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Paternal Lineage Detection of Pasundan Bulls Inferred from Partial Sex Region Y (SRY) Sequence

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ABSTRACT

The Pasundan breed is native cattle from West Java, Indonesia, that the farmers keep as beef cattle. This study aimed to detect the paternal lineage of Pasundan bulls inferred from the partial sex determining region Y (SRY) gene. The blood samples of Pasundan bulls (20 heads) and frozen sperm (straw) of Ongole grade bulls (10 heads) were used for DNA extraction analysis. In the present study, Pasundan and Ongole grade bulls were kept at the breeding station. The results showed that a g.15264C>T mutation point was detected in the SRY gene of animals under study. This mutation was shown as the genetic marker to classify them into *Indicine* and *Taurine* lineages. In conclusion, Pasundan cattle in this study have two paternal lineages of *Bos indicus* (80%) and *Bos taurus* (20%).

Key words: Paternal lineage, Mutation point, Pasundan cattle, SRY gene

INTRODUCTION

The Pasundan breed is the native cattle of West Java and has the potential for beef cattle in Indonesia. This cattle has been decided as the Indonesian native cattle since 2014 through the decision of the Ministry of Agriculture No. 1051/Kpts/SR.120/10/2014 (Said et al. 2017). The farmers keep the Pasundan cattle, also known as Rancah cattle, for meat production. Putra et al. (2019) reported that the average adult weight in Pasundan bulls was about 188.35±22.61 to 227.11±28.21kg. Meanwhile, Said et al. (2017) reported that Pasundan is humped cattle with the standard coat colors of solid reddish brown. Thus, a black coat color was observed in Pasundan cattle with low frequency. As native cattle, studying the origin of Pasundan cattle is important since these cattle have various phenotypic traits (Said et al. 2017). Previous studies reported that Pasundan cattle have a maternal lineage of *Bos javanicus* based on the mitochondrial Cytochrome-b (Cyt-b) region (Hartatik et al. 2019). Wulandari et al. (2019) reported that Pasundan cattle have a maternal lineage of Madura cattle (Bos indicus) based on the mitochondrial COI region. In addition, Salimah et al. (2022) reported that Pasundan has a maternal lineage of *Bos javanicus* and *Bos indicus* based on the mitochondrial D-loop region.

Unfortunately, a study to observe the paternal lineage of Pasundan cattle has not been reported before. The sex determining region Y (SRY) gene can be assessed to determine the paternal lineage of cattle. This gene is located at the non-recombining region of the Y chromosome (6,822,380bp) along 1.8kb (Olagunju et al. 2024). This region is found in a male-specific gene and has been investigated for determining animal genetic diversity and evolution studies (Kikkawa et al. 2003; Niu et al. 2024). Cheng et al. (2001) reported that the coding region of SRY gene in *Bovidae* family shares 84.7% of identity, and much higher identities (>95%) were observed within different subfamilies. Utomo et al. (2024) reported that the SRY gene can be used to characterize two different cattle breeds of Limousin (*Bos taurus*) and Madrasin (Limousin × Madura - *Bos indicus*).

In cattle, the SRY gene is located in the Y chromosome with a length of 15,842bp (GenBank: EU581861.1 and DQ336526.2). This gene has been used for detecting a paternal lineage in many native cattle breeds of Indonesia

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such as: Bali (Winaya et al. 2011), Madura (Prihatin et al. 2018) and Ongole grade (Putra et al. 2022). Moreover, the SRY gene was used to determine a paternal lineage in Banteng (*Bos javanicus*) of Thailand (Chaichanathong et al. 2021), extinct Kouprey (*Bos sauveli*) of Cambodia (Hassanin and Ropiquet 2007) and Gayal (*Bos frontalis*) of China (Guo et al. 2010). In addition, the SRY gene can differentiate many cattle sub-species (Hartatik et al. 2018; Hartatik et al. 2020). Moreover, the SRY gene is also used as the molecular selection for semen production traits in bulls (Gopinathan et al. 2015). Furthermore, this gene also has the potential as the genetic marker to influence twin-calving indirectly (Amin 2021).

