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Case report

CHARACTERIZATION OF SEX-DETERMINING REGION Y (SRY) GENE SEQUENCES OF ENDANGERED GEMBRONG GOAT (*CAPRA HIRCUS*) IN BALI ISLAND, INDONESIA

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Gembrong goat is an indigenous breed of Bali, Indonesia, currently threatened by high levels of inbreeding due to their declining population size. Genetic characterization is an important approach to support breed conservation and improve breeding management and selection measures. This study aimed to analyze the sex-determining region of the Y chromosome in bucks of the Gembrong goat breed. Blood samples from ten male bucks were collected, and DNA was extracted and amplified using polymerase chain reaction targeting a fragment of the sex-determining region of the Y chromosome. The amplified products were sequenced and analyzed to identify genetic variations. The results revealed two insertion-deletion mutations located in the non-coding region, which led to the identification of three gene variants. One variant was dominant, while the others were present at lower frequencies. In addition, a specific point mutation was detected which may serve as a potential breed specific genetic marker. Phylogenetic analysis showed that Gembrong goats belongs to a distinct genetic cluster compared to other goat breeds. These findings provide valuable new genetic information of the Gembrong goats breed highlighting their uniqueness. The results can contribute to the development of appropriate breeding strategies with the aim to reduce inbreeding and support long-term conservation processes. Furthermore, this study emphasizes the importance of genetic characterization studies in preserving endangered native livestock breeds.

Keywords: conservation genetics; genetic variation; Gembrong goat; inbreeding; phylogenetic analysis; sex-determining region Y gene.

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INTRODUCTION

Gembrong goat (*Capra hircus*) is an indigenous breed originating from Karangasem Regency, Bali Island, Indonesia [1]. The Gembrong goats have been registered as a native breed in Indonesia since 2013 through the decision of Indonesian Ministry of Agriculture No: 696/Kpts/Pd.410/2/2013. Nonetheless, the low population size of Gembrong goat in Bali Island has resulted in high inbreeding pressure [2,3]. As a result, the inbreeding coefficient and inbreeding rate values in Gembrong goats can attain 0.413 and 0.025, respectively [4]. Hence, both values indicated that the Gembrong goat is a threatened breed because of the high risk of inbreeding. One of the efforts to conserve this endangered breed is to investigate the sex-determining region Y gene (SRY), which is located on the Y chromosome [5-7]. According to the SRY gene information, the inbreeding risk of the Gembrong goats can be reduced with a good mating system. According to the NCBI database (www.ncbi.nlm.nih.gov), the length of SRY gene in goats is 6,512 bp with 1 exon (GenBank: EU581862). Unfortunately, there are no published studies focusing on the characterization of the SRY gene in Gembrong bucks. Hence, the present study was carried out to characterize the SRY gene sequence of Gembrong bucks with the ethical approval from Animal Ethics Committees, Faculty of Veterinary Medicine, Udayana University, Indonesia (Approval Issue: B/97/UN.14.2.9/PT.01.04/2025).

CASE PRESENTATION

Ten Gembrong bucks were included into the investigation and blood samples were collected. All the bucks were from Beraban Village, Kediri District, Tabanan Regency, Bali Province, Indonesia. Approximately 5mL blood samples were taken from the *jugular vein* using venoject vacutainer tube containing EDTA. A genomic DNA extraction kit (Geneaid, Taiwan) was used to collect the DNA samples following the manufacturer's instructions. According to the protocols, approximately 17.64 μL of DNA concentration and 1.71 of DNA purity were obtained in the present study. PCR analysis was performed in a total reaction of 30 μL per sample consisting of 6 μL of DNA template; 15 μL of PCR Mastermix (MyTaq™ HS Mix, Bioline, USA); 0.60 μL of each primer and 7.80 μL of nuclease free water. A specific primer pair was designed with a Primer3Plus program (www.primer3plus.com) as follows: SRY-F: 5' – GGT ATT GGG GGC GGA GAA AT – 3' and SRY-R: 5' – CGT GCC TTT GTT AGC GAG AG – 3'. Recently, this primer pair was registered as a Patent in Indonesia with the Application Number: S00202601883. The primer design in this study can amplify the exonic region of *caprine* SRY gene (GenBank: EU581862) along 823 bp. Therefore, the PCR program was operated with a mastercycler gradient (Eppendorf, Germany) in 1 cycle of pre-denaturation at 94 ºC for 5 minutes and followed by 35 cycles of denaturation at 94 ºC for 1 minutes; annealing at 54.4 ºC for 30 s; initial extension at 72 ºC for 1 minutes, 30 s and final extension at 72 ºC for 7 minutes. The electrophoresis analysis was performed on 1% agarose gel with 2 μL of FloroSafe DNA Stain (1^{st}

BASE, Malaysia) at 100 V for 30 minutes and captured with G-box Documentation System (UVITEC, UK). The sequencing analysis was performed by Apical Scientific laboratory in Malaysia. Therefore, the sequencing results were analyzed by BioEdit and MEGA packages [8,9].

