from 0.637 in Bonsmara cattle to 0.660 in Afrikaner (Table 1). The average number of alleles per locus varied between 5.50 (Afrikaner) and 7.00 (Bonsmara) with a mean of 6.25. It should be noted that observed heterozygosity was lower than the expected value in Afrikaner breeds. The PIC values varied slightly around a mean value of 0.600, from low values in Afrikaners of 0.598 to 0.602 in Afrikaner cattle, indicating the informativeness of these markers.

Table 1. Number of alleles, heterozygosity levels¹ and ⁴polymorphism information content (PIC) for each of the two cattle breeds analysed at four loci.

Population	N ¹	² He ± SD	³ H _O ± SD	⁴ PIC ± SD	⁵ No. alleles ± SD
Afrikaner	42	0.660±0.019	0.499±0.039	0.598±0.051	5.50±1.91
Bonsmara	58	0.637±0.131	0.673±0.032	0.602±0.252	7.00±2.16
Mean		0.649±0.075	0.586±0.031	0.600±0.152	6.25±2.04

¹N indicates the number of animals genotyped ²Expected heterozygosity (He) and ³Observed heterozygosity (H_O) where SD is the standard deviation and ⁵No. is the number of alleles.

The AMOVA test showed significant differentiation ($P < 0.05$) among populations, with 4.8% of the genetic variation due to differences between breeds while 95.2% was due to differences within breeds. All Bonsmara herds from Omusati and Khomas region were found to be polymorphic, with the number of alleles ranging between 5.50 in Omusati Bonsmara herd (BONO) and 6.50 in the Khomas Bonsmara (BONK) herd. Expected heterozygosity ranged from 0.616 in the Khomas Bonsmara (BONK) herd to 0.653 in the BONO herd with a mean of 0.635 while observed heterozygosity varied around an overall mean of 0.693. Although the BONO herd exhibited the lowest number of alleles (5.50), it showed a higher expected heterozygosity ($H_e = 0.653$) than the BONK herd ($H_e = 0.616$). PIC values varied from 0.581 (BONK) to 0.586 (BONO), with an overall mean of 0.584.

Table 2. Heterozygosity levels, polymorphism information content (PIC) and number of alleles of the two cattle populations based on their geographical locations in Namibia

² Population	N	Loci typed	¹ He \pm SD	¹ HO \pm SD	² PIC	³ No alleles \pm SD
BONO	20	4	0.653 \pm 0.082	0.744 \pm 0.052	0.586 \pm 0.170	5.50 \pm 1.91
BONK	38	4	0.616 \pm 0.156	0.642 \pm 0.039	0.581 \pm 0.292	6.50 \pm 1.73
Mean			0.635 \pm 0.119	0.693 \pm 0.046	0.584 \pm 0.231	6.00 \pm 1.82

¹He – Expected heterozygosity, H_o – observed heterozygosity, SD-Standard Deviation

²BONO - Omusati Bonsmara herd, BONK- Khomas Bonsmara herd

Results of the inbreeding coefficients (FIS) for each of the three populations across all loci are shown in Table 3. The Bonsmara herds showed negative FIS, ranging from -0.142 to -0.043 for the BONO and BONK herd respectively, suggesting excess of heterozygotes in the population. A clear deficit of heterozygote was observed in the Afrikaner cattle as evidenced by the positive FIS values of 0.247 suggesting high levels of inbreeding

Table 3. Inbreeding estimates (FIS) for the six herds of cattle based on their geographical location¹

Locus	AFR	BONO	BONK
DRB3	0.582	-0.286	0.131
DRBP1	0.313	0.010	-0.095
RM185	-0.056	-0.211	-0.072
BM1815	0.183	-0.162	0.006
Mean	0.247	-0.142	-0.043

AFR- Afrikaner, BONO- Omusati Bonsmara herd, BONK- Khomas Bonsmara herd.