This study aimed to determine the paternal lineage of Pasundan cattle from West Java, Indonesia, based on the partial SRY gene. The results are important for genetic conservation and provide basic genetic information for the breeding program of Pasundan cattle in the future.

MATERIALS AND METHODS

Ethical approval

The Indonesian Institute of Science (LIPI) has approved this study with letter No: 36/klirens/III/2021.

Animal and DNA extraction

The blood and sperm samples were used in this study and collected from 20 Pasundan and 10 Ongole cross bulls, respectively. Ongole bulls were used in this study as a comparison sample because of the common cattle breeds in the Java Island of Indonesia. The Pasundan bulls used in this study originated from the breeding center at Ciamis, West Java (BPPIB-SP Ciamis). Meanwhile, the Ongole cross bulls in this study were originated from the National Bureau of Artificial Insemination (BBIB) of Lembang (5 bulls) and Singosari (5 bulls). Amount 200µL of blood sample and 100µL of sperm sample were used for DNA extraction using Geneaid^{TM} Isolation Kit and gSYNC^{TM} DNA Extraction Kit, respectively. The DNA extraction in each material was performed following the protocol from the manufacturer. The blood samples were collected from *vena jugularis* and *vena coccygeal.* Meanwhile, the frozen sperms (straws) samples were collected from BBIB.

PCR amplification and sequencing

The amplification of SRY gene was performed in a total volume of 30µL consisting of 6µL of DNA template, 1.5µL of each primer, 15µL of PCR Mastermix, and 6µL water free nuclease. In this study, along 625bp of *bovine* SRY gene (GenBank: EU581861.1) was determined using a primer pairs of Forward: 5'- GCC TGG ACT TTC TTG TGC TTA -3' and Reverse: 5'- ACA GTG GGA ACA AAA GAC TAT -3' (Verkaar et al. 2003). The amplification of SRY gene fragment was performed with 1 cycle of pre-denaturation temperature at 95˚C for 5min and followed by 35 cycles of denaturation at 94˚C for 30s, annealing at 60˚C for 30s, extension at 72˚C for 30s, and the final extension was at 72˚C for 5min. DNA fragments were detected by electrophoresis analysis at 100 V for 30 min on 1.5% of agarose gel and visualized by G-Box Documentation System (Syngene-UK). Therefore, a commercial laboratory service performed the sequencing analysis of the partial SRY gene (1st BASE Laboratory-Malaysia).

Sequence Analysis

Gene sequence alignment was performed using a BioEdit program (Hall 2011). Haplotype diversity in the partial SRY sequences of Pasundan population was analyzed using a DNAsp program (Librado and Rozas 2009). Therefore, the phylogenetic tree was performed using MEGA-X program (Hall 2013) with UPGMA 1000 × bootstraps method. In addition, three SRY gene sequences from GenBank database (https://www.ncbi.nlm.hih.gov) such as: *Bos taurus* (DQ336526), *Bos indicus* (DQ336527) and *Bos javanicus* (DQ336528) were involved in the phylogenetic tree analysis. Despite this, a SRY gene sequence of Indonesian Madura cattle (Prihatin et al. 2018) was used for phylogenetic analysis because this breed is composed of *Bos indicus* and *Bos javanicus.*

RESULTS AND DISCUSSION

The SRY gene is inherited through the paternal lineage and it's widely used to trace the origins of a species from its proximity (Syed-Shabthar et al. 2013). Our study revealed that the SRY gene with a long 630bp in Pasundan bulls was successfully amplified in 1% agarose gel (Fig. 1). Compared with three *sub-species* of cattle in Indonesia (*Bos indicus*, *Bos taurus* and *Bos javanicus*), eight mutation sites were observed in the animal studies (Table 1). A mutation site of g.15264C>T was detected in animal studies with low frequency (Fig. 2). This mutation can be used as the genetic marker for differentiating *Indicine* and *Taurine* lineage in Ongole grade and Pasundan bulls.

