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Genetic Diversity Analysis and Determination of Specific Alleles of Kuantan Cattle Using Microsatellite Markers

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ABSTRACT

Kuantan cattle have an important role in Riau Province, Indonesia. Identification of the genetic diversity of these cattle is important to get the basic information for breeding and conservation strategies. The aim of this research was to identify the genetic diversity of Kuantan cattle using microsatellite markers. A total of thirty-nine DNA samples from three breeds were used in this study. The polymerase chain reaction was conducted using four labeled primers of microsatellite (INRA035, ILSTS06, HEL9 and ETH225). The data were analyzed using GenAlEx 6.41, Cervus 3.0, POPTREE, and STRUCTURE Software. A total of thirty-two alleles were found from microsatellite loci. Two alleles in INRA035 locus 112 and 118 occurred as specific allele candidates for Kuantan cattle. The mean of observed heterozygosity value of the Kuantan-2 population (0.602) was higher than Kuantan-1 (0.471) but lower than Madura (0.688) and Pesisir cattle (0.625). PIC value was higher in HEL9 loci. The dendrogram showed that Kuantan cattle existed at different clusters with Pesisir and Madura cattle. This finding indicated that microsatellite markers successfully distinguished clusters of the cattle and could serve as information for conducting conservation and breeding program.

Keywords: Kuantan cattle; genetic diversity; microsatellite marker

INTRODUCTION

Domestic database Animal Diversity-Information System (DAD-IS) FAO (2020) recorded about 206 local livestock breeds, including large ruminants, small ruminants, poultry, and pigs in Indonesia. The total number of local breeds cattle found are 14 breeds (1 native and 13 local breeds), and one of them is Kuantan cattle from Riau Province. These cattle were declared by the Ministry of Agriculture No. 1052/kpts/SR.120/10/2014. The population of Kuantan cattle spreads into two sub populations, i.e., Kuantan Singingi and Indragiri Hulu. These cattle play an important role in maintaining rural population living, social, religious, and traditional celebration. However, referring to data from the government of Riau, the population of Kuantan cattle has declined since 2014. The diminishing trend strongly relates to the scarce genetic information, reduced grazing areas, and lack of bulls (Misrianti *et al.*, 2018).

Conservation of Kuantan cattle is needed to maintain and improve its productivity. For this goal, it is important to identify and analyze the genetic diversity, population structure, and evolutionary origin of Kuantan cattle. Microsatellite marker has been used as one approach to identifying genetic diversity and phylogeny in animal. This information is needed for the breeding program because it has a high degree of polymorphism, easy to analyze, can calculate genetic diversity in the population (between and within), neutrality concerning selection, is cheap and highly accurate (Habimana *et al.*, 2020).

Genetic diversities based on microsatellite markers in indigenous and local cattle have been reported for many breeds of cattle, such as West African cattle (Alvarez *et al.*, 2021), East Eurasian cattle (Svishcheva, 2020); Russian cattle (Abdelmanova *et al.*, 2020), some of Indian local cattle (Vinod *et al.*, 2019); European cattle (Garkovenko *et al.*, 2018), Korean native cattle (Sharma *et al.*, 2020), Egyptian native cattle (Faid-Allah *et al.*, 2018), Lebedyn cattle (Ladyka *et al.*, 2019), Zimbabwean Sanga cattle (Gororo *et al.*, 2018), South African cattle (Westhuizen *et al.*, 2020); and Turkey cattle (Demir *et al.*, 2019). The technique has been applied to characterize Indonesia's local breed animals such as Aceh cattle (Abdullah *et al.*, 2011), Indonesian swamp local buffalo (Saputra *et al.*, 2020), Bali cattle (Jakaria *et al.*, 2012), and local Indonesian cattle (Sutarno *et al.*, 2016; Agung *et al.*, 2019;). Jakaria *et al.* (2020) successfully identified the genetic diversity of Bali cattle and its hybrids using four loci of microsatellite. Furthermore, Agung *et al.* (2019) investigated the genetic diversity of ten Indonesian local cattle breeds using microsatellite loci. However,

genetic investigation using the approach for Kuantan cattle is still absent. This provokes an attempt to complete genetic information using microsatellite loci for Indonesian local cattle breeds. The aim of this research was to identify the genetic diversity of Kuantan cattle using microsatellite markers.

