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GENETIC MUTATION AND DELETION IN MADURA CATTLE AS THE RESULTS OF CROSSBREEDING

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Abstract

Crossbreeding between Madura cattle with superior male female cattle other nations (exotic cattle) often occur in some area. These crosses would have consequences that germplasm conservation cow Madura Madura Island would be no longer valid, except in Sapudi Island. According to FAO (2000), that animal genetic resources native would tend to become extinct as a result of the new market demand (massive operations), uncontrolled crossing, turn breeds (replacement of the nation's cattle already exist with the nation's new cow) and the activities of agricultural mechanization (replacement of power use cow with engine power to cultivate farmland). Sapi Madura breed (race) local cattle that formed as a result of natural insulation and environmental influences, so as to have uniformity characteristics that stand out among other local breeds of beef cattle in Indonesia. With the contribution of genetic traits zebu cattle as tolerant of stress due to climate and durability against ticks as well as natural selection and rigorous environment over a period of time, then a cow cattle Madura become a nation that had very high adaptability to the environment. Besides, Madura cattle have a good response to the improvement of feed and resistant to feed with a high content of crude fiber (Soehadji, 1993).

Benefits of using mtDNA according Duryadi (1994), among others (1) As genetic markers in intraspecific variability studies (inter population) that could provide information both qualitatively and quantitatively; (2) Could be use to track the relatively new events such as the study of natural hybridization between two subspecies; (3) Could be use for a historical reconstruction of genealogy matrilinier a species or between existing populations; (4) to reconstruct phyilogenic of several species that were close together. This study used the D-loop region fragment Madura cattle to get the data so that it could determine the genetic diversity between individual nucleotide composition of Madura cattle and identify its phenotypes. Such data could be use to identify the genetic purity of Madura cattle that exist in the area of Sapudi island, Sumenep, Pamekasan Pamekasan, Sampang, Bangkalan.

Preliminary results of a DNA fragment size is 980 bp in D-loop region of mitochondrial DNA that was located in the area of individual mtDNA 15795-16341. Madura cattle had been successfully amplified by PCR using primers BIDLF and BIDLR. The process of sequencing with the Sanger dideoxy method using a primer BIDLF (foward) on the results of PCR successfully read the nucleotide sequence was different each individual cow Madura (BP 716; 756 BP; BP 964; 1098 BP; BP 1113).

Keywords: Germplasm, National Beef, Genetic Characters, mtDNA, Genetic Purity

Background

'World Watch List for Domestic Animal Diversity' report (3rd ed.) Reports that there are approximately 6300 race (breed) cattle in the world of about 30 species of animal domestication and most of the breed is now a local species originating from countries growing. The genetic diversity of local breeds owned a large role in the success of breeding programs in developing countries during the period of the 19th century to 20 (Schearf, 2003).

Food and Agriculture Organization (FAO) predicts that at least one traditional breeds become extinct every week and more than 30% of livestock in Europe is now estimated threatened with extinction (FAO, 1995). Many traditional breeds have disappeared as farmers focus more on the nation's new cow. Approximately 16% of the nation's traditional cattle have become extinct and less than 15% are rare (FAO, 2000)

As science and technology of cattle breeding, biotechnology, market demand, the mechanization of agriculture and livestock production, will encourage the exploitation of animals through crossbreeding to make new breed (Subandriyo and Setiadi, 2003; Sodhi et al., 2006).

Groups of cattle were included in the first category is Bali cattle because Bali cattle are known to be the result of domestication directly from Bull (MacHugh, 1996; Martojo, 2003; Hardjosubroto, 2004) and has the physical characteristics that only has a minor change compared to its predecessor (Handiwirawan and Subandriyo, 2004). The second group of cattle are Madura cattle because according to Payne and Rollinson (1976); Nijman et al., (2003); Verkaar et al., (2003), they are the result of cross between bull or Bali cattle with zebu cattle that has lasted more than 1,500 years ago, even though it is not well documented. (without obvious recording).

