Genetic Diversity and Relationship among Bali Cattle from Several Locations in Indonesia Based on ETH10 Microsatellite Marker

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ABSTRAK

Margawati ET, Volkandari SD, Indriawati, Ridwan M. 2018. Keragaman genetik dan hubungan genetik diantara sapi Bali dari beberapa lokasi di Indonesia berdasarkan Marker Mikrosatelit ETH10. JITV 23(4): 168-173. DOI: http://dx.doi.org/10.14334/jitv.v23i4.1915

Sapi Bali adalah salah satu sapi lokal Indonesia yang sampai saat ini mengindikasikan adanya *inbreeding*. Tujuan dari penelitian ini adalah untuk menganalisis keragaman genetik dan hubungannya diantara sapi Bali dari beberapa lokasi di Indonesia berdasarkan *marker* mikrosatelit ETH10. Sebanyak 94 sampel DNA yang terdiri dari 89 sapi Bali dan 5 Banteng dilakukan analisis. Sapi Bali berasal dari enam (6) lokasi di Indonesia yaitu Pulukan Bali (15), Nusa Penida Bali (15), Bima Nusa Tenggara Barat (14), Mataram (10), Riau (20), dan Kalimantan Selatan (15) sedangkan Banteng berasal dari Prigen Malang sebanyak 5 sampel. Marker mikrosatelit ETH10 dilabel HEX untuk digunakan dalam amplifikasi. Alel dianalisis menggunakan software Cervus 3.0.7 dan GenAlex 6. Hasil menunjukkan bahwa terdapat lima (5) alel pada marker ETH10 yaitu 209, 213, 215, 217, dan 219 pb. Rata-rata nilai heterosigositas teramati (Ho) dan harapan (He) adalah 0,46±0,05 dan 0,60±0,03. Lima dari enam lokasi sapi Bali telah terindikasi *inbreeding* kecuali sapi Bali dari Mataram. Jarak terjauh kekerabatan genetik yaitu antara sapi Bali dari Mataram dan Riau, sedangkan jarak terdekat yaitu sapi Bali dari Kalimantan Selatan dengan Mataram. Banteng sangat dekat dengan sapi Bali dari Nusa Penida dan berjarak sangat jauh dengan sapi Bali dari Kalimantan Selatan. Riset ini mengindikasikan terdapat *inbreeding* pada sapi Bali, karenanya diperlukan perhatian pada rotasi pejatan dan penyebaran semen untuk peningkatan performa sapi Bali.

Kata Kunci: Sapi Bali, Mikrosatelit ETH10, Keragaman Genetik, Hubungan Genetik

ABSTRACT

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Bali cattle is one of local beef cattle in Indonesia, up to present its performance indicated an inbreeding occurrence. This study was aimed to analyze the genetic diversity and relationship among Bali cattle from several locations in Indonesia based on ETH10 microsatellite marker. Ninety-four (94) DNA samples (89 Bali cattle; 5 Banteng) were analyzed. The Bali cattle samples were from 6 locations in Indonesia (15 Pulukan; 15 Nusa Penida; 14 Bima West Nusa Tenggara/WNT; 10 Mataram, WNT; 20 Riau; 15 South Borneo). DNA Banteng samples were collected from Prigen Malang of East Java. Microsatellite marker of ETH10 labelled HEX was used for amplification. Alleles were analyzed by using Cervus 3.0.7 and GenAlex 6.5. Result showed that there were five (5) alleles found in ETH10 marker *i.e.,* 209; 213; 215; 217; and 219 bp. Average of observed (Ho) and expected (He) heterozygosity were 0.46±0.05 and 0.60±0.03, respectively. Five (5) out of 6 locations were in breeding occurrence except Bali cattle from Mataram was not inbreeding. The longest genetic relationship was between Bali cattle from Mataram and Riau whereas the closest distance was Bali cattle from South Borneo with Mataram. Banteng was closest to Bali cattle from Nusa Penida and the longest was to Bali cattle from South Borneo. This finding indicates there is inbreeding in Bali cattle, therefore it needs to be concerned in bull rotation and semen distribution for increasing the Bali cattle performance.

