ASSESSMENT OF GENETIC DIVERSITY IN MALIN SHEEP USING MICROSATELLITE MARKERS

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ABSTRACT. Malin sheep is the indigenous sheep breed of Malaysia and mainly kept for meat production. A total of 48 individuals from the National Institute of Veterinary Biodiversity (NIVB) in Jerantut, Pahang were used. The objective of this study was to assess the genetic diversity in the Malin using microsatellite markers. Eleven microsatellite loci were successfully amplified in 48 Malin sheep. All loci were polymorphic. A total of 66 alleles were detected. The number of observed alleles per locus varied from 12 to 21, with mean observed number alleles per locus of 15.18±4.58. The observed heterozygosity and expected heterozygosity were 0.0189±0.01 and 0.8989±0.01, respectively. The mean polymorphic information content (PIC) value was 0.8970±0.01, indicating that the used markers were highly informative and could be used in parentage identification. Tests of genotype frequencies for deviation from the Hardy-Weinberg equilibrium (HWE), at each locus revealed depature from HWE due to loss in heterozygotes by high levels of inbreeding. The average inbreeding value for the 11 markers investigated was 0.9797±0.01 indicating a more homozygous nature of the population. This is the first report of microsatelitte based variations in Malin sheep breed and can be useful for development of a rational breeding strategy for genetic improvement of sheep in Malaysia which may benefit future conservation programmes.

Keywords: Microsattellite, inbreeding, genetic diversity, Malin sheep, indigenious breed

INTRODUCTION

The Malaysian indigenous sheep breed (a.k.a. Malin) is mainly kept for meat production, instead of wool production. Commonly, they are found in north-eastern and central part of Peninsular Malaysia especially in Kelantan, Terengganu, Pahang and Negeri Sembilan (Mastura *et al.,* 2014). A study of its genetic diversity is important but there is a lack of information about it.

Genetic studies on the genetic diversity of small ruminants based on microsatellite markers have been accelerated over the past decades (Musthafa *et al.,* 2012; Ahmed *et al.,* 2014). Microsatellite markers have been proven useful for genetic diversity studies, parentage test, linkage analysiss and population genetic testing such as construction of phylogeny and relationships among populations (Musthafa *et al.,* 2012; Ahmed *et al.,* 2014; Ivanna *et al.,* 2002; Yasemin *et al.,* 2014; Nokuthula *et al.,* 2014; El Nahas *et al.,* 2008; Pramod *et al.,* 2009; Qanbari *et al.,* 2007). A microsatellite

is a tract of repetitive DNA located in a non-coding area in the genome. Microsatellites are noted for their high mutation rates and high diversities in a population. They can be selected from a list recommended by the Food and Agricultural Organisation (FAO) and the International Society of Animal Genetics (ISAG) (FAO, 2004).

This preliminary study was carried out to assess the genetic diversity of Malin sheep at a government farm, the NIVB, of the Department of Veterinary Services, Malaysia. This nucleus herd has become a focal point in initiating genetic diversity studies on Malin sheep in Malaysia, so that effective conservation strategies could be implemented in future for this breed.

MATERIALS AND METHOD

Sampling and DNA extraction

This study, conducted in August 2017 until January 2018, collected 10 ml peripheral blood sample each from 48 Malin sheep. The animals were randomly selected from a nucleus herd at the NIVB. The herd was newly transferred from Pusat Ternakan Haiwan (PTH) Jeram Pasu, Pasir Putih Kelantan to NIVB. The selection was made based on the DairyChamp database. Genomic DNA was isolated using a commercial DNA extraction kit (Qiagen™) according to the manufacturer's instructions. The extracted DNA was appropriately labelled and stored at -20 °C for analyses.

Microsatellite amplification

A total of 11 microsatellite loci were used in this study: OARHH35, OARHH64, OARVH72, SRCRSP9, MAF209, OARJMP29, OARHH41, OARJMP8, MCM527, OARAE129 and OARCP20. These loci are a part of a list recommended by FAO) and ISAG (ISAG/FAO Standing Committee, 2004). Polymerase chain reaction (PCR) was carried out in a total volume of 25 µl containing 1× Gotaq Green Master Mix (Promega™), 1.25 µl each of forward and reverse primers and 2 µl of DNA template. PCR was accomplished by using the touchdown method. The PCR cycling conditions were as follows: initial denaturation for 2 min at 95 °C, followed by 35 cycles of denaturation at 95 °C for 45 s, annealing at temperatures ranging from 53 °C to 64 °C for 45 s and extension at 72 °C for 1 min, and final extension at 72 °C for 5 min.

Agarose Gel electrophoresis

The PCR product obtained was analysed by HyAgarose™, HE agarose™ (Hydragene™) gel 1.5% in 1× TBE buffer for 40 mins at 70 volt. The gels were stained with Fluorosafe™ (1st Base™) and visualised under UV light on a transilluminator and the PCR fragment size was determined using software GeneTools™ Ver. 3.08 (Syngene™).

Data Analysis

The results obtained from fragment analysis of each loci were used to determine the number of observed alleles (N_a), effective number of alleles (N_e), expected

heterozygosity (H_e), observed heterozygosity (H_o). The Hardy-Weinberg equilibrium (HWE) test was calculated using GenAlEx™ (Peakall and Smouse, 2006). The inbreeding coefficient (F_{IS}) (Weir and Cockerham, 1984) was determined using Genepop™, version 4.0.7 (Raymond and Rousset, 1995). The polymorphic content information (PIC) for each marker was calculated using the following equation:

$$\mathbf{PIC} = 1 - \sum_{i=1}^{n} p_i^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2p_i^2 p_j^2$$

Where *pi* is the frequency of the *i*th allele, and *n* is the number of alleles (Botstein *et al.,* 1980).