Exploitation of Madura cattle through crossbreeding increasingly broad and uncontrolled with exotic cattle which would give unfavorable impact on Madura cattle that have adapted to the local environment. Genetic studies in cattle Madura Madura is interesting because genetic variation is quite large. It is important related to business improvements and maintain the properties of the genetic code so that local livestock did not decrease even extinct genetic quality of Indonesian country.

Molecular tagging using DNA (Deoxyribonucleic Acid) both on the nuclear DNA and mitochondrial DNA (mtDNA) will get results that can reveal the difference with more

precision in differentiating intra and interspesies concerning the structure, composition and organization of the genome at the DNA level (Duryadi, 1994).

This study uses the D-loop region fragment Madura cattle to get the data so that it can determine the genetic diversity between individual nucleotide composition of Madura cattle and identify sightings fenotifnya. Such data can be used to identify the genetic purity of Madura cattle that exist in the area of Sapudi island, Sumenep, Pamekasan Pamekasan, Sampang, Bangkalan.

Materials Research

Sample : Venoject with 10% EDTAin it and filled with 5 ml of whole blood from each cattle taken from veinpuncture

Materials : 20 samples of whole blood from cattle, *WizardGenomic DNA Purification Kit* dari Promega (*cell lysissolution, nuclei lysis solution, protein precipitationsolution,* RNA*ase*, DNA *rehydration solution*), isopropanol, 70 % ethanol, PCR *Core System* I from Promega (MgCl 2, 10 X *buffer reaction Taq*DNA *polymerase*, PCR *nucleotida mix*, *Taq*DNA *polymerase*), restriction enzymes *Hind* III from Gibco BRL, agarose from Promega, 1X *tris acetic acid* EDTA (TAE), *ethidiumbromida*, aquades, primer D-Loop which consist of primers, primer DL-F:5'TTCTTCAGGGCCATCTCATC-3' and primer DL-R:5'GCATCTTGAGCACCAGCA TA-3', *blue loading dye*, tissue, parafilm, ice krystal, *ultra pure water* from Biotech, 50-2000 bp *marker* from Bio Rad.

Agarose Gel Electrophoresis : *agarose gel* 1.5% → solution of 0.5x TBE (Tris-Borate EDTA) 30 ml, 0.45 grams of agarose powder, EtBr 2.5 µl. Materials needed in agarose gel electrophoresis used a sample of DNA produced by PCR, loading dye (0.01 xylene Cyanol%, 0.01% Bromtimol Blue, 50% glycerol), danmarker 100 bp.

Research Instruments

Centrifuged (Hettich), a micropipette (size 20 mL, 200 mL, 1000 mL), tips 20µl, 200 µldan 1000 mL, 1.5 mL micro tube (Axygen), 0.6 ml PCR tube, a set of horizontal electrophoresis apparatus and power supply (Consort), microwave, incubators, GeneAmp PCR System 2400 thermocycler (Perkin Elmer), Gel Doc 2000 (Bio Rad), autoclave (Ogawa Saiki Co.), measurring cup, erlenmeyer, tube venoject, vortex mixer (Gemmy Industrial Corp.), gloves, water bath (Haake), freezer with temperature of 4 ° C, -20 ° C f, ice crystals maker (Cornelius), electric scales (Denver instrument). a set of tray scorer, digital scales, a

100 volt power supply, micro pipette, tips, glass beaker, microwave, stirrer, and UV Transilluminator.

Location and Time Research

Research was conducted at the Laboratory of Molecular Biology, Institute of Tropical Disease (ITD). Research was conducted in January and February 2015.

