Original Article

Haplotype variation of partial SRY gene in Ongole grade bulls (*Bos indicus*) of Indonesia

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Abstract

Ongole grade cattle (*Bos indicus*) are Indonesian beef cattle that adapt well in a tropical climate. This study was aimed to observe the genetic diversity of partial Sex-determining of the region Y chromosome (SRY) gene (625 bp) in Ongole grade bulls (*Bos indicus*) of Indonesia. A total twenty-three (23) DNA samples of animal studies were collected from frozen sperm (straws) produced by the National Artificial Insemination Centers (NAICs) of Lembang (13 straws) and Singosari (10 straws). Research showed that a total of four (4) insertion mutations were detected in the intronic region with the position of 1831th, 1844th, 1850th and 1859th nucleotides (GenBank: DQ336528.2). Hence, in total, six (6) haplotypes of SRY gene were detected in this study based on these mutations. However, the haplotype 1 (Hap.1) was observed as the common haplotype of animal studies (0.61). The phylogenetic tree revealed that all animal studies were classified into the *Bos indicus* cluster. In conclusion, the Ongole grade bulls in this study included the *Indicine* lineage with no genetic introgression from *Bos taurus* and *Bos javanicus.*

Keywords: Haplotype, indel mutation, phylogenetic tree, Ongole grade, SRY gene

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Received March 30, 2022
Accepted June 7, 2022
https://doi.org/10.14456/tjvm.2022.56

Introduction

Ongole grade cattle (*Bos indicus*) are one of Indonesian native cattle that have the potential for meat production. These cattle have been imported from India by the Dutch Colonial government as drought animals since the year 1900 and placed on Sumba Island. Despite for drought animals, these cattle have also been used for the grading up program with the native cows of Java Island. (Hardjosubroto, 1994). Recently, the graded-up Ongole cattle have become known as Ongole grade cattle and chosen as one of the native cattle of Indonesia through a decision of the Ministry of Agriculture No: 2907/Kpts/OT.140/6/ 2011 (Kementan RI, 2011). As beef cattle of Indonesia, the artificial insemination (AI) program with straw from selected Ongole grade bulls is important for genetic improvement.

Purebred Ongole grade bulls are important for selection in the purebreeding program at many Villager Breeding Centers (VBC) on Java Island. A selection of purebred Ongole grade bulls can be assessed with the phenotype characterization and molecular characterization. A Sex-determining region Y chromosome (SRY) gene has been used to characterize many Indonesian native cattle such as Bali (Winaya *et al*., 2014; Volkandari *et al*., 2017; Hartatik *et al*., 2018), Madura (Hartatik *et al*., 2017; Prihatin *et al*., 2018) and Ongole grade (Hartatik *et al*., 2017). The SRY gene has been known as the testes gene and male sex determination factor (Li *et al*., 2014). This gene is located at the non-recombining region of Y chromosome (Mburu and Hanotte, 2005; Mohammad *et al*., 2009). Its haplotypes and no recombination occurs during meiosis and have made it conserved and only inherited by the male lineage (Liu and Ponce de Leon, 2007). Hence, this gene can be used to observe the genetic diversity and evolution studies of animals (Kikkawa *et al*., 2003). However, this gene has a low diversity within species and has a high diversity between species (Syed-Shabtar *et al*., 2013).

Recently, study of genetic diversity in the SRY gene of Ongole grade bulls has been limited. Hartatik *et al.* (2017), did not find mutation sites on the exonic (coding) region of SRY gene in Ongole grade bulls at Kebumen Regency. Despite this, Prihatin *et al.* (2018), found many mutation sites on the intronic (non-coding) region of SRY gene in Madura bulls with normal semen production. However, Ongole grade was confirmed as *Bos indicus* type of cattle based on partial SRY gene (Hartatik e*t al.,* 2017). This study aimed to observe the haplotype variation of partial SRY gene (intronic region) in Ongole grade bulls from the National Artificial Insemination Centers (NAICs) of Lembang and Singosari. The results in this study can be used as basic information to evaluate Ongole grade bulls based on SRY gene diversity.

