Phylogenetic Tree Analysis for Ongole Grade (Kebumen Cattle) Based on Partial SRY Gene

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ABSTRACT

Ongole grade (PO Kebumen cattle) has been registered and regarded as a valuable animal genetic resources in Indonesia. As an initial step in assesing the genetic relationship to other breeds, therefore phylogenetic analysis and calculation of genetic distances have been conducted using partial SRY gene. To perform a phylogenetic tree, a total of 14 Kebumen cattles were fistly aligned to 43 Madura cattle and 12 genbank references *(Bubalus bubalis, Bos javanicus, Capra hircus, Bos taurus, and Bos indicus)* of 928 bp SRY gene sequences using MEGA ver.7.0 software and the phylogenetic tree was performed using Neighbor-Joining and UPGMA (Unweighted Pair Group Method with Arithmetic Mean) methods. All of the alignment sequences of Kebumen cattle, were in monomorphic as well as in Madura 1 and Madura 2. The main results indicated that Kebumen cattle have a close relationship to both *Bos indicus* and Madura cattle (Madura 1 and Madura 2) with the closest genetic distance 0.000, indicating no different sequences. Hence, it can be implied that they shared a common ancestor. Moreover, the Kebumen cattle shared a distant relationship to both *Bubalus bubalis* (0.030) and *Capra hircus* (0.065). In our suggestion, these result will provide useful information for preservation and conservation strategies for Kebumen cattle as an Indonesian animal genetic resources.

Keywords: Ongole grade (Kebumen cattle), SRY gene, Phylogenetic tree, and Genetic distance

INTRODUCTION

Cattle are the most important livestock sector in Indonesia. Some of local breeds are widely raised by local farmers. Peranakan Ongole (PO) Kebumen is one of Indonesian local cattle widely distributed in Kebumen and its surroundings area, Central Java. It is a crossbreed of PO and Brahman cattle. PO *(Bos indicus)* is a crossbreed of uncontrolled mating of Java cattle and Sumba Ongole cattle (Suyadi et al. 2014). Due to its big and strong body, high adaptability under different environmental condition, and able to grow in limited forage conditions, PO Kebumen is suitable as both draught and beef cattle. In addition, PO Kebumen is an important livestock species closely related in providing food and income to the farmers.

In order to protect this valuable local breed, identification and characterization must be concerned, including breed definition and their origin. Genetic characterization can be performed through phylogenetic analysis. Phylogenetic analysis will provide a valuable information in understanding the relationship among population and their origin based on their molecular basis data.

SRY gene, locating in non-recombining region of Y chromosome, is a male specific gene and has received considerable attentions in genetic diversity and evolution studies of animals (Kikkawa et al., 2003). Y-Chromosome is useful for analyzing the paternal lineages of the crossbred cattle population (e.g. Limousin x Madura) (Hartatik et al., 2014). Studies on SRY gene have been successfully carried out in Bali cattle (Mohamad et al., 2009; Rahayu et al., 2009; Winaya et al., 2014) and Aceh cattle (Hartatik et al., 2014). Meanwhile, phylogenetic studies focused on PO Kebumen cattle is rarely carried out.

Therefore, in order to perform a phylogenetic analysis, we collected of 14 Kebumen cattles based on their partial sequence of SRY gene and they were alignmented and compared to 43 Madura cattles sequences as well as 12 genbank references *(Bubalus bubalis, Bos javanicus, Capra hircus, Bos taurus, and Bos indicus)*. The results of the present study may be useful to provide a basic genetic information of PO Kebumen based on molecular data.

MATERIALS AND METHODS

Sample collection and DNA extraction

Fourteen PO Kebumen cattles were collected from Kebumen district. Reference sequences of SRY gene from 43 Madura cattles used in this study have been described previously (Hartatik et al., 2014). We used 12 genbank references namely from *Bos indicus* (DQ336527), *Bos taurus* (AF148462, EU581861.1, AB039748, E11537, DQ336526, DQ336526.2, U15569.1), *Bos javanicus* (DQ336528, AY079146), *Bubalus bubalis* (DQ417872), and *Capra hircus* (EU581862). The blood samples were collected from the jugularis vein. Genomic DNA samples were extracted from blood using gSYNC^{TM} DNA Extraction Kit Geneaid (PT. Genetika Science) following the manufacture's protocol.

