

Genetic Variation and Relationship among Indonesian Native Cattle Based on Mitochondrial DNA NADH Dehydrogenase Subunit 1 Gene

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DOI: 10.36347/sjavs.2019.v06i07.002

| Received: 02.07.2019 | Accepted: 11.07.2019 | Published: 30.07.2019

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Abstract

Original Research Article

Indonesia has various breeds of cattle which already domesticated and well-adapted to the environmental condition in Indonesia. The increase in cattle population year by year must be accompanied by the increase in genetic quality. The improvement of genetic quality can be done by knowing genetic variation and relationship as the basis of breeding, especially for Indonesian native cattle. This study aimed to determine the genetic variation and length of Pesisir, Pasundan, Madura, and Bali cattle based on mitochondrial DNA (mtDNA) in the area of NADH Dehydrogenase Subunit I (ND-1) gene. A total of 48 blood samples were taken from Pesisir, Pasundan, Madura, and Bali cattle (each of breed consisted of 12 cows). Three ml of each blood sample was stored in an EDTA-containing tube. The ND-1 gene amplification was done using the forward primer of 5'AATGGCCGCACGAGGGTTTTA-3' and reverse primer of 5'ATGGAGCTCGGTTTGTTCCTGC-3'. After that, the sequencing process was conducted in the 1st BASE Laboratory, Malaysia. The genetic variation was determined using MEGA 6.0 software, while the phylogenetic tree was constructed using the neighbor-joining method. The results showed that the genetic variation in Indonesian native cattle studied here based on ND-1 gene undergoes changes in the substitution base in the form of 16 nucleotide transitions and 1 nucleotide transversion. The farthest genetic length was 0.064 in Pesisir with Bali cattle, while the closest genetic length was 0.013 in Pasundan and Bali cattle. Based on the phylogenetic tree, there are three groups of Indonesian native cattle, the first group consisted of Pasundan and Bali cattle, the second group is Madura cattle, and the third group is Pesisir cattle.

Keywords: Indonesian native cattle, mtDNA, ND-1, genetic variation, phylogenetic tree.

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INTRODUCTION

Indonesia has various types of local cattle, such as Pesisir, Pasundan, Madura, and Bali cattle. Cattle are meat producers which have high economic value and have meaning in society. The demand for food from beef cattle continues to increase with the increase in cattle population in Indonesia. Based on data from the Directorate General of Livestock and Animal Health [1], the beef cattle population in 2013 was 12,686,000 and increased to 16,599,000 in 2017. The increase in cattle population should be followed by the improvement of livestock quality, which can be done through basic livestock breeding programs such as selection and mating.

The livestock quality is influenced by external factors (environment), which are temporary, and internal (genetic) factors, which are permanent and can be inherited to their offspring. If the parents have high

quality, it can be predicted that the quality can be inherited to their progeny [2]. The characters which are displayed either real or not is an expression of genes which are owned by the livestock or it could be stated that all information which can be observed in individual livestock is a genetic marker of that individual. The DNA analysis is a part of the development of DNA technology in finding the origin of livestock. Research on animal quality improvement can be carried out by observing the genetic relationship based on mitochondrial DNA (mtDNA). The study of genetic variation based on mtDNA is currently highly developed because it has an evolutionary rate about 5 to 10 times faster than the nuclear DNA and also has a copy number of 10^3 to 10^4 mtDNA molecules/somatic cell [3]. The proportions of the same genes which are owned by two or more animals are affiliated with their genetic relationship because it indicates that the livestock are originated from one family. The livestock

have a genetic relationship because one animal could distribute his genes to other livestock through gamete inheritance such as parent-child relationships. The genetic relationship is also related to inbreeding which could increase the decline in the livestock quality [4].

Mitochondria is a special organelle which is found in almost all eukaryotic cells. This organelle is basically related to cellular energy production. Mitochondrial DNA (mtDNA) is the only source of cellular protein which is located outside the cell nucleus. The mtDNA is arranged as a double-stranded DNA molecule in the form of a circle in most eukaryotes [5]. NADH Dehydrogenase Subunit 1 (ND-1) gene is one of the NADH Dehydrogenase gene groups which encodes enzymes involved in energy synthesis and is also involved in the oxidative phosphorylation [6]. Research using the ND-1 gene was carried out by Bochkarev *et al.*, [7] in Siberian white fish to see DNA morphology and variation. The ND-1 gene was also previously used to determine the evolutionary history of Chamois goats [8]. Recently, the mtDNA ND-1 gene analysis in Indonesian local cattle is still very limited. Therefore, this study was carried out to evaluate the genetic variation and relationship among Indonesian local cattle using ND-1 gene so that it can be used as the basis for livestock breeding.