Key Words: Bali Cattle, ETH10 Microsatellite, Genetic Diversity, Genetic Relationship

INTRODUCTION

Bali cattle is one of beef cattle gene pools in South East Asia and as one of local beef cattle in Indonesia (Margawati et al. 2015a). Bali cattle has been spread almost throughout the archipelago. Decreasing of performance (quantitative trait) and abnormal body color pattern is a sign of inbreeding occurrence. Abnormal pattern of body color occurred in several locations of Indonesia *i.e.*, Kupang, Nusa Tenggara Timur (NTT) (Tabun et al. 2013), Lombok, Nusa Tenggara Barat (NTB) (Sudrana et al. 2014), South Borneo (Lindell 2013), and Taro Gianyar, Bali island (Margawati et al. 2015b). The signs of abnormal color in Bali cattle are white spotted color, black color for cow, reddish body color for bull, reddish for soak leg color, and albino (Hardjosubroto & Astuti 1993).

Molecular approach technology could be used to analyze of genetic variation by using mitochondrial DNA and applying of microsatellite analysis. Investigation of that matter is very important for future monitoring of gene flow in populations, conservation and determining of the level of inbreeding within and between breeds (Hetzel & Drinkwater 1992). Microsatelitte marker is one of the most powerful means for studying the genetic diversity, calculation of genetic distance, detection of bottlenecks and admixture because of high degree of polymorphism (Sharma et al. 2015). (FAO 2011) recommended 30 microsatelitte markers for cattle to analyze genetic diversity and ETH10 is one of those microsatelittes. The range of ETH10 alleles is approximately 207-231 bp.

Utilization of marker microsatellite analysis could be used to investigate the level of inbreeding of Bali cattle in several locations. The objective of this study was to analyzed the genetic diversity and genetic relationship of Bali cattle from several locations in Indonesia based on ETH10 microsatelitte marker.

MATERIALS AND METHODS

Blood samples

A total of ninety-four (94) blood samples (89 samples of Bali cattle and 5 samples of Banteng) was collected (Table 1), from different areaas as presented in Figure 1. Fresh blood samples in 3 ml were taken from *vena jugularis* and collected into a vacuntainer containing anticoagulant (K_3EDTA).

Table 1. Samples of Bali cattle and Banteng in this study

Samples	Origin	N
Bali cattle	Pulukan, Bali	15
Bali cattle	Nusa Penida ,Bali	15
Bali cattle	Mataram, West Nusa Tenggara/WNT	10
Bali cattle	Bima, WNT	14
Bali cattle	South Borneo	15
Bali cattle	Riau	20
Banteng	Safari Garden2, Prigen, Malang	5
Total		94

DNA extraction and quantification

Genomic DNA was extracted using a High salt method (Montgomery & Sise 1990) for blood samples while DNA from tail hair was extracted using gSYNCDNA extraction kit (Geneaid). The quality of DNA samples were measured using a spectrophotometer (GeneQuant Pro, Amersham UK) to check DNA concentration and purity. DNA samples were prepared at 50 ng/µl.

Amplification of ETH10 microsatellite marker

Polymerase Chain Reaction (PCR) method was used for amplification of ETH10 marker. A pair primer of ETH10 marker was F: 5'-GTTCAGG ACTGGCCCTGCTAACA-3'; R: 5'-CCTCC AGCCCACTTTCTCTTCTC-3' and labelled with HEX fluorescent as recommended by (FAO 2011). A total volume of PCR reaction was 20 µl consisting of PCR master mix (K-2012, Bioneer) and mixed with17 µl free nuclease water, 1 µl primer *Forward* dan *Reverse* (10 pmol/µl), and 1 µl DNA template (50 ng/µl). The PCR reaction was run using a Thermal cycler (Eppendorft, Germany) which set up as initial denaturation at 95°C for 1 minute, followed by 30 cycles of 95°C for 1 minute (denaturation), 58°C for 1 minute (annealing) and 72°C for 1 minute (extension) then final extension at 72°C for 5 minutes (Sharma et al. 2015). The PCR product was checked using agarose gel 2% and visualized under UV light (MUV21, MajorScience, USA). The PCR products were then sent to sequence services (First BASE) for reading of DNA target fragment.

