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Comparison of horned, polled Bali cattle and Banteng based on microsatellite markers

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ABSTRACT

Bali cattle (*Bos javanicus*) are domesticated cattle from Banteng. Bali cattle have unique characteristics that is the white sock. In the Maiwa breeding center, Enrekang district, South Sulawesi Province, polled Bali cattle are being kept in large quantities, both male and female. The microsatellites are widely used in the study of population genetics and quantitative trait locus. Therefore, the study aims to observe genetic diversity and determine whether microsatellites can distinguish horned Bali cattle, polled Bali cattle, and Banteng as their wild relatives. DNA was isolated from blood samples of 57 Bali cattle from two different populations: horned Bali (n=20) from Nusa Penida Island Bali province, polled Bali cattle (n=37) from Maiwa Breeding Center, South Sulawesi province, and 20 samples of Banteng from Ragunan Zoo (n=10), Jakarta Province and Surabaya Zoo (n=10), East Java Province. Genotyping was done using 11 microsatellite markers. The size of each microsatellite marker was determined using GeneMapper version 5.0. The observed heterozygosity value (H_o), expected heterozygosity value (H_e), the heterozygote deficit within the breed (F_{is}), gene flow (Nm), Hardy-Weinberg equilibrium (HW), and allele frequency were performed using CERVUS version 3.0.7 program. The FSTAT 2.9.4 was performed to obtain F_{is} value from two different populations.Similarly, bayesian clustering assignments were analyzed using STRUCTURE version 2.2. The polymorphism information content of eleven microsatellite markers ranged from 0.390-0.879. Moreover, we found Fis values of all markers which depicted that there is no inbreeding in horned and polled Bali cattle populations. We also found that polled Bali cattle have more private alleles than horned Bali cattle. Using Bayesian analysis, we found different genetic structures between polled Bali and horned Bali cattle with the K optimal at K=3. Findings indicated that ILST6 allele 288, TGLA53 allele 132, and TGLA227 allele 70 can be considered as the private allele to differentiate between the horned, polled Bali cattle and Banteng.

Keywords: Genotyping, Horned Bali, Microsatellite, Polled Bali, Polymorphism

In Indonesia, cattle have an economic and socio-cultural role. Bali cattle is a unique germplasm from Indonesia that has high economic value and plays a role in Balinese culture. Bali cattle have an ancestor that still exists in Indonesia, namely the Banteng (Purwantara *et al*. 2012). Bali cattle is closely related to Banteng which forms a separate cluster from other Indonesian cattle (Agung *et al*. 2019). The existence of polled Bali cattle was reported in 1990. The benefit of polled Bali cattle is that they are easy to handle. This phenomenon inspired the farmers and the local Government to design a breeding program for the polled Bali cattle in South Sulawesi. Nowadays, polled Bali cattle are centralized in the Maiwa breeding center, Enrekang district, South Sulawesi Province (Baco *et al*. 2020).

The success of the breeding program can be affected by several factors, including determining the number of the initial (base) population in order to produce offspring consistent with the objectives of the breeding program (Agung *et al.* 2016). In order to determine the initial (base) population for the breeding program of the polled Bali cattle, a study about the genetic diversity of Bali cattle in South Sulawesi especially the polled Bali cattle needs to be done. Furthermore, horns are inherited as an autosomal recessive trait. For this reason, we suspect microsatellites can be a marker for hornless or polled in the Bali cattle. Microsatellites are commonly used to verify the Mendelian inheritance (Pupin *et al.* 2017). The objective of this study was to evaluate the genetic diversity of Bali cattle (horned and polled) and Banteng as their wild relatives based on DNA microsatellites.

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MATERIALS AND METHODS

Blood Sample and DNA collection: This study was conducted following the guidelines of research implementation included in The Indonesian Institute of Science Regulation number 08/e/2013 about the ethical clearance of research and scientific publication. The Ethical Clearance Committee of Hassanuddin University, Indonesia has approved all procedures related to the use of animals in this study (Register No. 302/UN4.6.4.5.31/PP36/2021). A total of 77 head of cattle including polled Bali cattle (n=37; from Maiwa Breeding Center, South Sulawesi Province), horned Bali cattle (n=20; from Nusa Penida island, Bali Province), and Banteng (from Ragunan zoo (n=10), Jakarta Province and Surabaya zoo (n=10) East Java Province) were used for blood sampling. The blood samples were used for the DNA extraction process using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following the producer's method.