Sampling and Data Collection

Sampling, Mitochondrial DNA Extraction, PCR reaction, D-lood amplification, *Fragment Length Polymorphism* (RFLP) analysis, electrophoresis and sequencing,

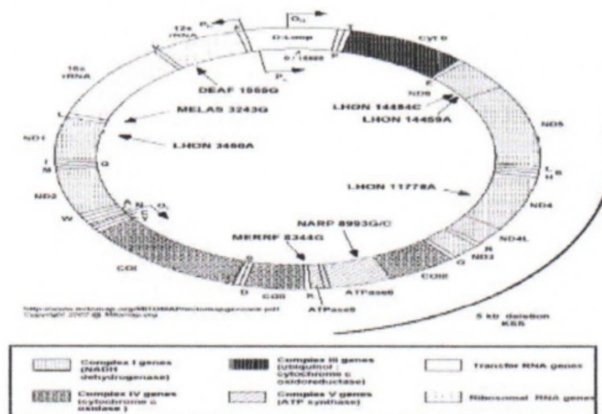

Figure 1 Diagram showed the location of the primary (DL-F, DL-R) which was used to produce the D-loop of mitochondrial DNA of cattle

Results analysis

Bases Nucleotide Composition In Madura Cattle Sapudi, Bangkalan, Sampang, Pamekasan, Sumenep.

PCR products were sequenced cattle's DNA from five lengths and gained different nucleotide bases that was 710 bp, 1110 bp, 964 bp, 756 bp, 716 bp. Analysis of nucleotide diversity arrangement made after the arrangement of mtDNA D-loop region Madura cattle Sapudi, Bangkalan, Sampang, Pamekasan, Sumenep aligned with MEGA application 6 with particular reference to the nucleotide composition of the island of Madura cattle Sapudi. The number of nucleotides of the five bulls was not the same after comparison. It was because there were some deletions of nucleotides in cattle Madura Sapudi, Bangkalan, Sampang,

Pamekasan, Sumenep. Results of multiple alignment could be analyzed as many as 156 bp (Partial) and derived nucleotide diversity of the site as much as 25 pieces. This proved that the D-loop region was an area with very varied nucleotide bases. According to Zhang et al. (2006), Luo et al. (2004), and Wilkinson-Herborts et al. (1996) note that the D-loop region of mitochondrial DNA had the nucleotide bases variation was high, so it was suitable to distinguish the differences between individuals both within the family and between families. Results of multiple alignment of the three cows could be seen in Table 1.

Table 1. Alignment of Nucleotide sequences of D-Loop Regions between Sapi Madura Sapudi, Bangkalan, Sampang, Pamekasan, Sumenep.

```
Sapudi ATG TAC ATA ACA TTA ATG TAA TAA AGA CAT GAT ATG TAT ATA GTA CAT 48
       TAA ATT ATA TAC CCC ATG CAT ATA AGC AAG                         78

SB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 48
       ... ... ... ... ... ... ... ... ... ...                         78

SP     .A. ... ... .T. ... ... ... ... ... ... A.. ... ... ... ... ... 48
       ..C ... .C. ... ... ... ... ... ... ...                         78

SS     .A. ... ... .T. ... ... ... ... ... ... A.. ... ... G.. ... ... 48
       ..C ... .CT ... .G. ... ... ... ... ...                         78

SSm    ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 48
       ... ... ... ... ... ... ... ... ... ...                         78

Sapudi TAC ATG ATC TCT ATA ATA GTA CAT AAT ACA TAC AAT TAT TAA TTG TAC 126
       ATA GTA CAT TAT ATC AAA TCC ATC CTC ACA                         156

SB     ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... 126
       ... ... ... ... ... ... ... ... ... .AC                         156

SP     ... T.. .A. ..C ... .C. ... ... .G. ... .TA .TC C.C ... .C. ... 126
       ... ... ... ..C ... ... ..T .C. ..T GAC                         156

SS     ... T.. .A. ..C ... .C. ... ... .G. ... .TA .TC C.C ... .C. ... 126
       ... ... ... ... ... ... ..T ... ..T GAC                         156

SSm    ... ... ... ... ... ... ... ... ... ... ... .-. ... ... ... ... 126
       ... ... ... ... ... ... ... ... ... .AC                         156
```

NB:

Sapudi : Sapi Madura Sapudi
SB : Sapi Madura Bangkalan
SP : Sapi Madura Pamekasan
SS : Sapi Madura Sampang
SSm : Sapi Madura Sumenep

Dot (.) Denotes nucleotide same Sapudi Madura cattle.