RESULTS AND DISCUSSION

Since this is the first preliminary report on genetic diversity of Malin sheep using microsatellite markers in Malaysia. All eleven microsatellites in this study were found to have polymorphic patterns and a total of 66 alleles (Table 1). According to Kalinowski (2004), the simplest measure of genetic diversity at a determined locus is the number of alleles (i.e. allelic richness). A considerable level of genetic variability for each tested loci must be greater than two in terms of number of alleles (Crawford *et al.,* 1995). The polymorphic informative content (PIC) is a parameter of the informativeness of a marker

Table 1. Parameter of genetic information content of eleven microsatellite loci estimated from the breeding herd of Malin sheep.

MARKER	Allele number, N_a	Effective Number of allele, N_e	Expected Hetero-zygosity, H_e	Observed Hetero-zygosity, H_o	Polymorphic Information Content, PIC	Hardy-Weinberg Equilibrium, HWE (significance)	Inbreeding coefficient, F_{IS}
OARHH35	17	12.6593	0.9210	0.1042	0.9197	0.001	0.8891
OARHH64	18	10.4727	0.9045	0.0417	0.9041	0.001	0.9549
OARVH72	14	10.4727	0.9045	0.0000	0.9031	0.001	1.0000
SRCRSP9	15	9.5207	0.8950	0.0000	0.8932	0.001	1.0000
MAF209	15	11.6954	0.9145	0.0417	0.9135	0.001	0.9554
OARJMP29	18	12.2553	0.9184	0.0000	0.9179	0.001	1.0000
OARHH41	12	9.9310	0.8993	0.0000	0.8971	0.001	1.0000
OARJMP8	12	6.4719	0.8455	0.0000	0.8407	0.001	1.0000
MCM527	13	10.0174	0.9002	0.0000	0.8988	0.001	1.0000
OARAE129	21	11.7252	0.9147	0.0208	0.9138	0.001	0.9777
OARCP20	12	7.7315	0.8707	0.0000	0.8654	0.001	1.0000
Mean	**15.18**	**10.27**	**0.8989**	**0.0189**	**0.8970**		**0.9797**
SE	**4.58**	**0.57**	**0.01**	**0.01**	**0.01**		**0.01**

and it ranges from 0 to 1. The value of PIC for the 11 microsatellite markers are shown in Table 1. The mean PIC was 0.8970±0.01, ranging from 0.8407 for OARJMP8 to 0.9197 for OARHH35. All the loci used showed PIC values higher than 0.5, which proved their utility in genetic diversity studies. The most desired loci for genetic diversity studies is when the PIC value is 1 or close to 1 (Bostein *et al.,* 1980).

The highest allele number was 21 (OARAE129) while the lowest is 12 (OARHH41,OARJMP8,OARCP20). The mean N_a and mean N_e were 15.18±4.58 and 10.27±0.57, respectively. However, the values were lower compared to Kivircik sheep (Yasemin *et al.,* 2014) with a mean of 16.27 and 11.89, respectively. Hence, the values in this study were higher than that reported for other sheep breeds (Al-Barzinji *et al.,* 2011; Musthafa *et al.,* 2012; Ivanna *et al.,* 2002; Nokuthula *et al.,* 2014; El Nahas *et al.,* 2008; Pramod *et al.,* 2009; Qanbari *et al.,* 2007).

Mean H_o and H_e were 0.0189±0.01 and 0.8989±0.01, respectively. 7 out of 11 loci showed zero value for H_o (Table 1). The values of mean H_e was almost similar to that reported by El Nahas (2008) for Egyption indigenous sheep breeds: Barki (0.86), Ossimi (0.811) and Rahmani (0.855). The higher value of mean H_e in this study indicated heterozygote deficiency within the breed. This also supported by HWE deviation for all the loci ($p<0.001$). Kivircik sheep (0.78) (Yasemin *et al.,* 2014), Hamdani sheep (0.76) (Al-Barzinji *et al.,* 2011), Nguni sheep (0.61) (Nokuthula *et al.,* 2014), Najdi sheep (0.75) (Musthafa *et al.,* 2012), Kail sheep (0.72) (Ahmed *et al.,* 2014) and Vembur sheep (0.73) (Pramod *et al.,* 2009), all showed lower mean H_e when compared to Malin sheep. The heterozygote deficiency is commonly related with high inbreeding coeffient (F_{IS}). In this study, the mean inbreeding coefficient (F_{IS}) was 0.9797±0.01. The potential factors that could contribute to heterozygote deficiency were the presence of null allele, small sample size, the Wahlund effect and relatedness mating (inbreeding) (El Nahas *et al.,* 2008; Al-Barzinji *et al.,* 2011). Moreover, the higher inbreeding coefficient (F_{IS}) indicated that the breeding herd was under selective pressure, which reflect an excess of homozygotes. From the herd structure of this breed, it showed that there was no proper breeding scheme applied. It suggests that the same rams were mated among ewes in the same group over few years. Appearently, this group of sheep were newly transferred from PTH to NIVB for a conservation programme involving selection for breeding and genetic improvement.

CONCLUSION

The findings in this study is initial step in assessing the genetic variability at the DNA level of Malin sheep, an indigenous breed of Malaysia. The microsatellite markers used in this study were good for genetic diversity studies (PIC>0.5). The genetic information gained from this study could be useful as a basis for development of a rational breeding strategy for genetic improvement of Malin sheep in Malaysia to benefit future conservation programmes.

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