MATERIALS AND METHODS

Ethic Statement

A qualified veterinarian collected blood samples for DNA extraction. There was no treatment and injuries to the cattle in this research. We ensure, no other types of tissue (meat or other) were used in this study. All experiments were approved by the Ethical Committee for Research of Faculty of Agriculture and Animal Science, State Islamic University of Sultan Syarif Kasim Riau (Ethical Clearance number: KE/KEP-FPP/07/09/2021).

DNA Samples

DNA samples used in this study were collections of Breeding and Genetics Laboratory in Faculty of Animal and Agriculture Science, Riau State Islamic University. DNA was extracted from 39 blood samples, including Kuantan cattle from two subpopulations, i.e., Kuantan Singingi (n=19), Indragiri Hulu (n=11), Pesisir cattle (n=5), and Madura cattle (n=4) (Table 1). The last two cattle used to compare the genetic profile since all cattle studied showed high similarity to Kuantan cattle. DNA was extracted using Geneaid DNA Mini Kit following producer's methods.

Primer, Amplification, Fragment Analysis

A total of four microsatellite primers labeled were used in Polymerase Chain Reaction (PCR) (Table 2). The primer was recommended by the International Society for Animal Genetics (ISAG) and FAO (Food and Agriculture Organization) (FAO, 2011). The final volume (28 mL) contained genomic DNA, 2 × GoTaq® Green Master Mix (Promega, United States), Primer forward and reverse, and nuclease-free water.

Amplification using *Applied biosystem* thermal cycler followed the procedures as follows: predenaturation at 95 °C for 5 min, amplification for 35 cycles (denaturation at 95 °C for 10 s, annealing at 55 °C for 20 s, extension at 72 °C for 30 s), and final elongation at 72 °C for 5 min. Visualization of PCR products conformed to the electrophoresis technique using 1.5% agarose gel. Multiplex fragment analysis was performed on fragment analysis services on 1st base (http://www.base-asia.com/fragment_analysis/). Allele size detection was performed by using Gene Mapper 5.0.

Table 1. Breed and origin of DNA sample

No	Breed	N	Origin
1	Kuantan-1	19	Kuantan Singingi, Riau, Indonesia
2	Kuantan-2	11	Indragiri Hulu, Riau, Indonesia
3	Pesisir	5	BPTU Padang Mangatas, West Sumatra, Indonesia
4	Madura	4	Sapudi Island, Madura, East Java, Indonesia

Note: N= number of sample.

Data Analysis

Identification of allele size followed the protocol of multiplex DNA Fragment analysis. GenAlEx 6.5 (Peakall and Smouse, 2012) was applied in the calculation of allele frequency, the observed number of alleles (Na), effective number of allele (Ne), observed heterozygosity value (Ho), expected heterozygosity value (He), F statistic (Fis, Fit, and Fst), and Genetic Distance. CERVUS VERSION 3.0.7 was used to identify Polymorphic Informative Content (PIC) and Hardy Weinberg (HW) equilibrium.

Furthermore, POPTREE was employed to construct a dendrogram using NJ (Neighbour Joining) method with 1000 bootstrap (Takezaki *et al.,* 2014). STRUCTURE version 2.2 was used for Bayesian clustering assignment, with 10 independent runs for each K between 2-4, followed by 1.000.000 iterations of the Markov Chain Monte Carlo Algorithm.

RESULTS

Genetic Variability

Thirty-nine DNA samples from three breeds were amplified using four labeled microsatellite markers. As a result, 32 alleles were found at four loci: 8 alleles in INRA035, 6 alleles in ILSTS06, 11 alleles in HEL9, and 7 alleles in ETH225. The frequency of alleles present per locus in each population was analyzed (Table 3). All populations showed a high allele number in HEL9

Table 2. Microsatellite loci, position in chromosome, sequence, and label of primer

