Global Veterinaria 9 (1): 113-116, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.1.64140

Genetic Diversity of Gembrong Goat Based on DNA Microsatellite Markers

[1]I. Nyoman Sulabda, [2]Ni Nyoman Werdi Susari, [2]Ni Luh Gde Surya Heryani and [2]I. Ketut Puja

[1]Departement of Veterinary Physiology,
Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia
[2]Departement of Veterinary Anatomy,
Faculty of Veterinary Medicine, Udayana University, Bali, Indonesia

Abstract: The present study was undertaken with primary objective to characterize Gembrong goat breeds. It is essential to characterize the germplasm for intragenetic variability, which will help in planning for conservation strategy as well as genetic improvement. DNA genome was isolated from hairs. Fourteen microsatellite were amplified by PCR. PCR product were run on 6% bis-Acrylamide gel in automated DNA sequencer. The result showed that all markers successfully amplified in Gembrong goat microsatellite loci. All the investigated microsatellites were found to be highly polymorphic, except SCRSP23, INRA063 and CSRD247. A total of 32 alleles were observed in the whole samples and the number of alleles per locus varied from one 1 (SCRSP23, INRA063 and CSRD247) to 5 (HSC) alleles. The allele sizes ranged from 85 bp (SCRSP23) to 302 bp (HSC). The PIC values ranged from 0,00 (locus SCRSP23, INRA063 and CSRD247) to 6162 (locus HSC). The average observed and expected heterozygosities ranged from 0.00 (locus SCRSP23, INRA063 and CSRD247) to 0,7308 (locus HSC) and 0.00 to 0,6765 (locus HSC), respectively.

Key words: Genetic Diversity · Gembrong Goat · Microsatellite Marker · Polymorphism

INTRODUCTION

The goat was the first animal domesticated to produce product for the mankind. The presence of the goat in all sectors of ancient societies has continued to the present, particularly in religion, economy, nutrition and tradition [1]. Goats are the most widely spread domestic species in the world and play an important economical role in developing countries [2]. Goats have been important for food and economic securities for countless years and their contributions to economic returns in developed countries has been rising as well [3].

There are numerous local goat breeds in Indonesia, which have a strong fitness and foraging capability under a wide range of habitats. Due to the habitat difference across Indonesia, it is natural that there are phenotypic differences among different native breeds. Currently, there are 7 breeds of indigenous goats from Indonesia which have been classified based on the conformation traits and geographical distributions.

Gembrong goat is the one of the indigenous goat from Indonesia. Gembrong goat are medium size animal and live only in Bali. Gembrong goat is a threatened breed whose actual population consists of 26 individuals and distributed only in restricted area in Karangasem regency, Bali. Indonesia. The number of goat decreased rapidly due to the introduction of modern commercial goat breeds in Bali, the loss of suitable habitat and uncontrolled intermixing. These process are leading to the endangered of breed purity and potentially important goat genetic material is being put to risk. It is essential to characterize the germplasm for intragenetic variability, which will help in planning for conservation strategy. Thus, information about the genetic profile of the Gembrong goat population is a first step in prioritization of breeds for conservation and necessary to develop strategies for conservation and effective long-term management for this species.

Microsatellite markers are widely accepted as a choiced marker for genetic characterization of populations as they are highly polymorphic single locus DNA sequences scaterred throughout the genome and are

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Corresponding Author: I. Ketut Puja, Faculty of Veterinary Medicine, Udayana University, Bali Indonesia.

readily adaptable to Polymerase Chain Reaction (PCR). Many authors have used microsatellites for molecular genetics characterization of goat [4]. However, the genetic characterization for Gembrong goats breeds has not been assessed and the present work will become the first study of the kind.

The aim of this study is to validate a panel of 14 microsatellites markers as a tool of genetic characterization of Gembrong goat in order to estimate the allelic frequency of different microsatellite gene loci and to establish a microsatellite gene profile for Gembrong goat and to estimate the percent heterozygosity and polymorphic information content (PIC) values for these microsatellite markers.

MATERIALS AND METHODS

Microsatellite DNA Markers: Twenty six (n=26) Gembrong goat samples were analyzed. Goat genomic DNA for polymerase chain reaction (PCR) amplification was extracted from hairs sample used the technique described by Veterinary Genetics Laboratory at the University of California-Davis. A total of 14 microsatellites markers were selected (INRA005, INRA023, INRA063, ILSTS19, ILSTS87, CSRD247, HSC, MAF65, McM527, OarFCB20, SPS113, SRCRSP5, SRCRSP8, SRCRSP23) and used to analyze Gembrong goat populations (Fig. 1).

Microsatellite Genotyping: To detect microsatellite polymorphisms, all markers were amplified with PCR. PCR amplification was carried out on PCT 100 (MJ Research, Inc, Watertown, Mass, USA). PCR products were run on 6% bis-acrylamide gel in automated DNA sequencer (ABI 377 DNA sequencer, PE Applied Biosystems). Fluorescent signals from the dye labeled microsatellite were detected using Genescan 3.1 sofware (PE Applied Biosystems).

