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COMPARISON OF GENOTYPE AND PHENOTYPE OF MADURA CATTLE TO OBTAIN THE GENETIC PURITY THAT CAN BE USED AS A LOCAL LIVESTOCK GERMPLASM CONSERVATION ON MADURA ISLAND

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Abstract

The crossbreeding between Madura cattle with superior bulls of other nations (exotic cattle) often occurs in an area. The crosses will have consequences that germplasm conservation of Madura cattleon Madura Island will be no longer valid, except in Sapudi Island. According to FAO (2000) that native livestock genetic resources will tend to become extinct as a result of the new market demand (large scale exploitation), cross uncontrolled, turnover breeds (replacement of the existingcattle breed with new cattle breed) and the activities of agricultural mechanization (replacement of cattle power with machines powerto cultivate agricultural land).Madura cattle become breed (race) of the local beef cattle that formed as a result of natural insulation and environmental influences, so as to have uniform characteristics that stand out among other local breeds of beef cattle in Indonesia. With the contribution of genetic zebu cattle such as tolerance to stress due to climate and durability against ticks as well as natural selection and rigorous environment within a period of time, Madura cattle become a breed that has very high adaptability to the environment. Besides that, 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 mt DNA according Duryadi(1994), are(1) As genetic markersin the study of intraspecific variability (inter population) that can provide qualitative and quantitative information; (2) Can be used to track the relatively new events such as the study of natural hybridization between the two subspecies; (3) Can be used for a historical reconstruction of genealogy matriliniera species or between populations that exist; (4) Can reconstruct phylogenic of several species that are close. This study uses the D-loop region fragment Madura cattle to get the data so as to determine the genetic diversity of the nucleo tide composition between individual Madura cattle and identify sightings of the phenotype. Such data can be used to identify the genetic purity of Madura cattle that exist in the region Sapudi island, Sumenep, Pamekasan, Pamekasan, Sampang, Bangkalan Temporarily results isa DNAfragmentsize980bpD-loop region of mitochondrial DNA that is located in thearea15795-16341mtDNA of individual cattle Madura has been successfully amplified by PCR using primers BIDLF and BIDLR. The process of sequencing a primer BIDLF (foward) on the results successfully read different nucleotide sequence of each individual Madura cattle (**716 BP; 756 BP; 964 BP; 1098 BP; 1113 BP**).

Key Words :Germplasm, Cattle breed, Genetic characteristic, mtDNA, Genetic purity

INTRODUCTION

Document'WorldWatch Listfor DomesticAnimal Diversity' report (3rd ed.) reports thatthere are approximately 6300race(breed) of cattlein the worldof about30species ofdomesticatedanimalsandmost oftoday'sbreedis anative speciesthat comes fromdeveloping countries.The genetic diversity oflocalbreedsownedmajor rolein the success ofbreedingprogramsindeveloping countriesduringthe period ofthe 19th century to20th. Thisclearly illustratesthat thelocal speciesis animportantsource of genetic resourcesand uniquetoanticipatethe needs oftoday'slivestockproductionand future(Schearf, 2003).

Food andAgriculture Organization (FAO) predictsthatat least onetraditionalbreedsbecome extincteveryweekandmorethan30% of livestock in Europeis now estimatedin a statethreatened with extinction(FAO, 1995). Manytraditionalbreedshave disappearedas farmers focusonthe newbreed cattle. Approximately16% ofthe nation'straditionalcattlehave become extinctandlessthan15% arerare(FAO, 2000). This situationcan be seen inbreed zebucattleinIndiaaresignificantlyhave losta very important economicandpopulation sizesdecreasemainlydue tothe widespreadcross(Sodhi *et al*., 2006)

As the development of scienceandtechnologycattle breeding, biotechnology, market demand, mechanization of agricultureandlivestock production, willencourage theexploitation oflivestockthroughcrossbreeding, replacement of new breed (SubandriyoandSetiadi, 2003;Sodhi*etal*., 2006), as well asthe depletion ofstocksin excess,andin turnwillthreaten thegenetic diversityof livestock. On theother hand,the conservationof animal geneticdiversitywill always be necessaryin breedingin the future,because ofthe absence ofgenetic diversity,breedingcattlewould not be possibleto anticipatefutureneeds(SubandriyoandSetiadi, 2003).