Locus*	Chromosome	Motif	Sequences	Label	Length
ETH225	9	$CA_{(14)}$	GATCACCTTGCCACTATTTCCT ACATGACAGCCAGCTGCTACT	HEX	131-185
ILSTS006	7	$GT_{(23)}$	TGTCTGTATTTCTGCTGTGG ACACGGAAGCGATCTAAACG	FAM	277-309
INRA035	16	$TG_{(16)}$	ATCCTTTGCAGCCTCCACATTG TTGTGCTTTATGACACTATCCG	FAM	98-124
HEL9	8	$GT_{(25)}$	CCCATTCAGTCTTCAGAGGT CACATCCATCCATGTTCTCACC	NED	141-173

Note: *microsatellite marker recommended by FAO.

loci, and a low number of the allele in ILSTS06 loci. The highest number of alleles in INRA035 and HEL9 loci was found at Kuantan-1, but the highest number of alleles in ILTS06 loci was found at Madura cattle despite Madura cattle showing a smaller sample size than the other breeds. New allele was found at INRA035 loci in Kuantan cattle population, i.e., 112 and 118, and ETH225 loci in Madura cattle, i.e., 159. The summary of private alleles by population is described in Table 4.

Heterozygosity of Microsatellite

The observed heterozygosity value ranged from 0.091 (ILSTS6 at Kuantan-2 population) to 1.000 (HEL9 at pesisir population), while the expected heterozygosity value ranged from 0.237 (ILSTS6 in Kuantan-1) – 0.840 (HEL9 in pesisir), as were presented in Table 5. The mean of expected heterozygosity was higher than the observed heterozygosity. We also found that HEL9 loci showed a high Na, Ne, Ho, and He in all studied populations.

Values of Fis, Fit, and Fst displayed a various range, i.e. -0.160 to 0.510, -0.025 to 0.632, and 0.086 to 0.248, respectively (Table 6). Meanwhile, the PIC values varied from 0.472-0.817, with a mean value of 0.663. Three loci existed with highly informative features (PIC value >0.5). The highest number of PIC was found in HEL9 loci (0.817), suggesting that the locus was highly recommended as a marker. Following the result of the Hardy Weinberg Equilibrium test, INRA035 locus fitted the equilibrium.

Table 3. Frequency of allele in each loci based on breed was analyzed

Locus	Allele (bp)/N	Kuantan-1	Kuantan-2	Pesisir	Madura
INRA035	N	17	11	5	4
	98	0.265	0.000	0.000	0.125
	100	0.059	0.300	0.250	0.500
	102	0.000	0.000	0.000	0.25
	106	0.118	0.200	0.000	0.000
	112	0.059	0.100	0.000	0.000
	116	0.471	0.400	0.500	0.125
	118	0.029	0.000	0.000	0.000
	120	0.000	0.000	0.25	0.000
ILSTS06	N	19	11	5	4
	275	0.000	0.000	0.000	0.125
	279	0.053	0.136	0.000	0.125
	291	0.000	0.136	0.400	0.500
	293	0.868	0.727	0.500	0.000
	295	0.000	0.000	0.000	0.125
	297	0.079	0.000	0.100	0.125
HEL9	N	19	11	5	4
	147	0.026	0.000	0.000	0.000
	149	0.053	0.000	0.200	0.250
	151	0.000	0.000	0.100	0.000
	153	0.211	0.136	0.200	0.250
	155	0.211	0.545	0.200	0.000
	159	0.000	0.045	0.000	0.000
	161	0.053	0.182	0.000	0.25
	165	0.026	0.000	0.100	0.125
	167	0.132	0.045	0.100	0.000
	169	0.211	0.045	0.100	0.125
	171	0.079	0.000	0.000	0.000
ETH225	N	19	11	5	4
	139	0.000	0.045	0.000	0.000
	145	0.105	0.227	0.200	0.375
	147	0.026	0.000	0.000	0.125
	151	0.316	0.409	0.100	0.000
	155	0.526	0.318	0.600	0.125
	159	0.000	0.000	0.000	0.125
	165	0.026	0.000	0.100	0.250

Note: N: Number of individual.

