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Genetic Variation and Phylogenetic Tree of Indonesian domestic Goat

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ABSTRACT: Indonesia has native domestic goat breeds as genetics resources, however so far there were limited informations of genetic diversity, population demographic history, and origin of Indonesian goats. The aim of this study was to examine the genetic diversity and phylogenetic tree of Indonesian domestic goats which compared with available data in Genbank. Polymerase Chain reaction from 20 individuals representing 4 indigenous breeds was performed to determine a 464-bp fragment of mitochondrial DNA (mtDNA) cytochrome b. Restriction fragment length polymorphism of 464 bp using Hinf and HaeIII show the similar fragment result in 2% Agarose gel. Two representative products PCR were sequenced for further analysis. The sequence results then aligned with 37 data genbank by using BioEdit version 7.0. These results pattern of genetic variation in goat's mtDNA sequences indicated 79 SNP contains substitution G → A (25 SNP), T → C (46 SNP), A → T (4 SNP) and 5 SNP of insertions/deletions. Phylogenetic tree analisis shows the distinct group of Indonesian goats compare with 37 individual data form genbank which clustered into two larger lineages. The genetic variation of domestic goats in Indonesia gives more contribution to the genetic diversity of animal in the world.

Keywords: Domestic goats; mtDNA; genetic variation, Phylogenetic tree

INTRODUCTION

The domestic goat (*Capra hircus*) is classified as Capra, Caprovinae, Bovidae, Ruminatia, Artiodactyle. Indonesia raises the number of goats (Bligon, Ettawa Grade, Kejobong, Gembrong, Marica, Samosir, Kosta, Muara, Benggala) and has a specific breed of goat breeds, called Kacang (Hartatik, 2014). There are around 300 breeds of goats identified in tropic and sub-tropic territory (Devendra and Burns, 1994). Mitochondrial DNA (mtDNA) is maternally inherited and changes in the nucleotide sequence occur faster than DNA (Brown, 1980) so mtDNA is ideally suited as a tool for studying population genetics (Bailey *et al*., 2000). Mitochondrial DNA (mtDNA) is a useful genetic marker for both intra- and interspecies studies (Brown *et al*., 1979; Kikkawa *et al*., 1995). Several studies on mtDNA RFLP of cattle have been reported. Cyt b gene is a gene that is often used to compare multiple phylogenetic species in the same genus or family, the diversity of the cyt b gene has been used to detect the source of milk derived from cattle (*Bos*), sheep (*Ovis*) and goats (*Capra*) and buffalo (*Bubalus*) (Lanzilao *et al*., 2005). Pfeiffer *et al*. (2004) has identified the diversity of the cyt b gene in the species of cattle (*Bos taurus*), sheep (*Ovis aries*), goats (*Capra hircus*), roe buck (*Capreolus capreolus*) and red deer (*Cervus elaphus*) (Wolf *et al*., 1999). Various molecular markers have been explored and commonly utilized in the studies of genetic diversity and phylogenetics of domestic goats based on studies of RFLP and the mtDNA genome. Li *et al*. (1999) deduced that the origin and evolution of modern Chinese goat breeds were independent of those of exotic goats and that the indigenous goats could be grouped into two main types, the North type and the South type. Since Indonesia has large amount of domestic goats, it is very interesting to understand the genetic variation and the origin of goats in Indonesia base on moleculer marker.

The aims of this study were to identify the genetic variation and phylogenetic tree of indonesian domestic goat compare with other goats in another country. Therefore we understand the contribution of Indonesia to promote the genetic diversity of animal in the world.

MATERIALS AND METHODS

Samples

Twenty Indonesia local goats consist of Bligon, Etawa Grade, Kacang and Kejobong were used in the study. Blood samples were collected from all animals by jugular vein that was saved in tubes containing ethylene diamine tetra-acetic acid (K2EDTA). Blood samples were stored at -20°C until DNA extraction.

DNA extraction and Polimerase Chain Reaction

Genomic DNA was isolated from whole blood by using Isolation DNA Kit (Genesync, 2015). The extracted DNA samples were stored at -20 °C and used later as a substrate for PCR reaction. The 464-bp fragment of cytochrome b (cyt b) gene was amplified using primers: forward primer of L14735 (5'-AAA AAC CAC CGT TGT TAT TCA ACT-3') and reverse primer of H15149 (5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3') (Wolf *et al*., 1999). DNA was amplified in a total volume of 20 µl containing 1 µl genomic DNA (10-100 ng), 1 µl each primers, 10 µl PCR KIT (KAPPA2GTM Fast, KAPABIOSYSTEMS, USA) and 7 µl aquabidest steril. PCR conditions were 2 min at 94°C, 36 sec at 95°C, 73 sec at 51°C, 84 sec at 72°C, 35 cycles and 3 min at 72°C (Prado *et al*., 2005). The PCR was carried out in Primus-25 Advanced (Germany) Thermal Cycler. The PCR products were visualized on 1% agarose gel buffered with 1X Tris-Boric-EDTA buffer (1XTBE), stained with ethidium bromide and visualized under UV light.