The results of the nucleotide alignment composition of Madura cattle in Sapudi, Bangkalan, Pamekasan, Sampang, Sumenep which could be analyzed by MEGA 6 along 156 bp. Of the five cows were studied, each of which had the nucleotide composition Thymine (T), Cytosin (C), adenine (A), Guanine (G) are different. This can be seen in Table 2

Table 2. Comparison of Nucleotide composition D-loop Region Partial Sapi Madura Sapudi, Bangkalan, Pamekasan, Sampang, Sumenep.

Sapi Madura	**%**					
	T	**C**	**A**	**G**	**T + A**	**C+G**
Sapudi	32.7	16.0	42.9	8.3	75.6	24.3
Bangkalan	32.7	16.0	42.9	8.3	75.6	24.3
Pamekasan	29.5	19.9	41.7	9.0	71.2	28.9
Sampang	31.4	17.9	40.4	10.3	71.8	28.2
Sumenep	32.9	16.1	42.6	8.4	75.5	24.5

From table 2, it could be seen from the table comparison of the percentage of nucleotide T (Thymine) D-loop region highest to lowest in a row owned by Sapi Madura Sumenep 32.9%, Sapudi 32.7%, 32.7% Bangkalan, Sampang and Pamekasan 31.4% 29.5%. Comparison of percentage of nucleotides C (Cytosin) D-loop region highest to lowest consecutive Pemekasan Madura cattle owned by 19.9%; 17.9% Sampang, Sumenep 16.1%, Bangkalan Sapudi 16% and 16%. A percentage of nucleotides (adenine) region D-loop highest to lowest consecutive Sapudi Madura cattle owned by 42.9%; Bangkalan 42.9%, 42.6% Sumenep, Pamekasan and Sampang 41.7% 40.4%. The percentage of nucleotide G (Guanine) D-loop region highest to lowest consecutive Sampang Madura cattle owned by 10.3%; 9% Pamekasan, Sumenep 8.4%, 8.3% and Bangkalan Sapudi 8.3%. Differences in the composition of the nucleotides A, T, G, and C showed the differences in composition of amino acids that it contained (Ridley, 1991).

The percentage of A + T nucleotides on all cattle studied were Madura cattle Sapudi, Bangkalan, Pamekasan, Sampang and Sumenep had a higher amount than the percentage of G + C nucleotides. The percentage of A + T nucleotides from the highest to the lowest, respectively were Cow Sapudi 75.6%; Bangkalan 75.6%; 75.5% Sumenep, Pamekasan, Sampang 71.8% and 71.2%. The percentage of G + C nucleotides from the highest to the

lowest, respectively were 28.9% Pamekasan Madura cattle; Sampang 28.2%.%, 24.5% Sumenep, Sapudi 24.3%, 24.3% Bangkalan. In this study, the composition of the nucleotide bases A + T had a higher frequency than the composition of the G + C.

Sites such diverse nucleotide bases due to mutations in the nucleotide bases. Mutations include substitutions transition mutations (replacement of a purine base by another purine base was A↔G or substitution of pyrimidine bases by another pyrimidine bases were T↔C) and transversion substitution mutations (replacement of a purine by a pyrimidine bases, namely A↔ C, A↔T, G↔C, G↔T, or replacement of a purine pyrimidine bases by which T↔A, T↔G, C↔A, C↔G). In addition there was also a deletion mutation, namely the reduction of base pairs that caused loss of base pairs that originally there. From these results, Pamekasan on Madura cattle there are 15 transition substitution (Si), 9 transversion substitution (ST). In cattle there were 13 mutations Sampang transitions, 8 transversions mutations. In cattle there were 2 Mutation Tranversi Bangkalan and Sumenep Cow tranversi there are two mutations, one deletion.