Materials and Methods

***Ethical approval*:** The following experiment was conducted under the guidelines of the Animal Ethics Committee of the Indonesian Institute of Science (LIPI) No: 36/klirens/III/2021

***Animals*:** Twenty-tree (23) Ongole grade bulls at the National Artificial Insemination Centers (NAICs) of Lembang (13 bulls) and Singosari (10 bulls) were used in the present study. Bulls were selected from Village Breeding Centers (VBCs) at Yogyakarta (1 bull), Central Java (9 bulls), West Java (1 bull) and East Java (12 bulls) Provinces of Indonesia. The bulls were selected according to characteristics standard for Ongole grade bulls (SNI 7651.5:2015) and sperm quality traits (SNI: 4869-1:2017).

***Sample collection and DNA extraction*:** Twenty-three (23) frozen sperms (straws) from each animal study were collected for DNA extraction. The DNA extraction was performed with 100 µL of sperm sample using gSYNC™ DNA Extraction Kit following the manufacturer's protocol.

***PCR amplification and sequencing*:** Along 625 bp of the SRY gene (GenBank: DQ336528.2) was amplified using primer pairs from Verkaar *et al.* (2003), *i.e.* SRY-F: 5'- GCC TGG ACT TTC TTG TGC TTA -3' and SRY-R: 5'- ACA GTG GGA ACA AAA GAC TAT -3'. The PCR was performed in a 30 µL mixture containing 6 µL of genomic DNA, 1.5 µL of forward and reverse primers, 15 µL of PCR Mastermix and 6 µL water free nuclease. PCR amplification was carried out under the following conditions: 95°C for 5 mins, followed by 35 cycles at 94°C for 30 secs, 60°C for 30 secs, 72°C for 30 secs and final extention at 72°C for 5 mins. A total of 30 µl of PCR product for each sample were then delivered to 1st Base Genetika Science for sequencing.

***Data analysis*:** All the SRY sequences were analyzed using BioEdit (Hall, 2001), DNAsp (Librado and Rozas, 2009) and MEGA-X (Hall, 2013) packages. A BioEdit package was used for alignment analysis with the similar sequences from another *Bovidae* species from GenBank such as *Bos taurus* (DQ336526), *Bos indicus* (DQ336527), *Bos javanicus* (DQ336528), *Bos gaurus* (DQ336529), *Bos frontalis* (DQ336530), *Bos grunniens* (DQ336531), *Bison bison* (DQ336532), *Bison bonasus* (DQ336533) and *Syncerus caffer* (DQ336534). A DNAsp package was used to determine the number of haplotypes. A MEGA-X package was used to reconstruct the phylogenetic tree with Neighbor-joining (NJ) and UPGMA methods (1000 × bootstrap).

Results

***SRY amplification*:** The partial SRY gene was successfully amplified in 1% of agarose gel as ilustrated in Figure 1. According to the sequence reference (GenBank: DQ336528.2), the amplification region of SRY gene in this study started from 1764th to 2388th nucleotides with size of 625 bp. Nevertheless, the amplified SRY gene in this study was not located at the coding sequence region (cds) that started from 1108th to 1797th nucleotides.

***Haplotype variation*:** A total of four insertion/deletion (indel) mutations were observed in this study and occured in 1831th, 1844th, 1850th and 1859th nucleotides as shown in Figure 2. According to these mutations, six haplotypes (Hap.) of the SRY gene were observed in

this study with a frequency of 0.61 (Hap.1); 0.09 (Hap.2); 0.01 (Hap.3, Hap.4, Hap.5) and 0.17 for Hap.6 as shown in Table 1

Compared to the other sub-species of cattle, three mutation sites at position 2104th, 2148th and 2376th were detected in the observed sequences as shown in Figure 2. Therefore, each mutation site in the observed sequence can be used as the genetic marker to discriminate among sub-species of cattle. Interestingly, the 2376th nucleotide of animal studies was different from the *Bos indicus* SRY gene reference but similar to *Bos javanicus* and *Bos taurus* cattle.

***Phylogenetic tree*:** The phylogenetic tree based on partial SRY gene with NJ and UPGMA methods revealed that all haplotype of Ongole grade bulls were classified into *Bos indicus* group (Figure 3). According to both methods, *Bison sp.* has close genetic relationship with *Bos sp.* However, *Bos indicus, Bos taurus* and *Bos javanicus* (domesticated animal) able to classified into separated cluster based on UPGMA method. Commonly, *Bubalus bubalus* and *Syncerus caffer* have a close genetic relationship according to both models.