Table 4. Summary of private alleles by cattle breed

Locus	Population (Allele/Freq)			
	Kuantan-1	Kuantan-2	Pesisir	Madura
INRA035	112 (0.059)	-	120 (0.250)	102 (0.250)
	118 (0.029)	-	-	-
HEL9	147 (0.026)	159 (0.045)	151 (0.100)	-
	171 (0.079)	-	-	-
ILSTS06	-	-	-	275 (0.125)
	-	-	-	295 (0.125)
ETH225	-	139 (0.045)	-	159 (0.125)

Genetic Distance

The values of genetic distance ranged from 0.267 to 0.842 (Table 7). Closed genetic distance was found at Kuantan-1 and Kuantan-2 populations, whereas the highest genetic distance was found at Kuantan-1 and Madura. The construction of the phylogenetic tree followed Nei Distance (Figure 1). As depicted in the dendrogram, three clusters of the cattle occurred, with Kuantan-1 and Kuantan-2 at the first cluster, Pesisir cattle at the second cluster, and Madura cattle at the third cluster.

Bayesian Clustering

Assessment of population structure complied with the Bayesian clustering approach based on the Markov Chain Monte Carlo (MCMC), and the result is presented in Figure 2. Pure ancestry was found at Madura cattle (red bar in K=2 and K=3), and Mixed ancestry was found at Kuantan-1, Kuantan-2, and Pesisir cattle.

DISCUSSION

Using four microsatellite loci, genetic diversity and phylogenetic relationship between Kuantan cattle and other Indonesian local cattle include Pesisir and Madura have been obtained. This present study becomes the first work discovering the genetic diversity of Kuantan cattle using microsatellite markers. Polymorphism of microsatellite loci can be identified by calculating the mean number of alleles and evaluating the PIC value. All loci occurred as polymorphic in Kuantan, Pesisir, and Madura. This result indicates that the genetic diversity of Kuantan, Pesisir, and Madura cattle were still high.

Table 5. Summary statistic of number of observed allele, number of effective allele, observed, and expected heterozigosity based on cattle breed

Pop	Locus	N	Na	Ne	Ho	He
Kuantan-1	INRA035	17	6.000	3.193	0.412	0.687
	ILSTS06	19	3.000	1.310	0.158	0.237
	HEL9	19	9.000	6.119	0.737	0.837
	ETH225	19	5.000	2.569	0.579	0.611
	Mean		5.750	3.298	0.471	0.593
	SE		1.250	1.019	0.124	0.128
Kuantan-2	INRA035	10	4.000	3.333	0.500	0.700
	ILSTS06	11	3.000	1.766	0.091	0.434
	HEL9	11	6.000	2.814	0.909	0.645
	ETH225	11	4.000	3.103	0.909	0.678
	Mean		4.250	2.754	0.602	0.614
	SE		0.629	0.346	0.196	0.061
Pesisir	INRA035	4	3.000	2.667	0.500	0.625
	ILSTS06	5	3.000	2.381	0.200	0.580
	HEL9	5	7.000	6.250	1.000	0.840
	ETH225	5	4.000	2.381	0.800	0.580
	Mean		4.250	3.420	0.625	0.656
	SE		0.946	0.946	0.175	0.062
Madura	INRA035	4	4.000	2.909	0.750	0.656
	ILSTS06	4	5.000	3.200	0.500	0.688
	HEL9	4	5.000	4.571	0.750	0.781
	ETH225	4	5.000	4.000	0.750	0.750
	Mean		4.750	3.670	0.688	0.719
	SE		0.250	0.379	0.063	0.029

Note: N=Number of sample, Na= Number of allele, Ne: Number of expected allele, Ho= Observed heterozygosity, He: Expected heterozigosity.

Table 6. Fixation indices (Fis, Fit, and Fst) based on four microsatellite loci

Locus	Na	Fis	Fit	Fst	Ho	He	PIC	HW
INRA035	8	0.190	0.287	0.120	0.486	0.761	0.719	**
ILSTS06	6	0.510	0.632	0.248	0.179	0.511	0.472	nd
HEL9	11	-0.095	-0.001	0.086	0.821	0.846	0.817	nd
ETH225	7	-0.160	-0.025	0.116	0.718	0.704	0.645	ns

Note: Na= Number of allele, Ho= Observed heterozigosity, He= Expected heterozigosity, PIC= Polymorphic information centre, HW= Hardy Weinberg.

