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Short communication

Evaluation of genetic variability in Brahman cattle breed in Malaysia using microsatellite markers

Abdelwahid H.H[1]. Panandam J.M[2]. Sharma R.S.K[3]. Yaakub H[4].

[1]*Department of Animal Breeding and Genetic, College of Animal Productions, University of Bahri,Alkaduro-Khartoum North P.O. Box 1660, Sudan*

[2]*Ecotone Worldwide Sdn. Bhd., Suite 912, Block A, Kelana Centre Point, 3 Jalan SS 7/19, Kelana Jaya, 47301 Petaling Jaya, Selangor D.E., Malaysia*

[3]*Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia*

[4]*Department of Animal Science, Faculty of Agriculture,Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.*

Corresponding Author: Abdelwahid H.H, University of Bahri Alkaduro-Khartoum, Sudan; **Email**: hhago2000@yahoo.com

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Abstract

The Brahman was one of the exotic breeds imported into Malaysia to help boost the local beef cattle industry. Due to its adaptability and the productive performance, it is considered as a suitable breed for the country, as well as a breed for use in crossbreeding. This study aimed to assess the genetic variability in the Brahman breed in Malaysia using microsatellite markers. Twenty microsatellite loci,BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH152, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILSTS005, ILSTS006, INRA005 and INRA023, were used to screen a random sample of 32 of Brahman cattle at the Brahman nucleus herd at DVS Livestock Center in Kuala Berang, Terengganu. A total of 151 alleles were detected. The number of alleles per locus varied between 6 and 10 with an average of 7.6. The effective number of alleles (Ne) range between 2 and 7 with average of 3.8. The observed heterozygosity values (0.26 - 0.83) were lower than the expected heterozygosity values for almost all loci. The overall mean heterozygosity was 0.54. The values of inbreeding coefficient (FIS) based on the individual locus were generally positive. Thirteen loci deviated from HWE equilibrium ($p<0.05$). This study showed that there was low genetic variability in the Brahman nucleus herd in Malaysia.

Keywords: Genetic variability, effective number of alleles, heterozygosity; nucleus herd

Introduction

The production performance of the indigenous Kedah Kelatan cattle breed (KK) was not able to meet the growing demand for beef in Malaysia; therefore, a number of exotic cattle breeds, both Zebu and Taurine breeds, were imported into Malaysia for crossing with KK or, in some cases, to replace it. Importation began in the 1970s; the main purpose being to increase the base beef cattle population with the aim to increase self-sufficiency in beef products (Jelan & Dahan, 1998). The imported breeds included Aberdeen Angus, Bali cattle, Brahman, Chinese Yellow cattle, Droughtmaster, Hereford, Nelore, Santa Gertrudis and Shorthorn. Except for Brahman, Bali cattle, Chinese Yellow cattle, Droughtmaster and Nelore, the other exotic breeds are no longer found in Malaysia. The poor reproductive performance and the low survival rates are the two main factors that contributed to the failure of most of the exotic breeds in Malaysia.

The Department of Veterinary Services Malaysia (DVS) had imported the Brahman from Australia (Sivarajasingam, 1984) and maintained a nucleus herd at the DVS livestock center in Kuala Berang, Terengganu. The Brahman is a popular Zebu cattle breed. It originated from India butis presently distributed all over the world. The Brahman proved to be well adapted to the Malaysian environment; based on its good productive performance the Brahman is considered as a suitable breed for the country as well as a breed for

use in crossbreeding in the formation of synthetic breeds. It has contributed the development of the local beef industry (Johari & Jasmi, 2009). Many owners of big farms have Brahman herds and smaller farm owners cross their cattle with the Brahman. To date there is little knowledge on the genetic variability of the Brahman cattle in Malaysia. The present study aimed to assess the genetic diversity and inbreeding in the nucleus herd of Brahman cattle breed in Malaysia using microsatellite markers.

Materials and methods

The Brahman nucleus herd at the DVS Livestock Center in Kuala Berang, Terengganu. was used as the study population. Blood samples from 32 animals were randomly selected for the study from blood collection during routine herd health screening. DNA was extracted from the whole blood using the QIAamp DNA Blood Kit (Qiagen) according to the manufacturer's instructions. The DNA quality and quantity was checked using spectrophotometry and agarose gel electrophoresis. The samples were screened for 20 microsatellite loci: BM1818, BM1824, BM2113, CSRM60, CSSM66, ETH3, ETH10, ETH152, ETH185, ETH225, HAUT24, HAUT27, HEL1, HEL5, HEL9, HEL13, ILSTS005, ILSTS006, INRA005 and INRA023.