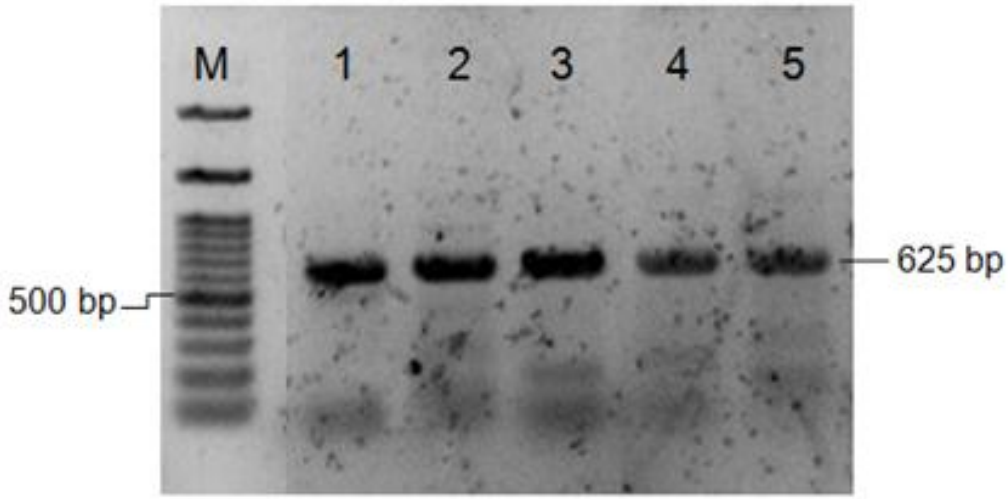

Figure 1 The amplification of partial SRYgene in Ongole grade bulls. M: DNA marker 100 bp; Line 1 - 5: DNA sample

Table 1 Haplotype frequency in partial SRY gene of Ongole grade bulls at different populations

Haplotype	Population (Freq.)				Total (Freq.)
	Yogyakarta	West Java	Central Java	East Java	
Hap.1	0 (0.00)	0 (0.00)	6 (0.43)	8 (0.57)	14 (0.61)
Hap.2	1 (0.50)	0 (0.00)	1 (0.50)	0 (0.00)	2 (0.09)
Hap.3	0 (0.00)	0 (0.00)	1 (1.00)	0 (0.00)	1 (0.04)
Hap.4	0 (0.00)	1 (1.00)	0 (0.00)	0 (0.00)	1 (0.04)
Hap.5	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.00)	1 (0.04)
Hap.6	0 (0.00)	0 (0.00)	1 (0.25)	3 (0.75)	4 (0.17)