Table 7. Genetic distance value based on nei genetic identity (above diagonal) and genetic distance (below diagonal)

Population	Kuantan-1	Kuantan-2	Pesisir	Madura
Kuantan-1	-	0.267	0.534	0.842
Kuantan-2	0.172	-	0.412	0.792
Pesisir	0.196	0.234	-	0.534
Madura	1.320	0.886	0.628	-

The mean number of allele in this research was higher than Bali cattle (Septian *et al.*, 2019) but lower than PO and Madura cattle (Jakaria *et al*., 2020).

Based on Svishcheva *et al*. (2020) specific allele denotes that the breed possesses a unique gene pool if frequency more than 0.01. The largest number of the specific allele was detected for INRA035 and ILSTS06 loci. There are two specific allele candidates found at Kuantan cattle, namely 112 and 118 (INRA035). Some previous studies did not detect this allele in Bali, PO (Peranakan Ongole), Madura Cattle (Jakaria *et al*. (2020), and Ciamis-West Java local cattle (Hilmia *et al.*, 2013). Therefore, detection of these new alleles is the earliest report in local Indonesian cattle. Specific allele candidates in HEL9 loci included 147, 171, and 159. In a former study by Jakaria *et al*. (2020), the allele was not found in Bali cattle. The distinct allele in Kuantan cattle may also suggest that the cattle exist in different groups from Bali cattle.

PIC values of the three microsatellite loci (INRA35, HEL9, and ETH225) indicated a high polymorphism (>0.5). The mean of PIC values in this study was higher than Egyptian Cattle (El-Sayed *et al.*, 2016), Punganur Cattle (Devi *et al.*, 2017), and lower than Turkey Cattle (Demir *et al.*, 2019). The high PIC value indicated that the microsatellite markers used are highly polymorphic, and it could be functional for analyzing the genetic diversity.

The observed heterozygosity value of Kuantan-1 and Kuantan-2 cattle was smaller than Madura and Pesisir cattle. The mean heterozygosity value in all populations was 0.597. Observed heterozygosity

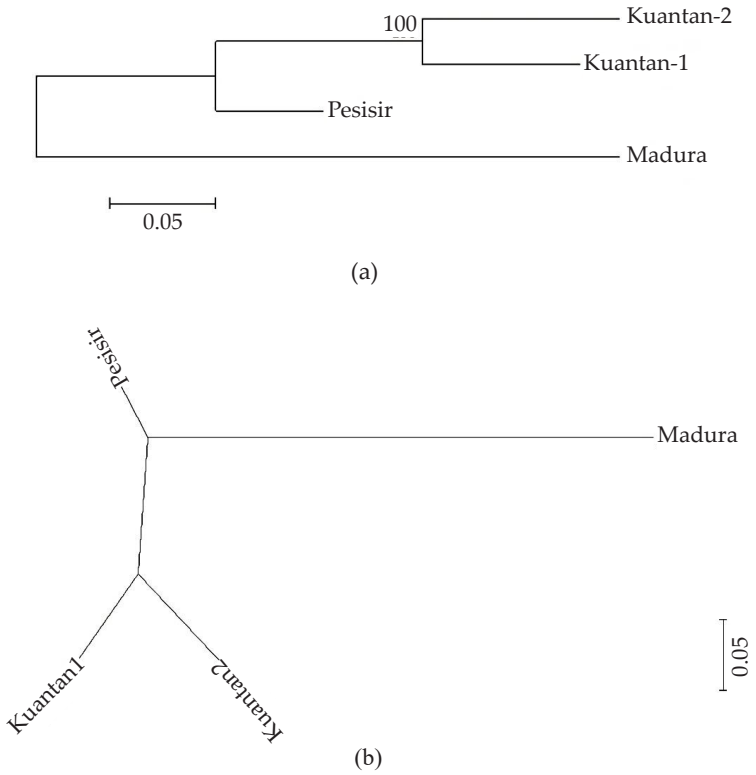

Figure 1. Dendrogram of Kuantan, Pesisir, and Madura Cattle using (a) UPGMA and (b) Neighbour Joining (NJ) method.