Primer and amplification: A total of 11 microsatellite labeled primers (part of the 30 primers recommended by FAO) were used in the polymerase chain reaction (PCR) process (primers sequence, annealing temperature, range of PCR product size, and label used were based on Agung *et al*. (2016)). The PCR reagent composition was as follows: KAPA2G Robust Hot Start Ready, Mix PCR master mix (Kapa Biosystems, Cape Town, South Africa) (18 µL), forward and reverse labeled primers (200 ng/µL), nuclease-free water, and DNA samples (5 to 30 ng/µL). Multiplex DNA fragment analysis was conducted afterward for allele identification in 1st BASE Laboratory, Malaysia.

Data analysis: The observed heterozygosity value (H_o), expected heterozygosity value (H_e), the polymorphism information content (PIC), and gene flow (Nm) were performed using CERVUS version 3.0.7 program (Kalinowski *et al.* 2007). The PIC value was estimated according to Botstein *et al*. (1980). The FSTAT 2.9.4 was performed to obtain F_{is} value from two different populations. Bayesian clustering assignments were analyzed using ten independent runs performed for each K between 1 and 10, with a burn-in period of 1,000,000 iterations followed by 1,000,000 iterations of the Markov Chain Monte Carlo algorithm. Bayesian clustering was performed using STRUCTURE version 2.2 (Pritchard *et al.* 2000). The Structure Harvester was used to identify the optimal groups (K) (Earl and VonHoldt 2012).

RESULTS AND DISCUSSION

The H_o, H_e, PIC values, and Nm for the Bali cattle (horned and polled) and Banteng populations are summarized in Table 1. The H_o and H_e ranged from 0.065 (TGLA227) to 0.948 (SPS113) and 0.456 (TGLA227) to 0.899 (TGLA53), respectively. The expected heterozygosity value was higher than the observed heterozygosity value in all populations studied except SPS113. PIC values ranged from 0.390 (TGLA227) to 0.879 (TGLA53). All microsatellite markers were considered to be highly useful in analyzing the polymorphism of Bali cattle except TGLA227. The highest gene flow value was found in SPS113 (6.6105). The higher value of gene flow, it is increasing the variability in populations.

Table 1. Characterization of the 11 microsatellite loci in this study

Locus	H_0	H_e	PIC	Nm
BM1824	0.584	0.615	0.587	4.8655
ILST6	0.486	0.829	0.800	0.7902
TGLA126	0.639	0.788	0.755	0.9426
TGLA53	0.629	0.899	0.879	1.8365
TGLA227	0.065	0.456	0.390	0.0457
TGLA122	0.622	0.786	0.748	1.7882
ETH225	0.645	0.829	0.799	1.1548
INRA23	0.268	0.559	0.485	1.6782
SPS113	0.948	0.803	0.770	6.6105
SPS115	0.632	0.815	0.786	0.9853
BM1818	0.493	0.729	0.682	0.8000

In general, this study reveals that microsatellite markers were informative for genetic diversity analysis in the Bali cattle population except for TGLA227 and INRA23. Jakaria *et al*. (2020) found PIC value of ETH225 is 0.8 in Bali, Madura, and Ongole grade cattle populations. The diversity of microsatellites will help the characterization and monitoring of animal genetic resources (Svishcheva *et al*. 2020). The PIC value for each locus was estimated according to Botstein *et al*. (1980). This study's PIC value was almost more than 0.5 (PIC>0.5) except for TGLA227 and INRA23. Hence, most microsatellite loci in this study were highly informative in detecting the genetic diversity level in the horned and polled Bali cattle population. The low value of PIC of TGLA227 and INRA23 in the Bali cattle in this study might be caused due to the number of sires and selection in the population (Agung *et al*. 2017). This limitation of sires number is caused by the restriction of crossing Bali cattle with other breeds based on government regulations.

The inbreeding coefficients (F_{is}) are shown in Table 2.

Table 2. F_{is} (heterozygote deficit within the breed) value of horned bali, polled bali cattle and banteng

Locus	Horned bali	Polled bali	Banteng
BM1824	-0.061	-0.046	0.240
ILST6	-0.060	0.085	1.000
TGLA126	0.125	-0.139	0.068
TGLA53	0.132	0.385	0.423
TGLA227	NA	0.406	0.000
TGLA122	0.066	0.150	0.113
ETH225	-0.286	0.067	0.578
INRA23	0.378	0.687	0.500
SPS113	-0.172	-0.202	-0.267
SPS115	-0.112	0.203	0.242
BM1818	0.220	-0.039	0.457
All	0.026	0.132	0.304

NA, Not Available.