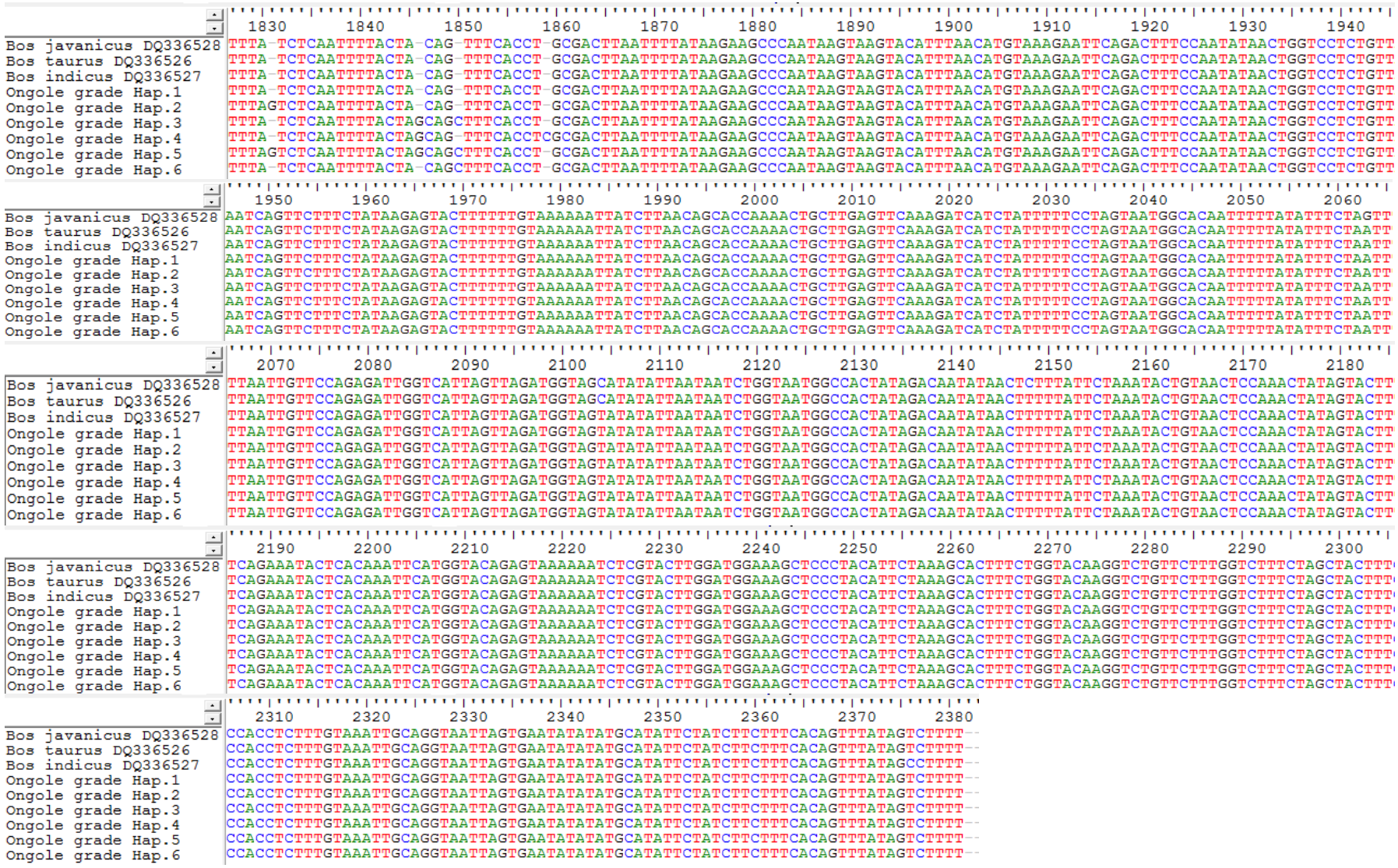

Figure 2 The sequence alignment among haplotype of partial SRY gene in Ongole grade bulls compared with another sub-species of cattle

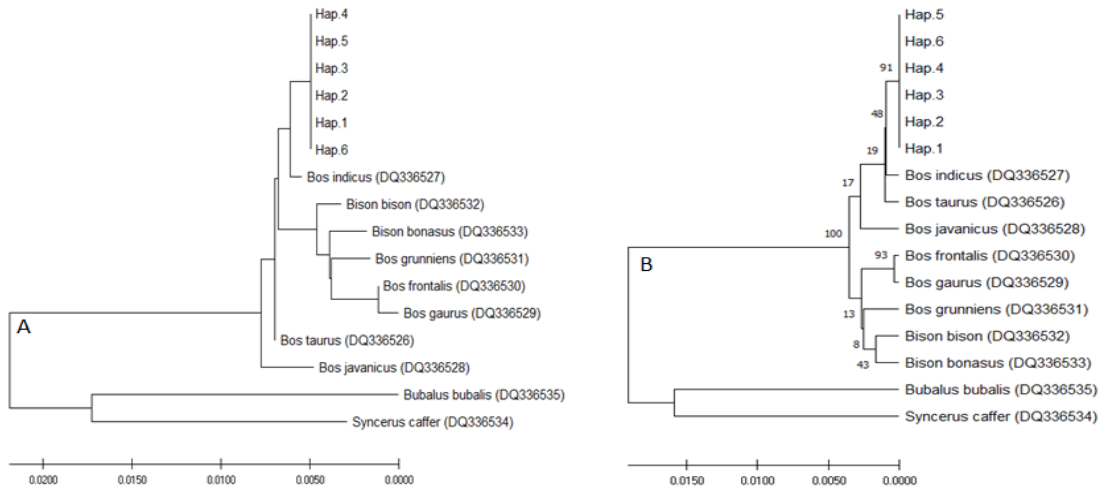

Figure 3 The phylogenetic tree among SRY haplotype of Ongole grade bulls and many *Bovidae* species based on NJ (A) and UPGMA (B) methods