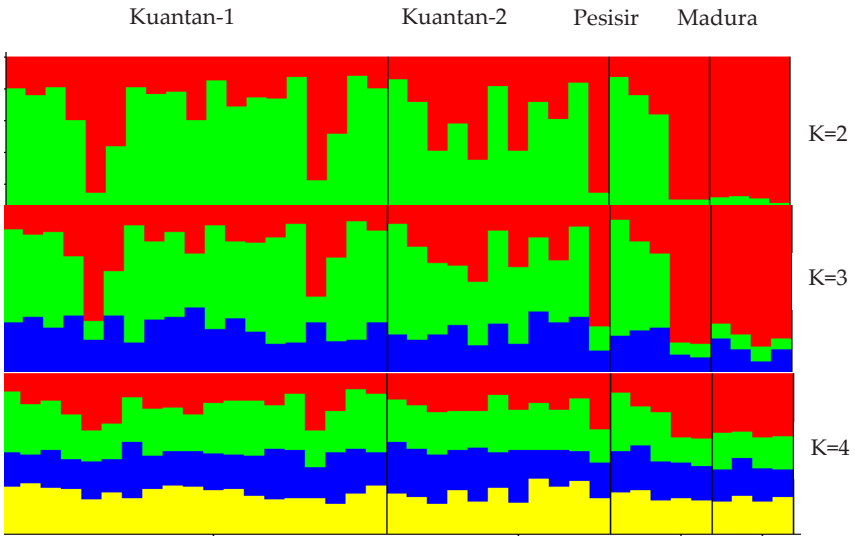

Figure 2. Genetic structure of Kuantan (green), Pesisir (blue), and Madura cattle (Red). Each color represents "one population," and the black line separates individual populations. K represents 3 optimal genetic structures.

value at Kuantan-2 cattle was higher than that found in other local cattle such as Bali cattle (Septian *et al.*, 2019), Pesisir, Pasundan, and Madura (Agung *et al.*, 2019). However, the Ho value was lower than East Eurasian Bos taurus Breeds (Svischeva *et al.*, 2020), Yugoslav Pied Cattle (Stevanovic *et al.*, 2010), and Algerian cattle breed (Rahal *et al.*, 2020). Observed heterozygosity in Kuantan cattle was lower than expected heterozygosity. Several factors can affect this condition, such as a higher rate of inbreeding due to the limited number of bulls (Unal *et al.*, 2021).

The fixation indices (Fis, Fit, and Fst) in INRA035 and ILSTS06 loci were positive. These values indicate the presence of the selection process in the population (Agung *et al.*, 2019). Fst values in three loci (INRA035 (0.120), HEL9 (0.086), and ETH225 (0.116)) also indicate a moderate genetic relationship between population.

Based on Nei genetic distance, Kuantan-1 and Madura cattle showed the highest value of genetic dis-

similarity and displayed a close genetic feature with Pesisir cattle. The result is consistent with the phylogenetic analysis. Based on morphological characteristic, especially in morphometric, Kuantan cattle has a unique performance, with a small body size like Pesisir cattle. As was reported by Misrianti *et al.* (2018), Kuantan cattle showed the smallest size as Pesisir cattle. Relevant to phenotypic and genetic diversity, Kuantan cattle closely relates to Pesisir cattle than Madura cattle.

Clustering analysis using STRUCTURE program revealed a combined cluster comprising Kuantan and Pesisir, while Madura cattle occurred at a specific cluster (Red color). This result follows the result Sutarno *et al*. (2015) reported that Madura cattle was distinct from PO, Aceh, and Bali cattle breeds. The structure analysis also supports genetic distance and dendrogram results. This result also indicated that Kuantan cattle have some origin with pesisir cattle, that is from *Bos indicus.*

CONCLUSION

All of the microsatellite loci used in this study were polymorphic and informative. Two alleles (112 and 118) in INRA035 loci occurred as the specific allele candidate for Kuantan cattle. All identified alleles could distinguish cattle breeds into three clusters, in which Kuantan cattle was arranged at a separated cluster from Madura and Pesisir cattle. The finding in the present study became important information in conducting conservation and breeding program in Kuantan cattle.

CONFLICT OF INTEREST

Cece Sumantri and Jakaria serve as editors of the Tropical Animal Science Journal, but have no role in the decision to publish this article. We certify no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

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