Indonesian native cattle are genetically and phenotypically generally are: (1) a derivative of Bulls (Bosjavanicus), which has been domesticated and can also (2) derived from the result of crosses between native Indonesia with exotic cattle were then domesticated and local adaptation. Groups of cattle were included in the first category are Bali cattle because Bali cattle is known as a direct result of domestication of the bull (MacHugh, 1996; Martojo, 2003; Hardjosubroto, 2004) and has the physical characteristics that only experienced minor changes compared to its great-grandparent (Handiwirawan and Subandriyo, 2004). The second group is Madura cattle because, according to Payne and Rollinson (1976); Nijman et al., (2003); Verkaar et al., (2003) is the result of crosses between bull or Bali cattle with zebu cattle that

has lasted more than 1,500 years ago, although it is not well documented in principle breeding (without clear recording).

State Gazette (staatsblad) number 226 year 1923, numbers 1465 year 1925, number 368 year 1927, number 57 year 1934, number 115 year 1937 and implicitly contained in Law No. 6 year 1967 set Madura cattle is germplasm protected and maintained purity in Madura Island. However, based on the latest reference, are (1) the decision of the Minister of Agriculture numbers: 208 / Kpts / DT210 / 4/2001, dated April 4, 2001 on Guidelines for the National Livestock Breeding; (2) the direction of the Director of the Nursery and the Director General of Livestock Production of Crosses cow Madura Madura Island; and (3) the submission of the Legal Opinion to get around the law or the *Staatblad*, has made cross-breeding between Madura cow with superior males of other breed (exotic cattle). The crosses will have consequences that germplasm conservation on the island of Madura Madura cow is no longer valid, except in the island Sapudi (isolated region that concentrated germplasm purification region with a capacity of ± 5000 cattles (Kutsiyah, 2012).

Exploitation of Madura cattlethroughcrossbreedingincreasingly widespreadanduncontrolled with exotic cattle breed would give unfavorable impactonMaduracattlesthat haveadapted tothe local environment. This concernhasoccurred innativecattleinLithuania(Eastern Europe) that is endangered(Malevičiūtė*etal*., 2002)due tointentionalcrossesbutunstructured. Even somenative cattle in Indiahas become extinctbefore the cattle is identified and exploitedas a result ofcrossbreedingwidespreadanduncontrolled(Sodhi*etal*., 2006). It thusis also confirmedby theFAO(2000) thatnativelivestock genetic resourceswilltend tobecome extinctas a result ofthe new market demand(large scale exploitation), crossuncontrolled, turnoverbreeds(replacement of the existing brred cattlewiththe new breed cattle)andfarmmechanizationactivities(replacement of cattle power with machines power to cultivate agricultural land).

Therefore, genetic studies ofMaduracattle in Madurabeinterestingbecausegenetic variationis quite large.This isimportant in relationto thenature ofthe business improvement and preservethe geneticcodeso thatlocal livestock is not decreased genetic quality even extinctfrom Indonesia.

Measurementsof genetic diversitythrough thedesignationof molecularusingDNA(Deoxyribonucleic Acid) either onnuclear DNAandmitochondrial

DNA(mtDNA) will getthe resultsthat couldrevealthe differencesmore carefullyin differentiatingintraandinterspeciesconcerning thestructure, composition and organization ofthe genomeat the level ofDNA(Duryadi, 1994).

Benefits of usingmtDNAaccordingDuryadi(1994), are (1) Asgeneticmarkersin the study ofintraspecificvariability(interpopulation) that can providequalitativeand quantitativeinformation; (2) Can be usedto track therelatively neweventssuch asthe studyof naturalhybridizationbetween thetwosubspecies; (3) Can be usedforahistoricalreconstructionofgenealogymatriliniera speciesor betweenpopulationsexist; (4) Can reconstructphylogenicofseveralspeciesthat are close.

In themitochondrial genome therearefragments ofprotein-codingandnon-coding proteins.Fragments ofprotein-codingareCytochromeOxidaseunitI (COX I), CytochromeOxidase unitII(COX II), CytohcromeOxidase unitIII(COX III), and theCytochromeb(Cyt. B). These sections areusedforresearch on the relationshipofthe genusorspecies ofthe same family, whilenotprotein-codingfragmentin the mitochondriawhichare oftenusedin studyinggenetic diversity andkinshipamong speciesisthe Displacementloop(D-loop) region. D-loop regionis interesting to studybecausetwoof the threedomainare HypervariableSegmentsI (HVS-I) andHVS-II has ahigh mutationthatchangesthe sequence ofnucleotidebasesoccurnot onlyonthe level interspeciesbutalsoat the levelintraspecies. Assessment ofthe D-loop regionis widely usedtostudypopulation biologyandevolutionof animals(Widayanti, 2006)

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

RESEARCH METODE

Sample and Data Procedure

1. Sample Taking

   5 ml of blood sample from Madura Cattle by venipuncture using 10 ml of 10% EDTA venoject tube. Vaccutainer tube for saving the sample was given a label for each Madura Cattle. Sample was stored in room temperature to take to the laboratorium.