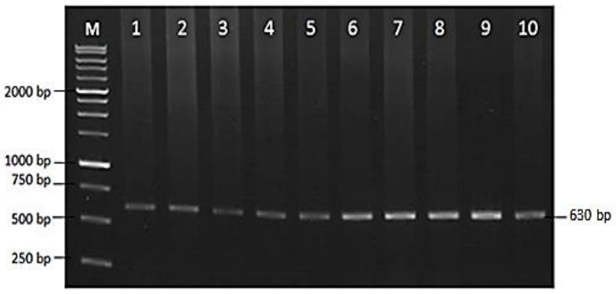

Fig. 1: The result of SRY gene amplification along 630bp in 1% agarose gel. M: DNA ladder 250bp; 1-10: DNA sample.

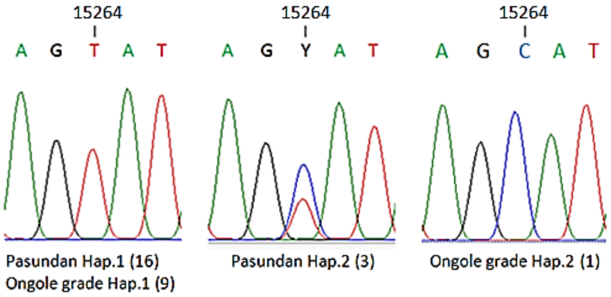

Fig. 2: A mutation point at 15264th nucleotide was detected in the *bovine* SRY gene (GenBank: EU581861.1) of animals under study. Y=C/T.

Table 1: Mutation site in the partial SRY gene of Ongole grade, Pasundan and Madura bulls compared to three sub-species of cattle

Breeds	Mutation site position							
	14974	14975	14976	14995	15223	15264	15308	15536
Bos taurus (DQ336526.2)	A	C	A	A	A	C	T	T
Bos indicus (DQ336527.2)	A	C	A	A	A	T	T	C
Bos javanicus (DQ336528.2)	A	C	A	A	G	C	C	T
Madura Hap.1 (N = 4)*	-	-	C	A	G	C	C	T
Madura Hap.2 (N = 1)*	-	-	C	-	G	C	C	T
Pasundan Hap.1 (N = 16)	A	C	A	A	A	T	T	T
Pasundan Hap.2 (N = 3)	A	C	A	A	A	Y	T	T
Ongole grade Hap.1 (N = 9)	A	C	A	A	A	T	T	T
Ongole grade Hap.2 (N = 1)	A	C	A	A	A	C	T	T

N: number of observation; Y=C/T. *Prihatin et al. (2018).

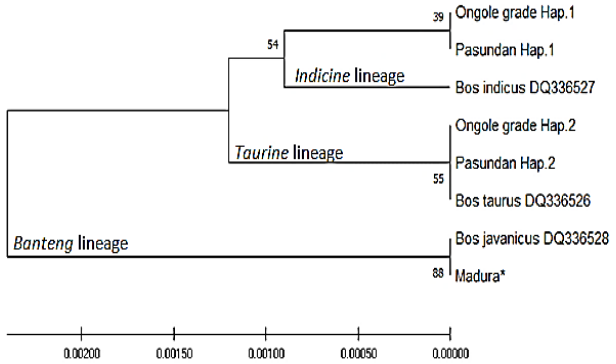

Fig. 3: UPGMA phylogenetic tree (1000 × bootstrap) among haplotype of Ongole grade and Pasundan bulls based on partial SRY gene. *Prihatin et al. (2018).

In addition, three insertion/deletion (indel) mutations were detected in Madura cattle (*Bos indicus*) of Indonesia at 14974th, 14975th and 14995th nucleotides (Table 1). According to mutation g.15264C>T, Pasundan and Ongole grade cattle can be classified to haplotype 1 (Hap.1) and haplotype 2 (Hap.2) clusters. The Hap.1 was typical of *Indicine* cattle and Hap.2 was typical of *Taurine* cattle (Fig. 3). The Pasundan and Ongole grade cattle in the present study were classified into Hap.1 cluster as the *Indicine* lineage.