Genetic distance between Madura Cattle Sapudi, Bangkalan, Pamekasan, Sampang, Sumenep.

Close genetic relationship between Madura cattle in Sapudi, Bangkalan, Pamekasan, Sampang and Sumenep were seen by measuring the genetic distance. Genetic distance was measured using Pairwise analysis indicated Distance Calculation matrix genetic differences between the three cows that had made sequencing the nucleotide at D-Loop region MtDNA.

The genetic distance from this model used to look at the level of substitution of transition and tranversi through many different nucleotides per couple (Abdullah, 2008). The cows that had the lower value of genetic distance, then the animal had a closer kinship. Instead of cattle that had high genetic distance, the relationships were more distant. Genetic history between Sapi Madura Sapudi, Bangkalan, Pamekasan, Sampang, Sumenep could be seen in Table 3.

Table 3. **Genetic Distance Based Method D Pairwise-loop Regional Distance between Sapi Madura Sapudi, Bangkalan, Pamekasan, Sampang, Sumenep.**

No	Sapi Madura	1	2	3	4
1	**Sapudi**				
2	**Bangkalan**	0.013	-		
3	**Pamekasan**	0.173	0.154	-	
4	**Sampang**	0.186	0.166	0.033	-
5	**Sumenep**	0.013	0.000	0.154	0.166

From table above showed that the genetic distance Madura cattle Sapudi (0.0000) with Beef Bangkalan and Sumenep (0:13) had a close kinship level. While in the cow Sampang Madura (0186) and Pemekasan (0173) had genetic distance farther. This was because of differences in nucleotide significantly in Madura cattle with cattle Sapudi Pemekasan and Sampang.

Discussion

Information on genetic diversity could be obtained in several ways, such as by using microsatellite DNA (Puja and Sulabda 2009), AFLPs, SNPs, Y-chromosomal DNA or analyzing the mitochondrial DNA (mtDNA) (Lenstra 2005). Mitochondrial DNA (mtDNA) had represented the most informative genomic elements for describing the origin of livestock. Until now, the mitochondrial sequences had been widely studied in cattle, pigs, sheep, horses, dogs, donkeys, and goats (Chen et al., 2005).

Mitochondrial deoxyribonucleic acid (mtDNA) in this study were isolated from cattle's blood cells with the same quality with mitochondrial DNA isolated from tissue / muscle. This was in line with the statement from Tapio and Grigaliunaite (2003), expressed the mitochondrial DNA could be isolated from the hair, bones, teeth, body fluids (saliva, semen, blood). According to Anderson et al. (1981) in Hartati and Infallible (2004), mitochondrial DNA found in cells or tissues that have the activity of metabolites high or in areas that require ATP in large quantities, such as the tail of sperm cells, epithelial cells were actively dividing the skin epidermal tissue and heart muscle cells.

MtDNA segments that could be used for the analysis of genetic diversity of an organism was the mtDNA control region or D-loop region, namely the non-coding part of the mitochondrial hypervariable (Elrod and Stansfield, 2007). The high rate of nucleotide polymorphisms or differences in the sequence of the second part of the non-coding hypervariable region used to distinguish between individuals of a species (Melton, 1999). In addition D-loop region analysis also used to see the variation among subspecies and between populations (Brown, 1986). MtDNA D-loop regions known to be rapidly developed compared with other parts of mtDNA. This was due to the accumulation of base substitution, insertion and deletion processes that speed was very fast when compared to nuclear of DNA (Foran et al., 1988).