Polymerase Chain Reaction - Restriction Fragment Length Polimorphism (PCR-RFLP)

The PCR-amplified DNA fragment of the cytochrome b was digested using HinfI and HaeIII restriction enzyme to identify of genetic pattern. Total volume of digestion was 15 µl containing 6 µl PCR product, 0,2 µl HinfI and HaeIII enzyme (1U), 1,5 µl Tango buffer and 7,3 µl aquabidest steril. The PCR product was digested at 37°C for three. The digestion products were separated on 2,5% agarose gels in 1XTBE buffer and run with 50 V for an hour for separation of the DNA fragments. The bands were stained with ethidium bromide to visualization by UV light. The size of DNA marker ϕX174 DNA/BsuRI (HaeIII) (Fermentas). The predicted size of PCR-RFLP product as follows:

Restricted Enzyme	Product size
Hinf I	266 dan 198 bp
HaeIII	230, 179 dan 35 bp

Sequencing

Two representative products PCR were sequenced for further analysis. DNA sequencing was performed by PT Genetika Science. The sequence results then aligned with 37 data genbank by using BioEdit version 7.0 in order to identify the single nucleotide polymorphism (SNP) and to construct the phylogenetic tree.

RESULTS AND DISCUSSION

The visualization of the PCR product of mt-DNA cytochrome b was performed by 1% agarose gel electrophoresis. Twenty samples of local goat show the same product size of PCR 464 bp. Restriction fragment length polymorphisms of 464 bp PCR product size using HinfI and HaeIII in 2% agarose gel electrophoresis show the similar fragment result for all samples. The same DNA fragments from PCR-RFLP restricted by HinfI restriction enzyme produce 266 and 198 bp. Restriction enzyme Hae III produce DNA fragments 230,179 dan 35 bp. Two or three samples of each indigenous breed of local goats were sequenced to clarify the result of PCR-RFLP

and to detect the SNPs in sequence product of mtDNA cytochrome b. The sequence result can be used to simulate the restriction mapping by using HinfI and HaeIII enzyme, as shown in Figure 1. The restriction site of HinfI restriction enzyme was take place in nucleotide number 266. The restriction site of HaeIII restriction enzyme was take place in nucleotide number 179 and 409.

A. HinfI

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BioEdit version 7.2.0 (4/30/2013) Restriction Mapping Utility
(c)1998,  Tom Hall

1814403_KJ4_Cytb_Forward Restriction Map
5/7/2015 2:24:26 PM
464 base pairs
Translations:  none

Restriction Enzyme Map:

1    AAAACCATCGTTGTCATTCAACTACAAGAACACTAATGACTAACATCCGAAAGACCCACCCATTAATAAAAATTGTAAACAACGCATTTATTGACCTCCCAACCCCATCAAACATCTCAT  120

121  CATGATGAAACTTTGGATCCCTCCTAGGAATTTGCCTAATCTTACAAATCCTGACAGGCCTATTCCTAGCAATACACTATACATCCGACACAATAACAGCATTTTCCTCTGTAACTCACA  240

161  TTTGTCGAGATGTAAATTATGGCTGAATCATCCGATACATACACGCAAACGGAGCATCAATATTCTTTATCTGCCTATTCATACATATCGGACGAGGTCTATACTATGGATCATATACCT  360
                                HinfI (266)

361  TTCTAGAAACATGAAACATTGGAGTAATCCTCCTGCTCGCGACAATGGCCACAGCATTCATAGGCTATGTTTTACCATGAGGACAAATATCATTCTGAGGGGC    464
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B. HaeIII

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1814403_KJ4_Cytb_Forward Restriction Map
5/7/2015 1:25:03 PM
464 base pairs
Translations:  none

Restriction Enzyme Map:

1   AAAACCATCGTTGTCATTCAACTACAAGAACACTAATGACTAACATCCGAAAGACCCACCCATTAATAAAAATTGTAAACAACGCATTTATTGACCTCCCAACCCCATCAAACATCTCAT   120

121 CATGATGAAACTTTGGATCCCTCCTAGGAATTTGCCTAATCTTACAAATCCTGACAGGCCTATTCCTAGCAATACACTATACATCCGACACAATAACAGCATTTTCCTCTGTAACTCACA   240
                                                          HaeIII (179)

241 TTTGTCGAGATGTAAATTATGGCTGAATCATCCGATACATACACGCAAACGGAGCATCAATATTCTTTATCTGCCTATTCATACATATCGGACGAGGTCTATACTATGGATCATATACCT   360