Gopinathan et al. (2015) obtained a mutation site of c.14907T>G on coding region of SRY gene in Holstein × Jersey bulls. Hartatik et al. (2018) obtained two (2) mutation sites in the promoter region of the SRY gene in *Bos indicus*. Despite this, Hartatik et al. (2020) obtained twenty-one (21) common mutation sites in the coding region of *bovine* SRY gene (study on Belgian Blue and Wagyu cattle breeds). The evidence of mutation g.15264C>T in Pasundan and Ongole grade cattle can be caused by crossbreeding with *Bos taurus* breed (Simmental and Limousine) through the Artificial Insemination (AI) methods. The crossbreeding program has likely led to the introgression of Taurine genes in Pasundan cattle, as indicated by paternal lineage. Consequently, many Pasundan bulls in West Java exhibit characteristics similar to Bos taurus, including being humpless (34.77%) and having white coloration on the muzzle (3.91%), horns (4.80%), and tail switch (2.73%) (Said et al. 2017). However, Agung et al. (2019) found that the Pasundan and Madura (*Bos indicus*) cattle share a similar genetic admixture based on twelve microsatellite markers. According to the morphometrical traits, Pasundan cattle have the genetic similarity with two *Bos indicus* breeds of Ongole grade (12.5%) and White Fulani (25.6%) cattle (Putra et al. 2020; Ahmed et al. 2021).

In the present study, the genetic introgression from Banteng was not observed in Pasundan and Ongole grade. Since the 1900, the Ongole cattle have been imported from India to Sumba Island by the Dutch colonial government for draught resources in Indonesia (Hardjosubroto 1994). Therefore, the Ongole bulls were delivered to Java Island for the grading-up program with Javan native cattle to produce draught cattle for plantations known as the Ongolization program. Bali cattle may not have been developed in the Java Island, with many small ruminants' livestock. Bali cattle was susceptible to Malignant Catarrhal Fever (MCF), a dangerous disease caused by Ovine Herpesvirus-2 (OvHV-2) with sheep and goat as reservoir animals (Damayanti 2016). Despite this, Madura and Bali bulls on Java Island may be castrated and culled for supporting the Ongolization program (Hardjosubroto 1994). Recently, the government of Indonesia produced the Pasundan straws from many selected bulls to increase the population and improve the genetic potency of these cattle.

Conclusion

The Pasundan cattle in the present study have two paternal lineages, Bos indicus and Bos taurus, based on a mutation site of g.15264Y at the partial SRY gene. Therefore, the Ongole was suggested as the paternal origin of Pasundan cattle. In the future, a deep study with whole genome sequencing (WGS) is important to determine the origin of Pasundan cattle accurately.

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Author's Contribution: WPBP, SDV conceptualized and designed the study; WPBP, SDV wrote and edited the manuscript; TLT and SA provided advice and and revise the manuscript. SDV funding research. All authors have seen the final version of the manuscript and approved for publication.

Data Availability Statement: The data that supports the findings of this study are available in the material of this article. There is no other supporting data.

REFERENCES

Agung PP, Saputra F, Zein MSA, Wulandari AS, Putra WPB, Said S and Jakaria, 2019. Genetic diversity of Indonesian cattle breeds based on microsatellite markers. Asian-Australasian Journal of Animal Science 32(4): 467-476. https://doi.org/10.5713/ajas.18.0283

Ahmed RO, Putra WPB, Arifin J and Bello SF, 2021. Morphometrics characterization in White Fulani, Muturu and Pasundan cows. Livestock Animal Research 19(3): 319-325. https://doi.org/10.20961/lar.v19i3.51661

Amin M, 2021. Early finding of twin and SRY genes to support the natural reproductive efficiency of local cattle breed in Indonesia. AIP Conference Proceedings 2353: 030039. https://doi.org/10.1063/5.0053055