RESEARCH MATERIALS AND METHOD

The materials used in this study were 48 blood samples, consisting of 12 samples of Pesisir cattle from West Sumatra, 12 samples of Pasundan cattle from West Java, 12 samples of Madura cattle from Madura Island, and 12 samples of Bali cattle from Nusa Penida, Bali. Blood samples were taken from each cow and stored in EDTA-containing tube with a capacity of 3 mL. DNA samples were obtained through DNA extraction process using DNA Kit (Geneaid, Taiwan) with the procedure according to the kit's manufacturer. The primers used in this study were adapted from DNA fragments targeting the NADH Dehydrogenase Subunit I (ND-1) gene based on NCBI GeneBank with access numbers AF492350.1 for *Bos indicus* and FJ997262.1 for *Bos javanicus*. The forward primer nucleotide sequence was ND-1, 5'AATGGCCGACGAGGGTTTAA-3' with a length of 21 pb, while the reverse primer nucleotide sequence was ND-1, 5'ATGGAGCTCGGTTTGTCTGTC-3' with a length of 22 pb, while the target of DNA

fragments size was 1256 pb [9]. The amplification process of DNA fragments was carried out using a PCR machine (Eppendorf) with the following conditions: initial denaturation of 94°C for 5 minutes, denaturation at 94°C for 50 seconds, annealing at 63°C for 50 seconds, extension process at 72°C for 50 seconds, then repeated until it reaches 30 cycles and ends with the final extension process at 72°C for 5 minutes. The results of PCR products were visualized on 1% agarose gel, followed by gel coloring process and captured using Gel Documentation System (G-Box). The results of PCR products were then also performed sequencing analysis using analysis services in the 1st BASE Laboratory, Malaysia. Sequencing results were then analyzed using MEGA 6.0 software [10] and used to reconstruct phylogeny tree by the neighbor-joining method [11].

RESULTS AND DISCUSSION

Genetic variation in Pesisir, Pasundan, Madura, and Bali cattle are presented in Table 1 and Table-2. Table-1 shows the nucleotide frequency based on mtDNA ND-1 gene found in Pesisir, Pasundan, Madura, and Bali cattle. The highest frequency of thymine (T) nucleotide was found in Madura G10 and Bali H11 cattle with 27.1% of each. The highest frequency of cytosine (C) nucleotide was found in Madura F10 cattle with 26.9%. The frequency of Adenine (A) nucleotide was mostly found in Bali cattle B12 with 33.0%, while the highest frequency of Guanine (G) nucleotide was found in Pasundan cattle F9 with 15.5%. The difference in the frequency of nucleotide base in each cow within the same breed had a chance of mutation. Mutation can change genes to a new allele or a variety of alleles produced [12], however, this case is very rare. Base change or base mutation in DNA with no phenotypic effect is called by silent mutation [13]. Mutation will give a significant change in allele frequency if mutations occur repeatedly and the mutants produced have the ability to adapt to the existing environment [13], although according to Lestari [14], DNA variation is not always expressed in the phenotypic variation (hidden variation), because of several things, not all genetic material is expressed and several variations in nucleotide sequences produce the same protein. Mutation which occurs in a pair of bases in DNA is referred as point mutations. One factor which causes point mutation is base substitution, in which it occurs in all cow samples in this study as can be found in Table-2.