2. DNA's Mitochondira Extracted

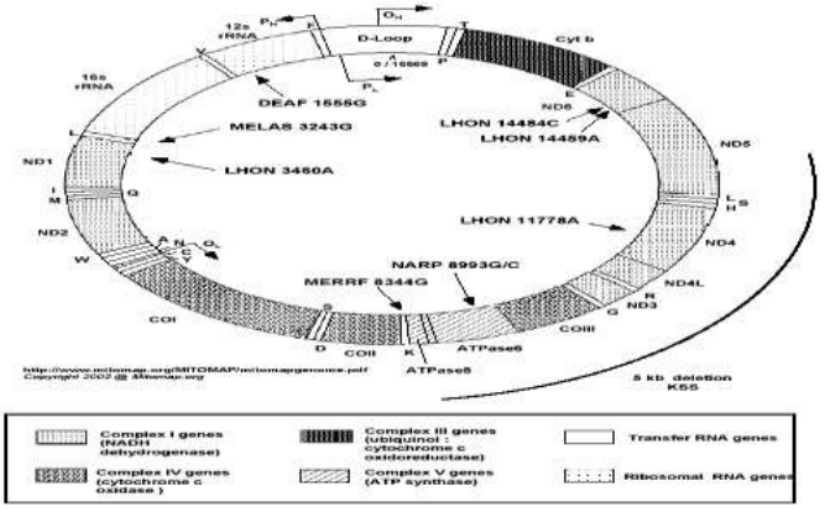
DNA was extracted from the total of the blood using Wizsrd Genomic Purification System. Total of the blood was 200 µl, put it in the 1,5 ml microcentrifuge tube with 450 µlpelisissel liquid (cell lysissolution), mix it by turn the tube 5-6 times then incubate it in room temperature for 10 minutes to make the blood cell lysis. If the pellet was still red, repeat the procedure until the pellet was clean (about 2-3 times). Centrifuge the tube 14000 rpm for 20 seconds in a room temperature to get the white pellet blood cell. Remove the supernatant and vortex it for around 20 seconds so it wouldn't lumpy. Add 150 µlpelisisinti (nuclei lysissolutin) into the white pellet blood cell and mix it by turn it over multiple times. Add uIRNAase into the core liquid and incubate in 37^{o}C for 15-20 minutes. Cool down the sample in the room temperature for about 3 minutes and add 60 µl protein precipitation solution, mix it with vortex for 10-20 seconds and centrifuge 14000 rpm for 3 minutes to making protein pellet. Take the supernatant with pipet and put it into 1,5 ml micro centrifuge tube with 150 µl isopropanol. Turn it over multiple times till it formed like white thread of DNA. Centrifuge it 14000 rpm for a minute in a room temperature. Remove the supernatant then add 300 µl 70% ethanol into the pellet then turn it over multiple times to wash the DNA pellet. Centrifuge it 14000 rpm for a minute in the room temperature. Remove the ethanol, turn over the micro centrifuge tuber over the strain paper in the room temperature for 15-20 minutes. After it dried, add 100 µl DNA rehydration liquid and DNA direhydration, incubate for a night in the room temperature. Save the DNA in the 2-8^{0}C temperature. Check the DNA with electrophoresis gel to know the existence of DNA extraction. DNA concentration could be measurable by compare it with the standard plasmid DNA into the electrophoresis gel.

3. PCR Reaction

The extraction of DNA could use to PCR reaction in a PCR machine (thermocycler). This reaction to amplify the DNA's mitochondria in the D-loop area. This reaction must do in a 25 µl mixed volume that contains 200 µM from each dNTPs, 2 µM $MgCl_2$, DNA template, primer DL-F and DL-R, each 0,15 µM, 10 times buffer reaction TaqDNA polymerase and 1,5 unit TaqDNA Polymerase in 0,6 ml PCR tube.

4. D-loop Amplification

D-loop area of DNA mitochondria was amplified with PCR amplification D-loop with primer DL-F or DL-R in a row.