These loci are part of the list recommended by Food and Agriculture Organization of the United Nations (FAO) for genetic diversity studies in cattle (FAO, 2004). Polymerase chain reaction (PCR) was carried out in a total volume of 15 µl containing 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM dNTPs (Promega), 0.4 µM each of forward and reverse primers, 1U Taq DNA polymerase (Promega) and 50 ng/µl of genomic DNA.

PCR was accomplished by using a touchdown programme. The PCR cycling conditions were as follows: initial denaturation for 8 min at 95 ^{o}C, followed by 40 cycles of denaturation at 95 ^{o}C for 45 s, annealing at temperatures ranging from 64 - 54 ^{o}C for 45 s, and extension at 72 ^{o}C for 1 min, and final extension at 72 ^{o}C for 10 min. The forward primers for each locus were 5'- labelled with three separate WellRED fluorescent dyes: black (D2), green (D3), and blue (D4). The PCR products were separated using capillary electrophoresis (CEQ 8000, Beckman Coulter).

In order to avoid any confusion or overlapping peaks within the common run, poolplexing technique was applied in this study. PCR amplicons for same sample but for different loci were combined together in a single tube and ran on single capillary. Poolplexing technique allows rapid identification of the DNA fragment sizes and minimizes miscalls in the scoring of allelic peaks. It is an essential element for decreasing the cost of the reagents and increasing the throughput.

The use of three different dyes in this study allowed for the poolplexing of samples during separation and allele sizing. Depending on the fragment size ranges, PCR products of three to six loci for one individual, labelled with a different dye (D2, D3 and D4) were pooled together. A 1.8 µl volume of each poolplexed (D4 = 0.3 µl, D3 = 0.6 µl and D2 = 0.9 µl) was added to 0.7 µl of 400 bp DNA size standard, labelled with D1 dye (red) and 37.5 µl of sample loading solution (Beckman Coulter, USA) in one of the wells of a 96 - well sample plate and overlaid with mineral oil. Each poolplexed sample was separated using the CEQ8000 system. Fragments were sized using the default fragment analysis protocol AE2.The capillary array was installed carefully in the CEQ 8000.

The wetting tray was filled with deionised water (the capillary ends sit in the wetting tray when they are not in use). The sample plate and buffer plate were installed in correct orientations, and the acrylamide gel cartridge was inserted. The sample information was entered corresponding to the sample plate setup. The separation method Frag-3 was used with the following running conditions: capillary temperature 50 OC, denaturation temperature 90 OC for 120 s, injection voltage 2.0 kV for 30 s, and separation voltage 6.0 kV for a run time of 35 min.

The POPGENE software version 1.31 (Yeh *et al.,* 1999) was used for data analysis.

Results and discussion:

Screening of the 32 Brahman cattle for 20 microsatellite loci detected, a total of 151 alleles with a mean number of alleles (MNA) of 7.6(Table 1). The most polymorphic loci were CSRM60 and CSSM66 with 10 alleles each, and the least polymorphic were BM1824, ETH3, ETH152, ETH185, ETH225 and HEL13 with six alleles each. The effective number of alleles (MNE) ranged from 2 at BM1824 to 7 at CSSM66; the mean was 3.8 alleles per locus. The MNE values were less than the MNA values for all 20 loci. The results of the present study showed that the Brahman herd exhibited low MNA (7.6). The smaller gene pool of the Brahman was probably due to the Brahman being an improved breed, and the local population being developed from an imported founder herd of limited size. Improved breeds often as a result of the selection process have a reduced number of alleles at some of the loci. The MNA of Brahman was lower than that reported for four Chinese native cattle breeds: Zaobei (MNA = 10.5), Guanling (MNA = 10.4), Enshi (MNA = 10.3) and Nanyang (MNA = 10.1) (Zhang *et al.,* 2007). Thelow MNA of the Brahman may also be attributed to the herd population size, the sample size and the sampling technique used in the present study. The MNA values observed in the present study, however, were higher than those reported for six Spanish native cattle breeds (4.9 - 6.7) (Marti´n-Burriel *et al.*, 1999). The sample size for the Spanish breeds ranged from 29 to 50 individuals.

Average heterozygosity is the best general measure of genetic variation (Allendorf & Luikart, 2007). The results of the present study showed that the Brahman herd had low observed heterozygosity (Ho), 0.54. Only three loci had heterozygosity above 70%. In addition, the values of Ho for all the loci, except ETH152, and the mean Ho were lower than the expected heterozygosity (He) values, indicating heterozygous deficiency. The low heterozygosity could be attributed to isolation of the nucleus herd and possible inbreeding due to small herd size, which would result in loss of alleles in low frequencies thus contributing to the low MNA. If uncheck this may subsequently result in loss of unexploited genetic potential (Ojango *et al.,* 2011). High heterozygosity values for a breed, on the other hand, would suggest possible mixed genetic background of the breed or herd, or historic gene flow between different populations.