Table-1: Nucleotide frequency in mtDNA ND-1 gene of Indonesian local cattle (Pesisir, Pasundan, Madura, and Bali cattle)

Sample	Nucleotide frequency (%)				Total	Sample	Nucleotide frequency (%)				Total
	T	C	A	G			T	C	A	G	
1. Pesisir H6	26.7	26.7	32.5	14.2	769.0	23. Pasundan F9	26.9	26.2	31.5	15.5	822.0
2. Pesisir A7	26.9	26.4	32.5	14.2	808.0	24. Madura H9	26.9	25.9	31.8	15.4	862.0
3. Pesisir B7	26.9	26.2	32.6	14.3	810.0	25. Madura A10	27.0	25.9	32.0	15.1	862.0
4. Pesisir C7	26.7	26.4	32.4	14.5	808.0	26. Madura B10	27.0	25.8	32.1	15.1	860.0
5. Pesisir D7	26.8	26.0	31.9	15.3	858.0	27. Madura C10	26.9	25.9	31.8	15.4	862.0
6. Pesisir E7	26.9	26.0	31.9	15.2	877.0	28. Madura D10	26.6	26.8	32.1	14.5	745.0
7. Pesisir F7	26.7	26.0	32.2	15.2	889.0	29. Madura E10	26.8	26.4	31.4	15.3	828.0
8. Pesisir G7	26.8	26.0	31.9	15.3	858.0	30. Madura F10	26.8	26.9	32.3	14.1	740.0
9. Pesisir H7	26.8	26.0	31.9	15.3	867.0	31. Madura G10	27.1	25.8	32.1	15.1	876.0
10. Pesisir A8	26.5	26.0	32.1	15.4	904.0	32. Madura H10	26.7	26.6	32.7	14.0	771.0
11. Pesisir B8	26.6	25.8	32.1	15.4	894.0	33. Bali D11	27.0	25.7	32.2	15.0	878.0
12. Pesisir C8	26.7	26.0	32.1	15.3	870.0	34. Bali E11	26.9	26.0	32.1	15.0	866.0
13. Pasundan D8	26.8	25.9	32.1	15.2	869.0	35. Bali F11	27.0	25.8	32.1	15.0	871.0
14. Pasundan E8	26.8	26.1	32.0	15.2	863.0	36. Bali G11	26.9	25.8	32.2	15.1	875.0
15. Pasundan F8	26.8	26.1	32.0	15.2	863.0	37. Bali H11	27.1	25.8	32.1	15.1	876.0
16. Pasundan G8	26.8	26.0	32.1	15.1	859.0	38. Bali A12	26.8	26.4	32.9	14.0	781.0
17. Pasundan H8	26.8	26.1	32.1	14.9	843.0	39. Bali B12	26.7	26.3	33.0	14.0	779.0
18. Pasundan A9	26.8	25.9	32.2	15.2	858.0	40. Bali C12	26.9	25.8	32.3	15.1	877.0
19. Pasundan B9	26.7	25.9	32.2	15.1	872.0	41. Bali D12	27.0	25.8	32.2	15.1	877.0
20. Pasundan C9	26.9	26.0	32.1	15.0	866.0	42. Bali E12	27.0	25.9	32.1	15.0	866.0
21. Pasundan D9	26.9	25.9	32.2	15.1	874.0	43. Bali F12	27.0	25.9	32.1	15.0	869.0
22. Pasundan E9	26.7	26.4	31.4	15.4	830.0	44. Bali G12	26.9	25.8	32.2	15.1	875.0

Table-2: Frequency of identical, transitional, and transversional pairs in mtDNA ND-1 gene of Indonesian local cattle (Pesisir, Pasundan, Madura, and Bali cattle)

Domain	Identical pairs	Transitional pairs	Transversional pairs
1st	259.00	2.00	0.00
2nd	260.00	1.00	0.00
3rd	247.00	13.00	1.00
Total	766.00	16.00	1.00

Table-2 presents the average frequency of total identical nucleotides reaching 766 nucleotides, while the transition occurs as many as 16 nucleotides and transversion as many as 1 nucleotide. The transition and transversal nucleotide changes include base mutation. According to Susanto [13], substitution mutation can be divided into 2, namely transition and transversion.

Transition occurs when purine substituted by purine (A and G) and pyrimidine substituted by pyrimidine (T and C), while transversion occurs when purine substituted by pyrimidine or pyrimidine by purine. In addition to the genetic variation, the genetic length of Indonesian local cattle is also elaborated in this study (Table-3).