All breeds had on average observable tick numbers of 10 under the tail in winter and an average observable tick numbers 30 in summer season (Table 4). According to the Herring (2014) tick scoring system, Bonsmara and Afrikaner cattle possess high resistance to ticks.

Table 4: Mean number of ticks in summer and winter and minimum and maximum number of ticks counted in this study

Breed	Mean \pm SD		Number of ticks	
	Summer	Winter	Minimum	Maximum
Afrikaner	17	6	0	45
Bonsmara	26	9	7	67

SD- Standard deviation

Analysis done on actual tick counts showed significant difference across summer and winter season ($P < 0.05$) and no significant difference between breeds ($P > 0.05$) in tick counts according to the mixed-design ANOVA results.

Discussion

Livestock adaptation is necessary to respond adequately to climate change, food security and livelihoods needs to feed the growing human population (Hoffmann, 2013). The livestock sector in Namibia face unprecedented challenges brought about by extreme environmental conditions. The Afrikaner and Bonsmara cattle exhibit high adaptive capacity particularly suitable for the ever changing harsh environmental conditions of Namibia characterized by frequent drought and poor

quality feeds. This is demonstrated by their high genetic diversity ranging from 0.660 in Afrikaner to 0.637 by Bonsmara cattle. The expected heterozygosity value ($He = 0.660$) is higher than the He values reported for 26 Afrikaner herds in South Africa (Pienaar, *et al.*, 2018). Although Afrikaner cattle exhibited high levels of gene diversity, they had a low number of alleles (5.50) compared to Bonsmara cattle (7.00). The mean number obtained in this study is higher than that obtained in Pienaar *et al.* (2018). However, it should be noted that the microsatellite markers genotyped in the two studies are different. High levels of inbreeding were detected in Afrikaners with an FIS value of 0.247. The level of inbreeding recorded here is an important observation as it exceeds the acceptable value of 0.15 (Blackburn *et al.*, 2011). This could be due to the inbreeding depression caused by Rinderpest pandemic which wiped out most Afrikaner cattle more than a hundred years ago (Porter, 1991).

The high level of inbreeding may also be attributed to paucity of pure Afrikaner bulls, small population sizes and strong selection as this is the main breed used in crossbreeding in Southern Africa and have to conform to the Afrikaner cattle breeder's society standards. Afrikaners have also been reported to be at risk of inbreeding in South Africa (Makina *et al.*, 2014) probably because of the selection pressure to breed pure Afrikaner animals for crossbreeding purposes. Inbreeding has negative effects which include reduced genetic diversity, milk production losses, reduced survival, decreased lactation length and decreased fertility as demonstrated in Holstein cattle (Thompson *et al.*, 2000; McPaland *et al.*, 2007). An excess of heterozygotes was observed in Bonsmara cattle with an inbreeding coefficient of -0.057, this can be interpreted as possible signs of outbreeding. This suggests a positive effect of the managed breeding taking place for this breed. Makina *et al.* (2014) also reported an excess of heterozygotes (FIS = - 0.017) in South African Bonsmara. Differences in genetic diversity observed in the two Bonsmara herds from Khomas and Omusati regions could be due to the different environmental conditions of the two regions. Omusati region is drier, constantly affected by droughts and is located in the northern communal areas (NCAs) which are considered disease-prone. Animals in this part (NCAs) of the country are constantly challenged with diseases, low quality feed and limited water which possibly explains their high genetic diversity.

The Bonsmara cattle exhibited the lowest genetic diversity ($He = 0.637$) in this study and had high tick burdens. It is probable that there is a genetic component of variation in host resistance to ticks. The differences in tick counts observed in the two breeds could also be due to morphological traits such as coat colour and skin thickness (Marufu *et al.*, 2011) along with environmental factors and vegetation composition (Schulz *et al.*, 2014) which affect the exposure to ticks as well as their survival on the host.