Picture 3.1 The Diagram Shows the Place of Primer (DL-F, DL-R) that Used to Produce D-loop from Cow's DNA Mitochondria

The primer that used to reaction amplification of D-loop from DNA mitochondria are : Primer D-loop : DL-F : 5'TTCTTCAGGGCCATCTCATC-3' and DL-R : 5'GCATCTTGAGCACCAGCATA-3' that get from nucleotide ordinal *Bosindicus*mitochondrial DNA, D-loop area (access code AB268575) that has published by Genbank. That primer was designed with using Primer3 Program (http://www-genome.wi.mit.edu/cgi-bin/primer/ primer3_www.cgi).

Condition of PCR's reaction amplification for D-loop are : a chain reaction early denaturation in 94^{o}C for 45 seconds, annealing in 58^{o}C for 45 seconds, and extention in 72^{o}C for a minute following with a step the end of polymeration in 72^{o}C for 6 minutes.

5. Restriction Fragment Length Polymorphism (RFLP) Analysis

Fragment from PCR amplification directly used in digestive reaction using restriction enzyme. D-loop area from mitochondrial DNA from PCR amplification was digested with HindIIILAliquot enzyme that contained about 100 ng DNA into sterile effendorf tube. Mix master is made from restriction

HindIII enzyme, 10 X React 2 buffer and ultra pure water. 8 µlMix master (1 unit HindIII enzyme, 3 µl 10 X React 2 buffer and the rest was ultra pure water) added in each tube contained amplification DNA and incubate it in 37°C for 6 hours.

6. Electrophoresis

DNA is seen by horizontal electrophoresis with 1% agarosa gel. Agarosa gel was made by mixing the agarosa into buffer 1 X TAE and boil it in microwave for 30 seconds then leave it until the temperature is 60°C and add 0,12 µl/ml ethidiumbromide so the DNA could be seen under the ultra violet light. Pour the agarosa liquid into the electrophoresis container till it set in firm (15-20 minutes). Do the electrophoresis for 90 minutes (depends on gel concentration and voltatio), 55 volt. DNA could be seen in a dark room with ultraviolet light and take the image with Gel Doc 2000 using red filter.

7. Sequencing

Determination of nucleotide sequence of DNA sequencing is done in a way that the final stages to obtain data from the nucleotide sequence of fragments of DNA fragments multiplication results. A single band on agarose gel as a PCR product used as template in the sequencing reaction using the forward and reverse primer, such as during the amplification. PCR products were sequenced is D-loop parsial.Proses sequencing performed in the Central Veterinary Institute.

RESEARCH RESULT

1.1. Mitochondrial DNA amplification Region Displacement Loop (D-Loop)

Mitochondrial DNA (mtDNA) has been able to be extracted by either of 5 Madura cattle's white blood cells originating from five regions on the island of Madura, namely Bangkalan, Sampang, Pamekasan, Sumenep and Sapudi Island. The entire mtDNA D-loop region Madura cattle tested can be amplified by PCR (Polymerase Chain Reaction) using a primer BIDL-F5'ACCCCCAAAGCTGAAGTTCT-3 'and primary BIDL-R 5'GTGCCT TGCTTTGGGTTAAG-3'. BIDLF primer pairs and BIDLR based base sequences Bosindicus. Based on the whole genome sequences of mitochondrial DNA Bosindicus (Nellore cattle) from GenBank, a DNA fragment of 980 bp sized cow is the result of amplification primer pairs BIDLF and BIDLR, consisting of 37 bp fragment tRNApro

gene at position 15,758 to 15,794 bases, 913 bp fragment of intact areas D-loop at the base position and the 30 bp fragment 15795-16341,1-366 tRNAPhe on base position 367-396 (Abdullah, 2008). BIDLF primer pairs and have BIDLR fragment measuring about 980 bp that is attached to the base to 30 to 49 genes tRNAPro (15758-15777) for primary BIDLF and bases 11 to 30 genes tRNAPhe (377-396) for primary BIDLR , Optimal viewing fragment amplification product primer pairs by using PCR machines 9800 Fast Thermal Cycler at 59 OC annealing conditions for 45 seconds. PCR amplification products electrophoresis results are presented in Figure 1.1. After the resulting PCR product sequenced mtDNA D-loop sequences MaduraCattle along 980 bp.