Fragment DNA analysis and phylogenetic tree construction

DNA fragment of ETH10 locus was calculated to analyze the genetic diversity. Parameters of this study were allele frequency, observed number of allele (N), expected number of allele (Ne), observed (Ho) and expected (He) Heterozygosity, Hardy Weinberg Equilibrium (HWE), Wright's *F*- statistics (Fis, F_{ST}, F_{IT}), and gene flow. Cervus 3.0.7 (Kalinowski et al. 2007) and GenAlex 6.5 (Peakall & Smouse 2012) software were used to estimate basic population of genetic descriptive statistic for each population. Phylogenetic tree was constructed by MEGA 5.0 software (Tamura et al. 2011) with a Neighborjoining (1,000 boostraps) method.

RESULTS AND DISCUSSION

Genetic diversity

Genetic diversity of Bali cattle and Banteng from several locations was established using an ETH10 microsatellite marker. Five variants of alleles were found *i.e.*, 209; 213; 215; 217; and 219 bp. According to (FAO 2011), allele ranges of ETH10 marker are 207-231 bp. Variation and number alleles of each of populations and locations are presented in Table 1. Distribution of alleles in this study was showed in Figure 2. Alleles of 213 and 217 bp were dominant alleles in all locations. (FAO 2007) has specified a minimum of four different alleles per locus for

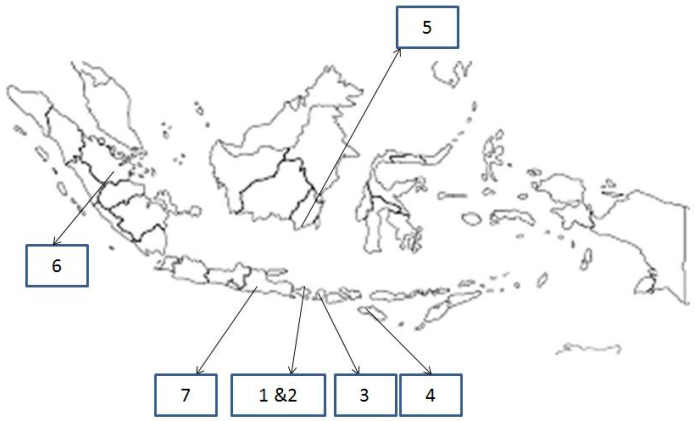

Figure 1. Location of samples collection. 1: Pulukan Bali; 2: Nusa Penida Bali; 3: Mataram WNT; 4: Bima WNT; 5: South Borneo; 6: Riau and 7: Prigen Malang East Java.

Table 1. Number of alleles in each population of Bali cattle and Banteng

Breed	Origin	Number of alleles	Variation of allele (bp)
Bali	Pulukan, Bali	3	209; 213; 217
Bali	Nusa Penida, Bali	5	209; 213; 125; 217; 219
Bali	Riau	4	209; 213; 217; 219
Bali	South Borneo	3	209; 213; 217; 219
Bali	Mataram, WNT	2	213; 217
Bali	Bima, WNT	3	209; 213; 217
Banteng	Prigen, Malang	3	213; 217; 219

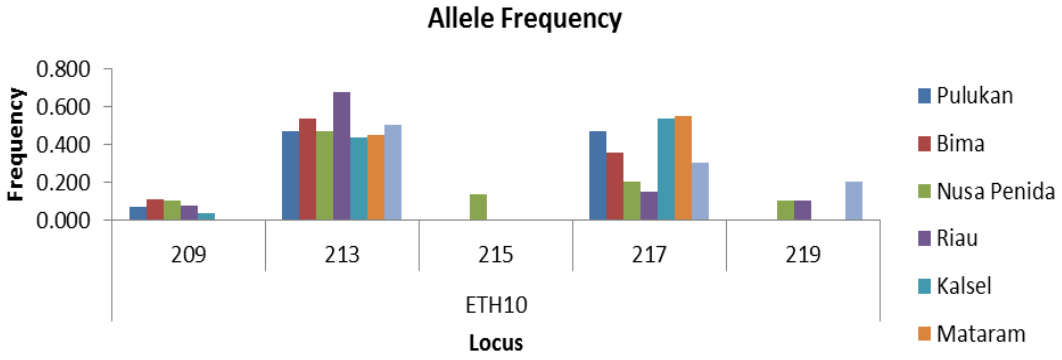

Figure 2. Distribution of alleles based on ETH10 locus.