361 TTCTAGAAACATGAAACATTGGAGTAATCCTCCTGCTCGCGACAATGGCCACAGCATTCATAGGCTATGTTTTACCATGAGGACAAATATCATTCTGAGGGGC   464
                                            HaeIII (409)
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Figure 1. Restriction mapping of enzyme HinfI (A) dan HaeIII (B) with BioEdit program

As shown in the Figure 1, the result from sequencing was firm with the result from PCR-RFLP. When the restriction sit take place in the position 266, the PCR-RFLP will produce 266 and 198 bp (Figure 1A). On the other result of restriction mapping, HaeIII restiction enzyme was found in the position 179 and 409. Therefore, the DNA product size of PCR-RFLP were 230,179 and 35 bp. Genetic variation of animal can be identified both using PCR-RFLP and sequencing. However sequencing gives more evidence if there is SNP in the position which cannot be recognized by restriction enzyme.

Two representative products PCR of each breed of local goat were sequenced by Genetika Science. The results of sequences were then analyzed by ClustalW in BioEdit. The totals of 9 samples (PE1, PE2, BG2, BG3, KAC1, KAC3, KEC2, KEC3 dan KJ4) were used in this study. The results indicate the same sequence order. Figure 4 shows the using BioEdit Sequence Alignment

Editor. Dot mark indicates the same sequence. The difference letters show the single nucleotide polymorphisms (SNPs). There are 18 SNPs between goat (9 samples) and cattle (J2). The same sequence order base on mtDNA cytochrome b indicate that the same origin of four breed of local goat. In mammalian, mitochondrial DNA is only passed down through the mother (maternal) without recombination (Manceau *et al*., 1999).

Since Kacang goat is the indigenous goat in Indonesia, the author suggest that all domestic breed of goat in Indonesia have the same maternaly origin from Kacang goat. Animals which were inherited from the same maternal breed will have similar type of mitochondrial DNA.

Figure 2. Sequence Alignment using BioEdit program

Phylogenetic analysis at Figure 3 shows the specific sequences of mtDNA cytochrome b of local goat in Indonesia. Separate groups of local goat appear at above site of phylogenetic tree. It seems clearly the differences of local goat of Indonesia compare with other goat from another country. The goat which was clustered within one branch of phylogeny was caused by the low sequence substitutions in Cytochrome b gene (Sultana *et al*., 2003). Oka *et al*. (2011) study about the three types of goat (Gembrong, Kacang and KacangxEtawah crossbred) had a very close genetic relationship base on mt DNA D-loop. The analysis of Cytochrome b gene of Kejobong goats originated from different area showed the high similarity and a close genetic relationship (Jiyanto *et al*, 2014).The registered mtNA in genbank give the evidence that domestic goat of Indonesia has the specific source of genetic. Therefore, the conservation of Kacang goat is very important as the local resources of genetic for the future study.

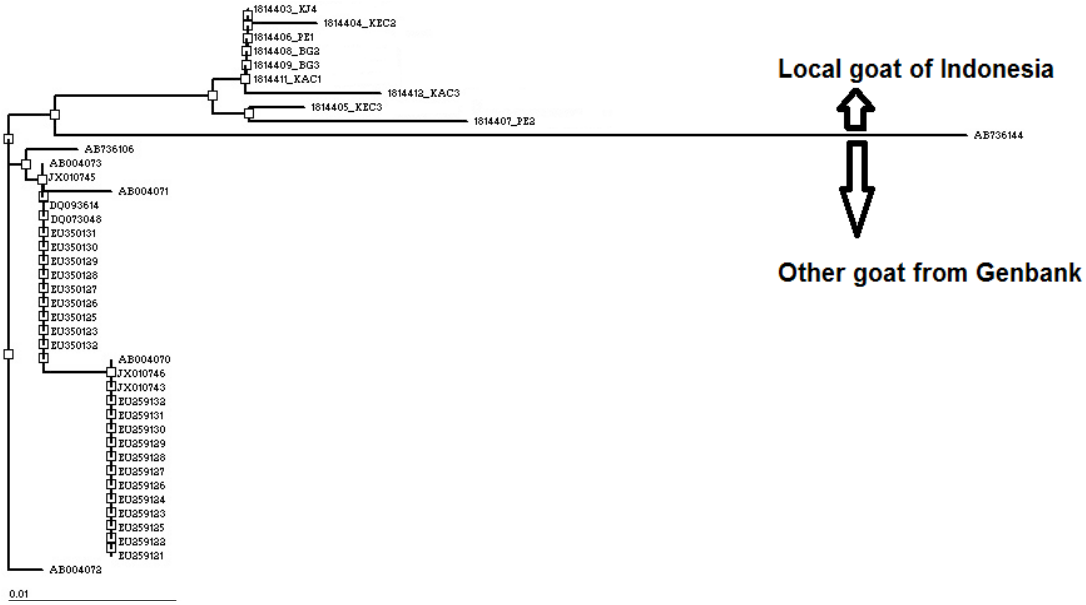

Figure 3. Phylogenetic tree

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