The results of comparative studies from Madura cattle in Bangkalan, Pemekasan, Sampang and Sumenep in this study had a high nucleotide diversity arrangement. From these data could be analyzed that nuklotida bases A (adenine) has a higher percentage of nucleotide bases C (cytosine). Studies Prusak and Grzybowski (2004) of 20 species of mammals discovered cases in humans and zebra have sequences with more base A compared to C on the strand mild mitochondrial DNA, sheep and cattle (Zebu) bases a lot more than the base C, and this phenomenon was common in mammals. The results also showed the same phenomenon. According to Irwin et al. (1991) This phenomenon had to do with the level of amino acid identity associated with a redox reaction center protein content (both Qo and Qi) which was involved in electron transfer (Hatefi, 1985; Howell and Gilbert, 1988).

Differences in the rate of mutation or variation in the mitochondrial regions in this study may be influenced by many factors (Kimura, 1987; Mindell and Thacker, 1996). Studies to determine the barrier effect of the mutation rate of the level of replication, DNA repair efficiency, and exposure to mutations were not done immediately, and it was generally associated with biological variables, such as body size, generation time and SMR (Standard Metabolic Rate). According to the theory, the individual taxa with large body size, a long life and value of low metabolic, usually had low mutation rate, but according to the results of research on ribosomal genes and P12, mtDNA mutation was not related to body size and SMR (Gissi, et al. 2000). Laird, et al. (1969), Martin and Palumbi (1993), Rand (1994) argue that the effect of the rate of metabolism was proposed as a factor affecting the level of mitochondrial mutations, because the pollutants contained in the free radicals of oxygen could affect the metabolism rate. Although discovered a correlation between mutation rate

and SMR, but only found in the case of an improved model of mitochondrial damage (DNA repair) due to insufficient oxidation. This indicated that mitochondria in higher organisms had been equipped excellent mechanism through excision bases within the system repair oxidative damage (Bogenhagen, 1999; Sawyer and Van Houten, 1999), as happened in the nuclear genome of the cell (Sawyer and Van Houten, 1999), hence the existence of a specific DNA repair efficiency in the taxon had never been proven. Reyes et al. (1998) reported that the composition of mtDNA was not associated with oxidative damage but more on spontaneity during the replication process. Therefore, the discovery of cases of mutations in this study was likely to occur at the level of DNA replication. This was in line with the opinion of Wibowo (2001) that genetic diversity could arise due to natural selection, environmental influences, mutation and mating. The genetic diversity and a high mutation rate in this study were not interpreted as being detrimental. By Rhymer (1999) maintain the genetic diversity of a population was very important in conservation because of the high genetic diversity will greatly help a population to adapt to the changes that occur in the surrounding environment. Polymorphic high level in the D-loop region of mtDNA did not cause changes in third phenotype cows were tested. This was because the D-loop region was non-coding regions that were not expressed. This area only play a role in the regulation and initiation of replication and transcription of mtDNA (Boore, 1999).

Conclusion

1. Mitochondrial Deoxyribonucleic acid (mtDNA) region Diplacement Loop (D-Loop) Sapudi Madura cattle, Bangkalan, Pamekasan, Sampang, Sumenep could be amplified well with the size of 980 bp.
2. From the sequence analysis of mtDNA D-loop region found 50 sites of genetic diversity that was Madura cattle from Pamekasan substitution mutation, there were 15 transitions, 9 transversions substitution mutation. Sampang Madura cattle have 13 substitution mutations transitions, 8 transversions substitution mutation. Madura cattle from Sumenep had 2 substitution transitions mutation, 2 transversions substitution mutations and 1 deletion. Despite this high level of variation in this study did not result in a change in phenotype as D-Loop region is non-coding regions that are not expressed.

Recommendation

1. It was necessary to do further research to find out the main cause genetic changes in cattle Madura and its influence on the genetic quality of Madura cattle.
2. In further research could be done on the DNA sequencing Mitochondrial cyt area b, to be compared with Mitochondrial DNA D-Loop region.

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