Discussion

In total, four (4) indel mutations were detected in the intronic region of the partial SRY gene of Ongole grade bulls revealing six (6) haplotypes. In a Madura bull, one indel mutation was detected in the intronic region (1836th nucleotide) of SRY gene (Prihatin *et al*., 2018). Hence, the evidence of indel mutation in Ongole grade bulls was higher than in the Madura bull. The intronic region of SRY gene in Ongole grade bulls was shown to be polymorphic and has potency as the genetic marker for sperm quality. Meanwhile, there are no mutation sites in the exonic region of SRY gene in Ongole grade bulls at Kebumen Regency (Hartatik *et al*., 2017). The 2376th nucleotide of animal studies was similar to *Bos javanicus* and *Bos taurus* cattle. Hence, this poin mutation can be used as genetic marker to discriminate between Ongole grade and *Bos indicus* purebreed cattle. Commonly, the SRY gene has low genetic diversity. This gene is located in the male-specific region of the Y chromosome (MSY) that can not be recombined during meiosis. Hence, the diversity within a species was relatively very small due to the low rate of mutations in Y chromosome genes, including SRY (Mburu and Hanotte, 2005). Moreover, the genetic markers on the MSY, which is paternally inherited in a haploid way, have been used for studying the origin of species, range expansion, admixture of populations and migration in animals (Pidancier *et al*., 2006).

Winaya *et al*. (2014), reported that the genetic diversity of SRY gene in Bali bulls from Singosari and Baturiti have a close genetic relationship and similar to Ongole grade bulls from Lembang and Singosari in the present study. Hence, the SRY gene is not able to charactrize Ongole grade and Bali bulls from different populations. Despite, this gene also cannot able to characterize Egyptian and Pakistani buffalo bulls (Hasanain *et al*., 2022). Commonly, Hap.1 was suggested as the common haplotype for Ongole grade bulls. Ongole grade bulls Lembang have a higher haplotype variation rather than in Singosari. In Madura cattle, the genetic mutation in the mitochondrial D-loop gene can be caused by crossbreeding (Utomo, 2017). Nonetheless, all haplotypes in Ongole grade bulls were included in *Indicine* lineage. Hence, the indel mutations in the SRY gene of animal studies were not given a high genetic distance within population.

Selection of bulls based on the maternal or paternal lineage is important to detect their origin lineage. In the purebreeding program, conserving the original traits including phenotype and genotype are important for developing breed standardization. In Indonesia, two (2) *Bos indicus* bulls of Ongole grade and Brahman have been used for straw production and distributed to many VBC's. This can be allowed by the farmers because of similar phenotype characteristics. However, the crossbred cattle produced from Ongole grade and Brahman breeds have a higher body size than purebred Ongole grade cattle (Utomo *et al*., 2015). However, the genetic introgression of Brahman can be detected with the evidence of a mutation site at exonic region (T1707G) of SRY gene (GenBank: MN727883) mainly for Wagyu × Brahman and Belgian Blue × Brahman cattle (Hartatik *et al*., 2020). The phylogenetic tree with NJ and UPGMA methods revealed that Ongole grade bulls in this study included *Bos indicus* type. Hence, there is no genetic introgression from *Bos javanicus* and *Bos taurus* observed in animal studies.

The unweighted pair-group method with arithmetic mean (UPGMA) refers to a straightforward approach to constructing a rooted phylogenetic tree from a distance matrix while neighbor-joining (NJ) tree refers to the new approach for constructing a phylogenetic tree, which is unrooted through a star tree. Moreover, UPGMA is an agglomerative hierarchical clustering method based on the average linkage method while the NJ tree is an iterative clustering method based on the minimum-evolution criterion (Michener and Sokal, 1957; Kuhner and Felsenstein, 1994).

In conclusion, the Ongole grade bulls in this study were confirmed as *Bos indicus* lineage without genetic introgression from *Bos taurus* and *Bos javanicus*. In the future, study to investigate the sperm quality in each haplotype is important for obtaining the genetic marker for sperm quality in Ongole grade bulls.

Acknowledgements

The authors thank all staff at the National Artificial Insemination Centers (NAICs) of Lembang and Singosari for their help in providing the straw sample for this research.

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