evaluation of genetic differences between breeds. Using the (FAO 2007) criterion, therefore, polymorphic was only detected in Bali cattle from Nusa Penida (5 alleles) and Riau (4 alleles). Previous study of (Sharma et al. 2015) reported that the number of alleles in Indian breeds cattle was found 14 alleles with allele range 185-221 bp in ETH10 locus. Furthermore, (Cervini et al. 2006) found that Nellore cattle has 8 alleles (205-219 bp), whereas four alleles on ETH10 locus has been found in Sudanese cattle (Hussein et al. 2015) and five alleles in Buśa cattle from Bosnia (Rogic et al. 2011).

The observed number of alleles (Na) was 2 in Bali cattle from Mataram WNT to 5 in Bali cattle from Nusa Penida Bali and the average alleles of populations was 3.29±0.36 (Table 2). While the expected number of alleles (Ne) varied from 1.980 (Mataram, WNT) to 3.383 (Nusa Penida, Bali). The result showed a lower expected number of alleles (Ne) compared to the observed number of alleles (Na) (Na>Ne) in all of Bali cattle from all locations.

Table 2. Genetic diversity of Bali cattle and Banteng

Breed	Origin	N	Na	Ne	Ho	He	Fis
Bali	Pulukan, Bali	15	3	2.273	0.400	0.579	0.286
Bali	Nusa Penida, Bali	15	5	3.383	0.467	0.704	0.338
Bali	Riau	20	4	2.025	0.500	0.506	0.012
Bali	South Borneo	15	3	2.113	0.200	0.527	0.620
Bali	Mataram, WNT	10	2	1.980	0.500	0.495	-0.010
Bali	Bima, WNT	14	3	2.347	0.571	0.574	0.004
Banteng	Prigen, Malang	5	3	2.632	0.600	0.620	0.032
Mean±SD		13.43±188	3.29±0.36	2.40±0.19	0.46±0.05	0.60±0.03	0.18±0.10

N= number of individuals; Na= observed number of alleles; Ne= expected number of alleles; Ho= observed heterozygosity; He= expected Heterozygosity; Fis= inbreeding coefficient

Table 3. PIC value and global F-statistics of ETH10 locus

Locus	PIC	Fis	Fit	Fst	Nm
ETH10	0.533	0.188	0.237	0.060	3.896

PIC= Polymorphic Information Content, Fis= coeficient of inbreeding, Fit= deviation of Hardy Weinberg proportion in total population, Fst= Wright's standardized variance, Nm= gene flow

Table 4. Pairwise population matrix of Nei genetic distance

Pulukan	Bima	Nusa Penida	Riau	South Borneo	Mataram	Banteng	
0.000							Pulukan
0.021	0.000						Bima WNT
0.126	0.066	0.000					Nusa Penida
0.178	0.080	0.052	0.000				Riau
0.007	0.052	0.181	0.254	0.000			South Borneo
0.010	0.058	0.188	**0.257**	**0.001**	0.000		Mataram WNT
0.091	0.070	**0.067**	0.073	**0.119**	0.116	0.000	Banteng

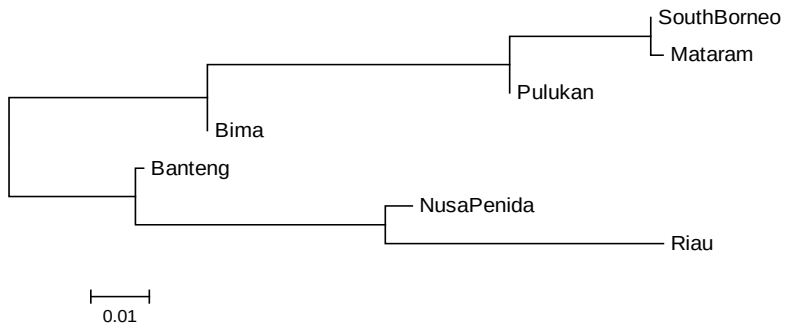

Figure 3. Genetic relationship of Bali cattle and Banteng from several locations.