Table-3: Genetic length of Indonesian native cattle (Pesisir, Pasundan, Madura, and Bali cattle) based on mtDNA ND-1 gene

	Pesisir cattle	Pasundan cattle	Madura cattle	Bali cattle
Pesisir cattle				
Pasundan cattle	0.055			
Madura cattle	0.046	0.030		
Bali cattle	0.064	0.013	0.026	

Table-3 shows the genetic length of 4 Indonesian local cattle. The farthest genetic length (0.064) was found in Pesisir with Bali cattle, while the

closest genetic length (0.013) was found in Pasundan with Bali cattle. The genetic length of 4 Indonesian local cattle in this study is also illustrated in Figure-1.

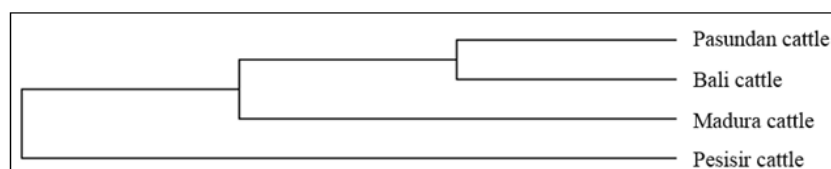


Fig-1: Genetic relationship of Indonesian native cattle (Pesisir, Pasundan, Madura, and Bali cattle) based on mtDNA ND-1 gene

Figure-1 shows the genetic length among 4 Indonesian local cattle studied here which was illustrated in the phylogenetic tree using the Neighbor-Joining method. As can be seen in the phylogenetic tree, there were 3 groups of Indonesian local cattle, the first group consisted of Pasundan and Bali cattle, the second group was Madura cattle, and the third group was Pesisir cattle. In the first group, Pasundan and Bali cattle were within the same group, which can also be seen from the closest genetic length of 0.013 (Table-3).

The close genetic relationship between Pasundan and Bali cattle may be due to the similar body color of these two breeds, moreover, both of Pasundan and Bali cattle also experiencing changes in body color from red brick to black when reaching sexual maturity [15]. According to the Decree of Minister of Agriculture Republic of Indonesia Number 1051/Kpts/SR.120/10/2014 [16], Pasundan cattle was originated from *Bos sondaicus*, Javanese cattle, and Madura cattle, for that reason, Pasundan cattle had typical genetic information with their ancestors. In the second group, there were Madura cattle which had a genetic relationship with Pasundan and Bali cattle. Based on genetic length in Table-3, Madura cattle have the closest genetic length with Madura cattle (0.026) and followed by Pasundan cattle (0.030). This finding is the same as the genetic information of Pasundan cattle published in the Decree of the Minister of Agriculture of the Republic of Indonesia Number 1051/Kpts/SR.120/10/2014 [16], likewise, Madura cattle also the result of the crossing between Bali cattle (*Bos sondaicus*) with zebu cattle (*Bos indicus*). In the third group, there were Pesisir cattle, which was separated from the other three breeds. In Table-3, Pesisir cattle had the farthest relationship with Bali cattle (0.064), followed by Pasundan cattle (0.055) and Madura cattle (0.046). This result may be because of Pesisir cattle was originated from *Bos indicus*, which typically had particular characteristics in the form of hump and dewlap [17], while Bali, Pasundan and Madura cattle were originated from *Bos sondaicus*, which had no dewlap. In a study by Mohamad *et al.*, [18], it was found that Pesisir cattle had zebu mtDNA, Madura cattle had the mixture of zebu and banteng mtDNA, while Bali cattle had banteng mtDNA.

The high genetic diversity will greatly help a population to deal with the changes in their surrounding environment, including being able to overcome disease in nature [14]. Genetic diversity is also important to anticipate unpredictable conditions related to population growth and climate change [19]. In another study,

Istiqomah [20] showed that genetic progress has occurred in the local cattle so that the method of breeding to improve genetic quality was effectively carried out through selection program and assortative mating.

CONCLUSIONS

It could be concluded that the genetic variation is found among Pesisir, Pasundan, Madura, and Bali cattle based on the change in nucleotide frequency. Nucleotide transition is occurred on 16 nucleotides, while nucleotide transversion occurs on 1 nucleotide. The farthest genetic length is 0.064 in Pesisir with Bali cattle, while the closest one is 0.013 in Pasundan with Bali cattle. According to the phylogenetic tree, there are 3 groups of Indonesian native cattle, the first group consists of Pasundan and Bali cattle, the second group is Madura cattle, and the third group is Pesisir cattle.

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