PCR amplification and sequencing

According to the sequence of bovine SRY gene (GenBank Acc. No. AB039748), a pair of primers was used to amplify 928 bp of SRY gene. The primers pair were SRY-F: 5'-GTT GAT GGG TTT GGG CTGACT-3' and SRY-R: 5'-AAA TTG AGA TAA AGA GCG CCT-3'. The PCR was performed in a 25 µl mixture containing 1 µL genomic DNA (50 ng), 1,5 mM MgCl2, 0,2 mM dNTP dan 1,25 U Taq DNA polymerase (Promega, Madison, WI USA). PCR amplification was carried out under the following conditions: 94°C for 5 minutes, followed by 35 cycles at 95°C for 1 min, 60°C for 1 min, 72°C for 1 min, and final extention at 72°C for 7 minutes. A total of 30 µl of PCR product for each samples, then were delivered to 1st Base Genetika Science for sequencing.

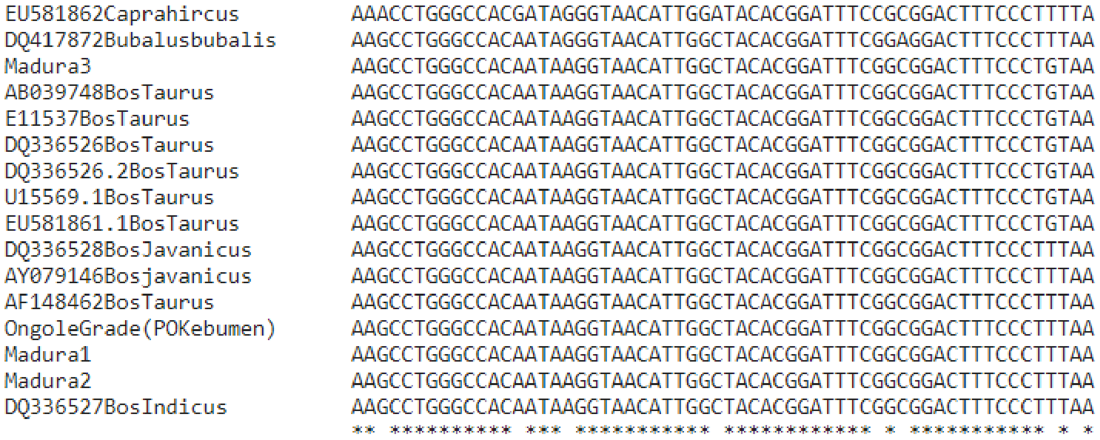
Data analysis

All the sequences including reference and GenBank sequences were aligned using Bioedit 7.7 software. The neighbor-joining (NJ) and UPGMA dendograms were generated using MEGA software ver.5.0 (Kumar et al., 2001) with a Kimura 2parameter model (Bootstrap value is 1,000). We used *Capra hircus* as an outgroup.

RESULTS AND DISCUSSION

With the sequence of AB039748 (GenBank Acc. number), all the samples of PO Kebumen cattle were monomorphic based on 928 bp of partial SRY gene (Fig.1). Figure 2 shows a phylogenetic tree of PO Kebumen cattle together with Madura cattle and 12 GenBank references that was constructed from D_A genetic distance by using the Neighbor-Joining (NJ) and unweight pair group method (UPGMA) dendogram. Two phylogenetic tree were constructed based on the Kimura 2-parameter model with 1,000 bootstrap replication

(Fig.2). There is no different clusters resulted from both methods. All of cattle sequences were clustered in one group that different with *Bubalus bubalis* and *Capra hircus* as an outgroup. The result indicated that PO Kebumen cattle have a close relationship to both *Bos indicus* and Madura cattle (Madura 1 and Madura 2) with the closest genetic distance 0.000, indicating no different sequences. The results showed that PO Kebumen have a genes flow from *Bos indicus*. It is also in agreement that Ongole Crossbred *(Bos indicus)* is the result of genetic improvement of Javanese zebu cattle, which has been existed since the beginning of the first century AD, with Sumba Ongole (SO) from Sumba Island (Sutarno and Setyawan, 2016). PO Kebumen cattle is bigger that PO purebreed cattle since the farmers mated PO with Brahman breed. Utomo (2015) reported that Pure PO cattle began rare because many breeders cross them with Brahman cattle. Their mating produces fertile calf and usually is also called PO because of its smaller size. In Kebumen, Central Java, PO cattle are also known as Madras cattle which are the origin of zebu cattle in East India (Sutarno, dan Setyawan, 2015).