Table 3. Comparison of the size range, the heterozygosity, and the PIC of microsatellite loci in the several studies

Locus	Parameter	A	B	Horned bali*	Polled bali*	Banteng*
BM1824	Range (bp) [n_A]	183-193 [9]	176–200 [12]	178-196 [6]	178-194 [6]	178-216 [5]
	H_o	0.933	0.90	0.5500	0.7027	0.4000
	PIC	0.78	0.78	0.4710	0.6223	0.4780
ILSTS6	Range (bp) [n_A]		267–309 [22]	282-294 [5]	281-303 [6]	285-295 [4]
	H_o		0.94	0.6500	0.6111	0.0000
	PIC		0.87	0.5374	0.5939	0.5925
TGLA126	Range (bp) [n_A]	113-129 [9]	115–139 [13]	105-119 [4]	104-118 [3]	104-118 [4]
	H_o	0.904	0.96	0.6000	0.6389	0.6875
	PIC	0.76	0.88	0.5995	0.4798	0.6579
TGLA53	Range (bp) [n_A]	151-183 [13]		129-163 [10]	130-164 [8]	129-147 [5]
	H_o	0.935		0.7000	0.5714	0.5000
	PIC	0.80		0.7634	0.8181	0.7551
TGLA227	Range (bp) [n_A]	75-107 [13]	71–107 [19]	71 [1]	71-115[7]	70-76 [2]
	H_o	0.959	0.86	0.0000	0.1081	0.0500
	PIC	0.86	0.90	0.0000	0.1759	0.0476
TGLA122	Range (bp) [n_A]	138-182 [12]		150-166 [7]	150-164 [6]	136-162 [5]
	H_o	0.934		0.7500	0.5882	0.5500
	PIC	0.80		0.7495	0.6425	0.5268
ETH225	Range (bp) [n_A]	131-183 [10]	131–167 [16]	134-164 [5]	134-164 [9]	130-164 [7]
	H_o	0.929	0.99	0.9000	0.7222	0.2500
	PIC	0.80	0.85	0.6340	0.7277	0.5363
INRA23	Range (bp) [n_A]	195-229 [12]	193–223 [16]	192-200 [3]	192-208 [4]	192-200 [3]
	H_o	0.959	0.91	0.4000	0.2000	0.0909
	PIC	0.86	0.91	0.5446	0.5009	0.1626
SPS115	Range (bp) [n_A]	242-254 [7]	158–262 [16]	244-252 [5]	242-264 [7]	243-247 [3]
	H_o	0.889	0.42	0.8000	0.5946	0.4545
	PIC	0.73	0.61	0.6513	0.6881	0.5035

n_A, number of alleles; A, Viryanski *et al*. (2022); B, Rahal *et al*. (2020); *, current study.

The low value of F_{is} in the BM1824, TGLA126, SPS113, and BM1818 indicates no inbreeding in the polled Bali cattle population. This shows that polled Bali cattle are not in a state of inbreeding with an overall value of 0.132. Bakae *et al*. (2022) found the inbreeding coefficient in Tswana and Tuli populations was 0.200±0.002 and 0.332±0.001, respectively. Therefore, microsatellite is an appropriate genetic marker to estimate the coefficient of inbreeding in a population. There are some differences between our findings and those of other studies that used the same microsatellite loci. The PIC value, the number of observed alleles and the minimum and maximum allele sizes could all be different (Table 3). The natural high polymorphism of the microsatellite was a major factor in the variation in both the observed number of alleles and their size range. In this study, the locus TGLA227's PIC value and observed heterozygosity (H_o) value were low. This condition was the same in Sumba Ongole (SO) cattle (Agung *et al*. 2015), but this is in contrast to several studies (Agung *et al*. 2016, Demir and Balcioglu 2019, Viryanski *et al*. 2022, Bigirwa *et al*. 2019 and Teneva *et al*. 2020), that reported the locus TGLA227 with higher PIC and H_o values. Kesvulu *et al.* (2009) and Agung *et al*. (2015) reported that the TGLA53 has a low value for the PIC and H_o which was in contrast with the present study.