Heterozygosity value is a suitable used to measure the genetic diversity within populations (Hanslik et al. 2000). The estimates of observed (Ho) and expected (He) heterozygosity of Bali cattle from all locations and populations were 0.46±0.05 and 0.60±0.03, respectively. The calculation showed that Ho value was lower than He value. The highest Ho value was found

in Banteng population whereas the lowest Ho value was found in Bali cattle from South Borneo.

In this study, polymorphic information content (PIC) showed a considerable high variation (0.533). (Botstein et al. 1980) classified PIC into three groups, ie those are low (PIC<0.25), moderate (0.25<PIC<0.50) and high (PIC>0.50) variation. Based on Wright's *F*- statistics analysis, Fis value detected that populations were in random (Sodhi et al. 2008). Fit value is greater than Fst value (Fit>Fst), therefore Fis value would be positive. It interpreted that there was inbreeding occurrence (Weir 1996); Sharma et al. 2015). Average of Fis value in this study was positive (0.188) (Table 3). Fis value in all populations and locations were mostly positive. The negative value was only found in Bali cattle from Mataram WNT (Table 2). According to (Nei 1987) Fis>0 showed deficiency of heterozygotes and excess of homozygotes. It could be influenced by a number of factors *i.e*., assertive mating, presence of population substructure within the populations (Wahlud effect) or null alleles.

The number of migrants per generation (gene flow or Nm) was observed to be 3.896% (Table 3) which perhaps was not influenced by genetic structure in all of populations. It seems due to of using similar bull for matings in quite long time (Sharma et al. 2015) reported that Nm value in Indian cattle reached 5.608%.

Genetic relationship

Based on Pairwise population matrix of Nei genetic distance analysis (Table 4) within population, the longest genetic distance was investigated between Bali cattle from Mataram WNT (0.257) and Riau whereas the closest genetic distance was between Bali cattle from South Borneo (0.001) with Bali cattle from Mataram WNT. Banteng represented as indigenous cattle of Indonesia. Banteng or *Bos javanicus* is known as a wild cattle (Syed-Shabtar et al. 2013) and Bali cattle is domesticated cattle from Banteng 3,500 BC (Mohamad et al. 2009). Findings of this present study showed that the closest genetic distance with Banteng was Bali cattle from Nusa Penida Bali and the longest genetic distance was Bali cattle from South Borneo. This result was proved by Phylogenetic tree analysis (Figure 3) which found that Banteng was at the same group to that Bali cattle from Nusa Penida and Riau but was in different group to that Bali cattle from South Borneo, Pulukan Bali, Mataram WNT and Bima WNT. It might be related with the trade market and distribution of Bali cattle among islands in Indonesia. (The Agency of Livestock and Animal Health of NTB Province 2014) reported that demand of Bali cattle breeding stock originated from West Nusa Tenggara reached 14,651 heads and 1,718 out of 14,651 heads was transported to South Borneo.

This recent study proved that inbreeding already occurred in Bali cattle population from several locations in Indonesia, it was also happened in Banteng from Safari Garden 2 Prigen Malang of East Java. Rotation of bulls or semen distribution (for artificial insemination needs) for mating is important to be properly managed. Those managements are necessary and very important to avoid inbreeding occurrence in Bali cattle population throughout the country.

CONCLUSION

The genetic diversity and relasionship was detected in the study of on ETH10 microsatelitte marker with five-variant alleles. Polymorphic was found in two locations of Bali cattle population *i.e* Nusa Penida Bali and Riau. The occurence of inbreeding detected in Bali cattle from Pulukan Bali, Nusa Penida Bali, Mataram WNT, Bima WNT, and Riau and Banteng from Prigen 2 Malang of East Java. The longest genetic relationship was found between Bali cattle from Mataram WNT and Riau while the closest distance was Bali cattle from South Borneo to Mataram WNT. The closest genetic distance to Banteng was showed by Bali cattle from Nusa Penida Bali and the longest genetic distance was Bali cattle from South Borneo.

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