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EU581862Caprahircus          AAACCTGGGCCACGATAGGGTAACATTGGATACACGGATTTCCGCGGACTTTCCCTTTTA
DQ417872Bubalusbubalis       AAGCCTGGGCCACAATAGGGTAACATTGGCTACACGGATTTCGGAGGACTTTCCCTTTAA
Madura3                      AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
AB039748BosTaurus            AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
E11537BosTaurus              AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
DQ336526BosTaurus            AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
DQ336526.2BosTaurus          AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
U15569.1BosTaurus            AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
EU581861.1BosTaurus          AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTGTAA
DQ336528BosJavanicus         AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
AY079146Bosjavanicus         AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
AF148462BosTaurus            AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
OngoleGrade(POKebumen)       AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
Madura1                      AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
Madura2                      AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
DQ336527BosIndicus           AAGCCTGGGCCACAATAAGGTAACATTGGCTACACGGATTTCGGCGGACTTTCCCTTTAA
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Figure 1. Multiple sequences alignment of PO Kebumen cattle, Madura cattle and Genbank references using ClustalW online software

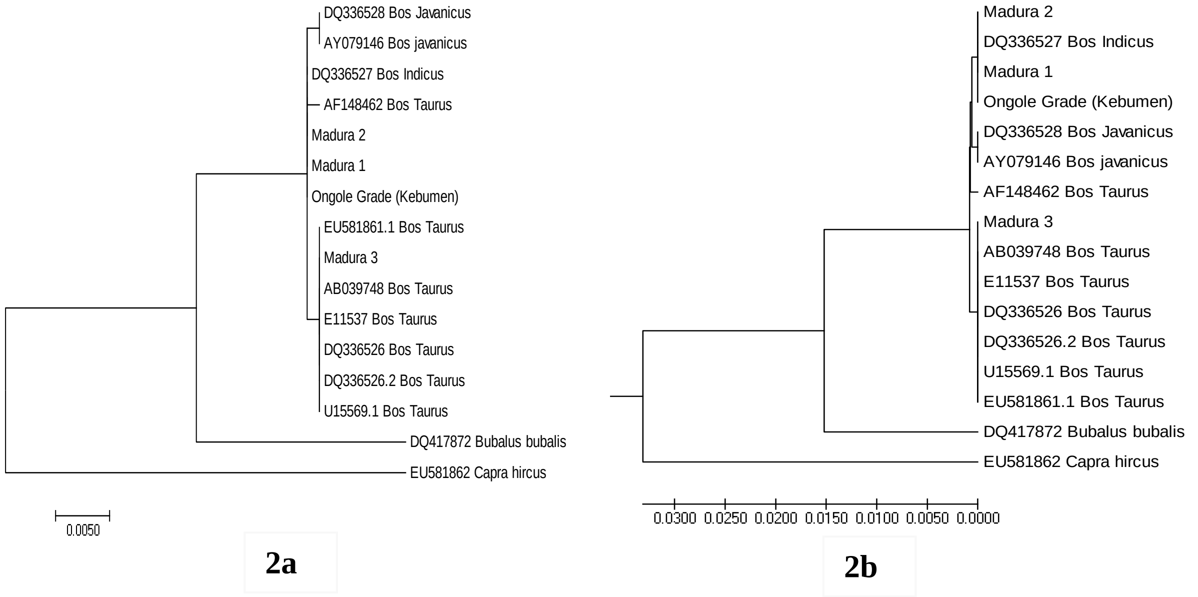
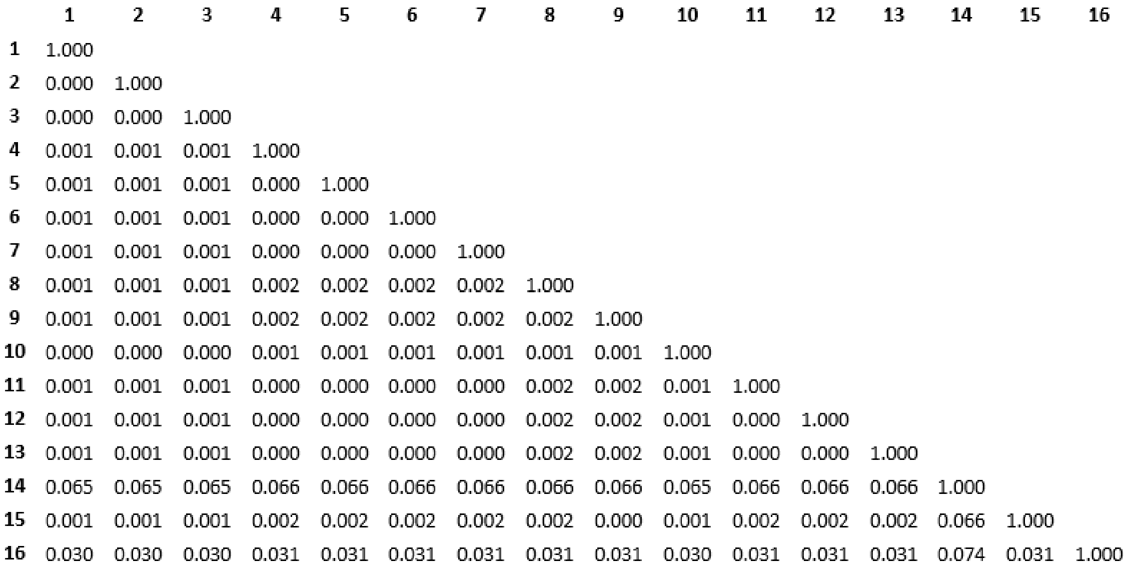