Chaichanathong W, Klinsawat W, Sukmak M, Sakulthai A, Wajjwalku W, Sripiboon S, Kaolim N, Nakbhun S, Tunpradit B, Nipanunt T, Tipkantha W, Yindee M and Thongtip N, 2021. Genetic characterization of banteng (*Bos javanicus*) populations in Thailand for conservation. Thai Journal of Veterinary Medicine 51(4): 647-654. https://doi.org/10.14456/tjvm.2021.78

Cheng H, Shi H, Zhou R, Guo Y, Liu L, Liu J, Jiang Y, Kudo T and Sutou S, 2001. Characterization of Bovidae sex-determining gene SRY. Genetics Selection Evolution 33(6): 687-694. htpps://doi.org/10.1186/1297-9686-33-6-687

Damayanti R, 2016. Malignan catarrhal fever in Indonesia and its control strategy. Wartazoa 26(3): 103-114. https://doi.org/10.14334/wartazoa.v26i3.1391

Gopinathan A, Sivaselvam SN and Karthickeyan SMK, 2015. Molecular association of Y-chromosome specific genes with semen production traits in crossbred Jersey and crossbred Holstein Friesian bulls. Indian Journal of Applied Research 5(8): 388-393.

Guo X, Wang YQ, Yang S, Deng W and Mao HM, 2010. Genetic diversity and origin of Gayal and cattle in Yunnan revealed by mtDNA control region and SRY gene sequence variation. Journal of Animal Breeding and Genetics 127(2): 154-160. htpps://doi.org/10.1111/j.1439-0388.2009.00807.x

Hall GH, 2013. Building phylogenetic trees from molecular data with MEGA. Molecular Biology and Evolution 30(5): 1229-1235. https://doi.org/10.1093/molbev/mst012

Hall T, 2011. BioEdit: an important software for molecular biology. GERF Bulletin of Biosciences 2(1): 60-61.

Hardjosubroto W, 1994. Aplikasi Pemuliabiakan Ternak di Lapangan. Gramedia Widiasarana, Jakarta, INA.

Hartatik T, Bintara S, Ismaya I, Panjono P, Widyobrotobp, Agus A, Budisatria IGS and Leroy P, 2020. Single nucleotide polymorphism of sex determining region-Y gene coding sequences in Belgian Blue bull and Wagyu bull crossbred cattle. IOP Conference Series: Earth and Enviromental Science 478(1): 012020. https://doi.org/10.1088/1755-1315/478/1/012020

Hartatik T, Hariyono DNH and Adinata Y, 2019. Genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on cytochrome b gene sequences. Biodiversitas 20(1): 17-22. https://doi.org/10.13057/biodiv/d200103

Hartatik T, Priyadi DA, Agus A, Bintara S, Budisatria IGS, Panjono, Ismaya, Widyobroto B P and Adinata Y, 2018. SRY gene marker differences in native and crossbreed cattle. Bulletin of Animal Science 42(3): 179-183. https://doi.org/10.21059/buletinpeternak.v42i3.29940

Hassanin A and Ropiquet A, 2007. Resolving a zoological mystery: the kouprey is a real species. Proceedings Biological Science 274(1627): 2849-2855. https://doi.org/10.1098/rspb.2007.0830

Kikkawa Y, Takada T, Sutopo K, Nomura K, Namikawa T, Yonekawa H and Amano T, 2003. Phylogenies using mtDNA and SRY provide evidence for male-mediated introgression in Asian domestic cattle. Animal Genetics 34(2): 96-101. https://doi.org/10.1046/j.1365-2052.2003.00956.x

Librado P and Rozas J, 2009. DnaSP v.5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25(11): 1451-1452. https://doi.org/10.1093/bioinformatics/btp187