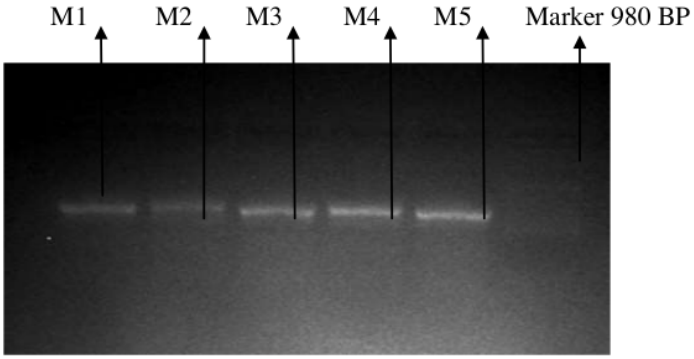


Figure 1.1ResultsVisualizationof PCR(Polymerase Chain Reaction) MitochondrialDNADisplacementLoopRegion.
Description: M1=BangkalanMadura Cattle
M2=SampangMaduraCattle
M3=Pamekasan Madura Cattle
M4=SumenepMadura Cattle
M5=SapudiIsland Cattle

1.2. Mitochondrial DNA sequencing Region Displacement Loop (D-Loop).

Mitochondrial DNA D-loop region were successfully amplified, once that is done on the sequencing of the PCR product direction and carried foward alignment between Madura cattle genome sequences derived from Bangkalan, Sampang, Pamekasan, Sumenep and Sapudi Island. Results of such sequencing electropherogram readout scanner results against fragments in polyacrylamide gel. Each nucleotide produce a peak with a different color on the electropherogram is nucleotide A green, black G nucleotide, the nucleotides C in green and red T nucleotides.

The sequencing results obtained are less good because many nucleotide sequencing is lost in the process, for it can not be analyzed properly so it is necessary to see the results of

sequencing PCR products from the reverse direction so that the missing nucleotide can be known. At this time sequencing of direction revese still under construction.Mitochondrial DNA (mtDNA) has represented the most informative genomic elements to describe the origin of livestock. Until now, the mitochondrial sequences have been extensively studied in cattle, pigs, sheep, horses, dogs, donkeys, and goats (Chen et al., 2005).

In this study mitochondrial DNA can be isolated from blood cells of Madura cattle with quality similar to mitochondrial DNA isolated from tissue / meat or beef liver Madura. This is in line with the statement Tanio and Grigaliunaite (2003), which states mitochondrial DNA can 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 tissue which has an activity of metabolites highest or in areas that require ATP in large quantities, such as the tail of sperm cells, epithelial cells that actively divide in the skin epidermal tissue and heart muscle cells. MtDNA segments that can be used for the analysis of genetic diversity of an organism is the mtDNA control region or the D-loop region, which is part of the mitochondrial hypervariable noncoding.

Mitochondrial DNA (mtDNA) has been able to be extracted by either of 5Madura cattle's white blood cells originating from five regions on the island of Madura, namely Bangkalan, Sampang, Pamekasan, Sumenep and Sapudi Island. The entire mtDNA D-loop Madura cattle tested can be amplified by PCR using primers BIDL-F5'ACCC CCAAAGCTGAAGTTCT-3 'and primary BIDL-R 5'GTGCCTTGCTTTGGGTTA AG-3'. BIDLF primer pairs and BIDLR based base sequences Bos indicus. Based on the whole genome sequences of mitochondrial DNA Bos indicus (Nellore cattle) from GenBank, a DNA fragment of 980 bp sized cow is the result of amplification primer pairs BIDLF and BIDLR, consisting of 37 bp fragment tRNApro gene at position 15,758 to 15,794 bases, 913 bp fragment of intact areas D-loop at the base position and the 30 bp fragment 15795-16341,1-366 tRNAPhe on base position 367-396 (Abdullah, 2008).

BIDLF primer pairs and amplification BIDDLE has upgraded the size of about 980 bp fragment attached to the base to 30 to 49 genes tRNAPro (15758-15777) for primary BIDLF and bases 11 to 30 genes tRNAPhe (377-396) for primary BIDLR. Optimum display fragments of the primer pair amplification product using a Perkin Elmer 2400 PCR machine at 59 OC annealing conditions for 45 seconds. PCR amplification products electrophoresis results are presented in Figure 1.1. After the resulting PCR product sequenced mtDNA D-loop sequences Sapi Madura along 980 bp.

CONCLUSION

Tentative conclusion that can be derived from this study are:

1. DNA fragment size of 980 bp D-loop region of mitochondrial DNA that is located in the region of mtDNA from 15,795 to 16,341 from individuals Madura cattle have been successfully amplified by PCR using primers BIDLF and BIDLR.

2. The process of sequencing at the Sanger dideoxy method using a primer BIDLF (foward) on the results of PCR successfully read different nucleotide sequence of each individual Madura cattle (BP 716; 756 BP; BP 964; 1098 BP; BP 1113).

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