Figure 2. Phylogenetic tree anaylsis among PO Kebumen, Madura cattle and 12 Genbak references using a). UPGMA and b). Neighbor-Joining (NJ) methods



	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.000															
2	0.000	1.000														
3	0.000	0.000	1.000													
4	0.001	0.001	0.001	1.000												
5	0.001	0.001	0.001	0.000	1.000											
6	0.001	0.001	0.001	0.000	0.000	1.000										
7	0.001	0.001	0.001	0.000	0.000	0.000	1.000									
8	0.001	0.001	0.001	0.002	0.002	0.002	0.002	1.000								
9	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	1.000							
10	0.000	0.000	0.000	0.001	0.001	0.001	0.001	0.001	0.001	1.000						
11	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.002	0.001	1.000					
12	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.002	0.001	0.000	1.000				
13	0.001	0.001	0.001	0.000	0.000	0.000	0.000	0.002	0.002	0.001	0.000	0.000	1.000			
14	0.065	0.065	0.065	0.066	0.066	0.066	0.066	0.066	0.066	0.065	0.066	0.066	0.066	1.000		
15	0.001	0.001	0.001	0.002	0.002	0.002	0.002	0.002	0.000	0.001	0.002	0.002	0.002	0.066	1.000	
16	0.030	0.030	0.030	0.031	0.031	0.031	0.031	0.031	0.031	0.030	0.031	0.031	0.031	0.074	0.031	1.000

Figure 3. Genetic distance among PO Kebumen, Madura cattle and 12 Genbak references. 1) Ongole_Grade_(Kebumen); 2) Madura_1; 3) Madura_2; 4) Madura_3; 5) AB039748_Bos_Taurus; 6) E11537_Bos_Taurus; 7) DQ336526_Bos_Taurus; 8) AF148462_Bos_Taurus; 9) DQ336528_Bos_Javanicus; 10) DQ336527_Bos_Indicus; 11) DQ336526.2_Bos_Taurus; 12) U15569.1_Bos_Taurus; 13) EU581861.1_Bos_Taurus; 14) EU581862_Capra_hircus; 15) AY079146_Bos_javanicus; 16) DQ417872_Bubalus_bubalis

CONCLUSIONS

The PO Kebumen cattle was closely related to *Bos indicus.* The current mating system of PO Kebumen cattle with Brahman cross may cause an increase of gene flow from Brahman. It is needed an effort for preservation and conservation strategies for Kebumen cattle as an Indonesian animal genetic resources.

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