Niu P, Huang F, Liu X, Liu B, Wang J, Fang D, Khan A and Gao Q, 2024. Sorting effects of X/Y sperm on in vitro fertilization of Belgian blue bulls. Asian Journal of Agriculture and Biology 2024(1): 2023166. https://doi.org/10.35495/ajab.2023.166

Olagunju TA, Rosen BD, Neibergs HL, Becker GM, Davenport KM, Elsik CG, Hadfield TS, Koren S, Kuhn KL, Rhie A, Shira KA, Skibiel AL, Stegemiller MR, Thorne JW, Villamediana P, Cockett NE, Murdoch BM and Smith TPL, 2024. Telomere to telomere assemblies of cattle and sheep Y-chromosomes uncover divergent structure and gene content. Nature Communications 15: 8277. https://doi.org/10.1038/s41467-024-52384-5

Prihatin KW, Maylinda S and Hakim L, 2018. The SRY gene variations amongst selected Madura cattle populations. Jurnal Kedokteran Hewan 12(4): 101-103. https://doi.org/10.21157/j.ked.hewan.v12i4.9674

Putra WPB, Agung PP, Anwar S and Said S, 2019. Polymorphism of bovine growth hormone receptor gene (g.3338A>G) and its association with body measurements and body weight in Pasundan cows. Tropical Animal Science Journal 42(2): 90-96. https://doi.org/10.5398/tasj.2019.42.2.90

Putra WPB, Anwar S, Volkandari SD and Said S, 2022. Haplotype variation of partial SRY gene in Ongole grade bulls (*Bos indicus*) of Indonesia. The Thai Journal of Veterinary Medicine 52(3): 493-498. https://doi.org/10.14456/tjvm.2022.56

Putra WPB, Hilmawan F and Arifin J, 2020. Characterization of two Indonesian *Bos indicus* cattle breeds based on morphometrical measurements. Turkish Journal of Veterinary Research 4(1): 29-32.

Said S, Putra WPB, Anwar S, Agung PP and Yuhani H, 2017. Phenotypic, morphometric characterization and population structure of Pasundan cattle. Biodiversitas 18(4): 1638-1645. https://doi.org/10.13057/biodiv/d180443

Salimah I, Agung PP, Said S, Farajallah A and Perwitasari D, 2022. Origin and phylogenetic analysis of Pasundan cattle based on D-loop of mitochondrial genome. IOP Conference Series: Earth and Enviromental Science 1001: 012041. https://doi.org/10.1088/1755-1315/1001/1/012041

Syed-Shabthar SMF, Rosli MKA, Mohd-Zin NAA, Romaino SMN, Fazly-Ann ZA, Abas-Maszni MCO, Zainuddin R, Yaakop S and Md-Jain BM, 2013. The molecular phylogenetic signature of Bali cattle revealed by maternal and paternal markers. Molecular Biology Report 40(8): 5165-5176. https://doi.org/10.1007/s11033-013-2619-y

Utomo B, Fadholly A, Margawati ET, Ibrahim and A, Belgania RH, Amrullah MF, Rimayanti and Ratnani H, 2024. *SRY* gene based kinship relationship of Limousin and Madrasin cattle. Pakistan Journal of Zoology 56(1): 491-494. https://doi.org/ 10.17582/journal.pjz/20220715080754

Verkaar ELC, Vervaecke H, Roden C, Romero-Mendoza L, Barwegen MW, Susilawati T, Nijman IJ and Lenstra JA, 2003. Paternally inherited markers in bovine hybrid populations. Heredity. 91(6): 565-569. https://doi.org/10.1038/sj.hdy.6800359

Winaya A, Rahayu ID, Amin M and Herliantin, 2011. The genetic variation in Bali cattle (*Bos javanicus*) based on sex related Y chromosome gene. Animal Production 13(3): 150-155.

Wulandari A, Nurgiartiningsih VMA, Kaswati and Susilorini TE, 2019. Kinship of several Indonesian local cattles by using DNA mitochondrial COI (Cytochrome Oxidase Sub-unit I). International Research Journal of Advanced Engineering and Science 4(3): 165-167.