Inbreeding coefficient (F_{IS}) shows the deficiency of heterozygotes in a population. A negative F_{IS} value indicates an excess of heterozygosity that could be a cue of outbreeding. A positive value indicates a deficit of heterozygosity, which implies inbreeding. In the present, study the mean F_{IS} value was positive for the Brahman (0.240), indicating occurrence of inbreeding in the herd. This may be due to non random mating; some non-stringent selection and controlled mating were practised in the farm concerned. Although some record keeping was practiced, this was not complete; pedigree records were often not available. The F_{IS} values observed in the present study was higher than that reported for 27 native cattle breeds in China (0.007- 0.147) (Zhang *et al*., 2007), and eight native Ankole populations in Uganda (-0.040 – 0.054) (Kugonza *et al*., 2011).These populations were also screened using the same 20 microsatellite loci used in the present study. However, in these reported studies each breed was not represented by animals from a single farm as was the case in the present study.

A population is said to be in Hardy-Weinberg equilibrium (HWE) when the gene and genotype frequencies remain constant from generation to generation, and the latter is of a definite proportion (Falcorner & Mackay, 1996). In the present study the deviation from HWE was observed in the Brahman breed (13 loci). Deviation from HWE could be attributed to many causes, among which are selection, assortative mating, migration and small population size, all of which may have contributed to the deviation from HWE observed for this population. The numbers of loci that deviated from HWE was high compared to that reported for 10 Ethiopian native cattle using the same 20 microsatellite loci (3 - 6 loci) (Dadi *et al.,* 2008) and for 10 native Portuguese cattle breeds (1- 5 loci) (Mateus *et al.,* 2004). In general, the low genetic diversity observed in the present study in terms of low mean number of the alleles, heterozygote deficiency and deviation from

HWE could be attributed to many reasons, but the most probable reasons are inbreeding, small population sizesand assortative mating.

Table 1. Observed (Na) and effective (Ne) number of alleles, observed (Ho) and expected (He) heterozygosity, inbreeding coefficient (FIS) and probability values of the Chi-square test for deviation from Hardy-Weinberg equilibrium (HWE) for 20 microsatellite loci in the Brahman cattle breed**.**

		No. of Alleles		Heterozygosity			
No.	locus	Na	Ne	Ho	He	FIS	HWE (p-value)
1	BM1818	7	3.10	0.56	0.70	0.179	0.00*
2	BM1824	6	2.00	0.43	0.50	0.124	0.29
3	BM2113	9	3.80	0.50	0.75	0.323	0.06
4	CSRM60	10	4.10	0.71	0.77	0.066	0.03*
5	CSSM66	10	7.00	0.83	0.87	0.027	0.40
6	ETH3	6	2.60	0.39	0.62	0.357	0.00*
7	ETH10	7	4.30	0.76	0.78	0.011	0.70
8	ETH152	6	3.00	0.70	0.68	-0.048	0.00*
9	ETH185	6	2.60	0.41	0.63	0.341	0.00*
10	ETH225	6	3.20	0.42	0.70	0.382	0.04*
11	HAUT24	8	4.30	0.52	0.78	0.324	0.06
12	HAUT27	7	3.40	0.45	0.72	0.359	0.01*
13	HEL1	8	4.00	0.57	0.77	0.236	0.23
14	HEL5	7	5.50	0.09	0.84	0.889	0.00*
15	HEL9	9	5.00	0.70	0.81	0.119	0.00*
16	HEL13	6	2.90	0.69	0.67	-0.051	0.54
17	ILSTS005	8	4.50	0.46	0.79	0.407	0.00*
18	ILSTS006	9	2.30	0.26	0.57	0.531	0.00*
19	INRA005	7	4.30	0.70	0.78	0.081	0.00*
20	INRA023	9	3.90	0.64	0.76	0.144	0.00*
mean		7.55	3.79	0.54	0.72	0.240	
SD		1.39	1.19	0.18	0.09		

*P < 0.05

Conclusion

The results of this study suggest low genetic variability and presence of inbreeding in the nucleus herd of Brahman cattle at the DVS Livestock Center in Kuala Berang, Terengganu. However, more microsatellite loci (30) as recommended by ISAG/FAO advisory group and a larger number of animals must be studied. In addition, other Brahman populations should also be investigated to obtain better understanding of the genetic structure of the Brahman cattle in Malaysia. This information may then be utilized to control the inbreeding and maintain the genetic diversity of the Brahman nucleus herd, which are vital for genetic improvement of the production performance of the Brahman breed in Malaysia.

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