Fig. 1: A Gembrong goat

Computation: Alleles were designated according to PCR product size and allelic frequencies, number of alleles and both observed and expected heterozygosity, the polymorphism information content (PIC) were calculated using Microsatellite Toolkit V.3.1 [5].

RESULTS

The result showed that all 14 markers successfully amplified in Gembrong goat microsatellite loci. All marker microsatellite loci examined were polymorphic in all populations except SCRSP23, INRA063 and CSRD247. A total of 32 alleles were observed in the whole samples and the number of alleles per locus varied from one 1 (SCRSP23, INRA063 and CSRD247) to 5 (HSC) alleles, with a mean of 2.57. The allele sizes ranged from 85 bp (SCRSP23) to 302 bp (HSC). The PIC values ranged from 0.00 (locus SCRSP23, INRA063 and CSRD247) to 6162 (locus HSC). The average observed and expected heterozygosities ranged from 0.00 (locus SCRSP23, INRA063 and CSRD247) to 0,7308 (locus HSC), with an average value of 0,3352 and 0.00 to 0.6765 (locus HSC), with an average value of 0.3181, respectively. The results of the microsatellite analysis in terms of number of alleles observed, alleles size, polymorphism information content and heterozygosity of Gembrong goats were presented in Table 1.

DISCUSSION

The number of alleles identified in our study varied from one to 5, with mean of 2.57. The avarage alleles values for Gembrong goat were lower compared to those values obtained for Croatian spotted goat and Markhoz goat, which number of alleles of 8.1[4, 6], for Barbari goats from India was 6.3 [7], Raeini goat from Iran was 7.8 [8] and for Egyptian and Italian goat breeds was 6.5 [9].

The average observed heterozygosity (Ho) and expected heterozygosity values for the Gembrong goat populations were 0.3352 and 0.3181. Gene diversity estimated in this breed was in the range (0.3 to 0.8) to be useful for measuring genetic variation [10]. This value was lower to those reported for Portuguese goat breeds (HO=0.636, HE=0.702) [11], it is also lower than Croatian spotted goat with means for expected gene diversity of 0.771 and observed heterozygosity of 0.759 [4]. According to Ho, the level of genetic variability of Gembrong goat is smaller compared to other breed. Locus HSC exhibits the highest level of heterozygosity and was the most informative locus for Gembrong goat breed.

Table 1: Details on microsatellite markers used, number and size of the alleles, polymorphism information content and heterozygosity in Gembrong goat

				Heterozigosity	
Microsatellite Marker	No of Alleles	Allele size range (bp)	PIC	Observed	Expected
INRA005	2	115-117	0.2453	0.3462	0,.2919
SCRSP23	1	85	0.00	0.00	0.00
MAF65	3	119-139	0.5291	0.6538	0.6184
OarFCB	2	99-101	0.037	0.0385	0.0385
SRCRSP8	2	230-240	0.1319	0.1538	0.1448
ILSTS19	4	152-158	0.5691	0.6923	0.6463
McM527	2	156-158	0.3262	0.4231	0.4186
SRCRSP5	3	167-177	0.2593	0.3462	0.2979
INRA023	4	195-209	0.5382	0.6154	0.595
INRA063	1	175	0.00	0.00	0.00
CSRD247	1	243	0.00	0.00	0.00
ILSTS87	4	141-151	0.4372	0.5	0.4879
HSC	5	267-302	0.6162	0.7308	0.6765
SPS113	2	140-142	0.2058	0.1923	0.2376
Avarage	2.57		0.2782	0.3352	0.3181

The polymorphic information content (PIC) estimated in the present study was 0.2782. It was observed that these values lower compared to those values obtain in other breed. The average of PIC value for the Croatian Spotted goat was 0.743 [4]. Moreover, PIC values for Korean were 0.350, Chinese (0.620) and Saanen (0.570) goats [12], Tali goat (0.704) [13], Lori goat (0.725) [14] and Raeini goat (0.778) [8]. An average PIC value of 0.48 was reported in three Indian goat breeds (Sirohi, Jamnapari and Barbari) using cattle microsatellite markers [15] and in Markhoz goat from Iran were 0.767 [16]. The lower PIC value in the Gembrong goats indicated that low genetic diversity and the breeds are homogenous. Also low genetic diversity may indicate high levels of inbreeding. Based on the PIC values, the microsatellite markers can be well utilized for molecular characterization of goat breeds.

CONCLUSION

This work has demonstrated that 11 microsatellite markers from 14 markers used in the present study were shown to be polymorphic. The Gembrong goat showed low levels of genetic diversity. The markers used in this study are useful for the molecular characterization of Gembrong goats. The information elucidated through the present study would be useful for the formulation of effective conservation strategies.

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