Private allele candidates are shown in Table 4. It was observed that allele 70 in TGLA227, the private allele in the Banteng population with the highest frequency (0.975). Seven of the eleven microsatellites in polled Bali cattle showed the presence of private alleles with allele frequencies ranging from 0.014 (ILST6, TGLA227, ETH225, SPS113, SPS115) to 0.286 (TGLA53). The TGLA53 allele 132 in polled Bali cattle needs to be validated in the larger population as well as the ILST6 allele 288 in horned Bali cattle due to its frequency among other private alleles. Jakaria *et al*. (2020) reported that allele 149 in ETH225 and allele 283 in ILST6 loci were private alleles in Bali cattle but those alleles were not found in both horned or polled Bali cattle in present study.

Bayesian clustering with K optimal at 3 is shown in Fig. 1. The result showed genetic differences among two Bali cattle populations using microsatellites. The difference in population structure between the two Bali cattle populations is caused by the presence of private alleles, thus creating two clusters. Hence, microsatellites can distinguish horned and polled Bali cattle. The population structure was used to estimate the number of groups that were assigned to the group surveyed (Yun *et al.* 2022). Furthermore, Mueller *et al*. (2021) suggested gene editing to increase the number of polled animals compared to

Table 4. Private allele distribution

Locus	Allele	A	B	C	Locus	Allele	A	B	C
BM1824	190	0.075	-	-	TGLA227	70	-	-	0.975
	196	0.025	-	-		73	-	0.027	-
	216	-	-	0.050		76	-	-	0.025
ILST6	281	-	0.028	-		77	-	0.014	-
	282	0.025	-	-		79	-	0.014	-
	286	0.050	-	-		85	-	0.014	-
	288	0.550	-	-		103	-	0.014	-
	292	0.075	-	-		115	-	0.014	-
	294	0.300	-	-	TGLA122	136	-	-	0.025
	303	-	0.014	-		160	-	-	0.025
TGLA126	105	0.300	-	-		166	0.025	-	-
	113	0.425	-	-	ETH225	130	-	-	0.025
	114	-	-	0.250		138	-	0.014	-
	115	0.025	-	-		142	-	0.014	-
	119	0.250	-	-		144	-	0.028	-
TGLA53	130	-	0.143	-		150	-	-	0.025
	132	-	0.286	-		154	-	0.056	-
	133	0.075	-	-		160	-	-	0.075
	134	-	0.071	-		164	-	-	0.050
	135	-	-	0.250	INRA23	208	-	0.050	-
	137	0.075	-	-	SPS113	122	-	-	0.075
	141	-	-	0.250		126	-	0.014	-
	142	-	0.071	-		128	-	-	0.050
	144	-	0.143	-		132	-	0.014	-
	148	-	0.071	-		168	-	0.014	-
	149	0.125	-	-	SPS115	242	-	0.014	-
	153	0.050	-	-		243	-	-	0.227
	154	-	0.143	-		245	-	-	0.182
	159	0.025	-	-		247	-	-	0.591
	161	0.050	-	-		264	-	0.216	-
	163	0.025	-	-	BM1818	258	0.050	-	-
	164	-	0.071	-		278	-	-	0.056

A, Horned; B, Polled; C, Banteng

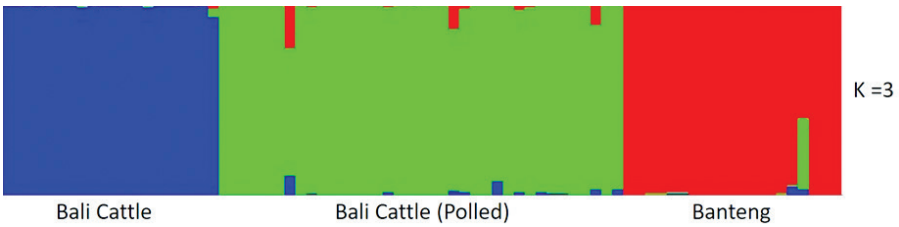

Fig. 1. Bayesian clustering of horned bali, polled bali cattle, and banteng.

conventional breeding.

It can be concluded that the ILST6 allele 288, TGLA53 allele 132 and TGLA227 allele 70 can be considered as the private allele to differentiate between the horned, polled Bali cattle and Banteng. However, backcross must be done to find out the genotype of polled Bali cattle. Nonetheless, further study using the SNP array is needed to explore the gene that affected polled and horned cattle.

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