Conclusion and recommendations

This study presents a detailed analysis of the genetic diversity and differentiation of two beef cattle breeds in Namibia from different regions. The study revealed that locally adapted breed exhibit high levels of genetic diversity based on the results of four microsatellite markers analysis. It will be worthwhile to assess the genetic diversity of more Afrikaner and Bonsmara herds from different localities to fully understand the genetic diversity within this breed. The high genetic diversity of these breeds suggests that there is a great need for the conservation of these breeds, as they hold potential for production in harsh and fluctuating Namibian environments characterised by drought. Excessive inbreeding detected in the Afrikaners breed suggests the need for appropriate measures to be taken to avoid the negative effects of inbreeding. It is recommended that more sites be sampled to find out if all Afrikaner populations are at risk of inbreeding as reported in South Africa. Differences in genetic diversity between the Bonsmara herds from different geographical areas reflect how these animals have evolved to adapt to their different environments. Indiscriminate crossbreeding should be avoided to preserve the genetic diversity and to prevent the irreversible effects which can put these breeds at risk of extinction. A more detailed investigation using SNP markers may provide

more definite results. Further research on the association of genetic diversity with disease resistance is recommended. Overall, this study provides results that can be useful in breeding strategies for parasite and disease resistance.

Acknowledgement

This study was carried out with the funding support by the National Commission on Research Science and Technology (NCRST) of Namibia. The authors are very grateful for the financial support received from the NCRST. We are also thankful to the bilateral collaborators from the University of Pretoria, South Africa: Professor Este van Marle Koster, Dr. Karina Visser and Mrs. Lydia Bosman for excellent technical assistance during the research, particularly the genotyping. We also acknowledge the support by the Department of Animal science, Faculty of Agriculture & Natural Resources, University of Namibia. This paper is a contribution to the Fifteenth RUFORUM Annual General Meeting held 2-6 December 2019 in Cape Coast, Ghana.

References

- Bowles, D., Carson, A. and Isaac, P. 2014. Genetic distinctiveness of the Herdwick sheep breed and two other locally adapted hill breeds of the UK. *PLoS One* 9 (1): 87823.
- Herrings, A. D. 2014. Beef cattle production systems. Wallingford, UK: CABI.
- Hoffmann, I. 2013. Adaptation to climate change – exploring the potential of locally adapted breeds. *Animal* 7(2): 346–362.
- Makina, S. O., Muchadeyi, F. C., van Marle-Koster, E., MacNeil, M. D. and Maiwashe, A. 2014. Genetic diversity and population structure among six cattle breeds in South Africa using a whole genome SNP panel. *Frontiers in Genetics* 5: 333-339.
- Marufu, M. C., Qokweni, L., Chimonyo, M. and Dzama, K. 2011. Relationships between tick counts and coat characteristics in Nguni and Bonsmara cattle reared on semiarid rangelands in South Africa. *Ticks and Tick-borne Diseases* 2(3): 172-177.
- Mcparland, S., Kearney, J. F., Rath, M. and Berry, D. P. 2007. Inbreeding effects on milk production, calving performance, fertility and conformation in Irish Holstein-Friesians. *Journal of Dairy Science* 90 (9): 4411-4419.
- Muchenje, V., Dzama, K., Chimonyo, M., Raats, J. G., and Strydom, P. E. 2008. Meat quality of Nguni, Bonsmara and Aberdeen Angus steers raised on natural pasture in the Eastern Cape, South Africa. *Meat Science* 79 (1): 20-28.
- Pienaar, L., Grobler, J.P., Scholtz, M.M., Swart, H., Ehlers, K., Marx, M., MacNeil, M.D. and Nester, F.W. 2018. Genetic diversity of Afrikaner cattle in southern Africa. *Tropical Animal Health and Production* 50 (2):399-404.
- Porter, V. 1991. Cattle. Marlborough, England: Crowood Press.
- Untalan, P. M., Pruett, J. H. and Steelman, D. C. 2006. Association of the bovine leukocyte antigen major histocompatibility complex class II DRB3 *4401 allele with host resistance to the Lone Star tick, *Amblyomma americanum*. *Veterinary Parasitology* 145 (1-2): 190-195.