In this study, the primer design is able to amplify the SRY gene of Gembrong goat along 823 bp (Figure 1).

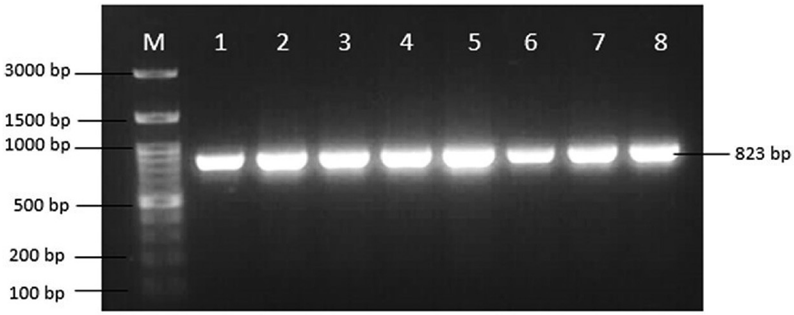

Figure 1. Amplification of SRY gene in Balinese Gembrong goat (*Capra hircus*) along 823 bp on 1% agarose gel. M: DNA ladder 100 bp. Line 1-8: DNA sample.

Therefore, the sequence alignment analysis reveals two insertion-deletion (indel) mutations between 3447th and 3451th nucleotides (Figure 2).

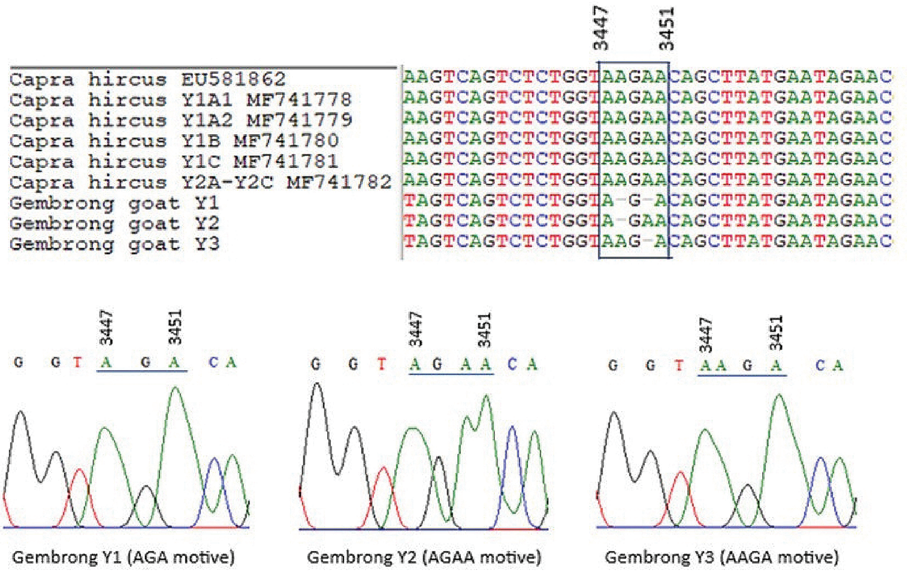

Figure 2. Detection of mutation sites in the SRY gene sequence of Balinese Gembrong goats (*Capra hircus*) between 3447th and 3451th nucleotide position (box) reveals three motives of *caprine* SRY gene sequence.

According to both mutations, the SRY gene sequence in Gembrong goat can be classified as Y1 (AGA motive), Y2 (AGAA motive) and Y3 (AAGA motive) variations.

Most of the Gembrong bucks under study were identified as Y1 (0.80), Y2 (0.10) and Y3 (0.10) variants. The variants of SRY gene sequence in Gembrong goat were deposited in the NCBI database accession number of LC907682 (Y1), LC907683 (Y2) and LC907684 (Y3). These motives found are located in the 5'UTR region of the *caprine* SRY gene and absent in global goat breeds. Therefore, the AAGAA motive was confirmed as the wildtype SRY motive of global goat based on two indel mutations.

Moreover, a transversion mutation of g.3433A>T was found in the Gembrong goat under study and possibly for the genetic marker in Gembrong goat breed. Interestingly, the phylogeny analysis explained that the Gembrong goats is classified in the separated cluster with the global *caprine* SRY cluster (Figure 3).

Figure 3. Phylogenetic tree of Balinese Gembrong goat (*Capra hircus*) based on SRY gene sequence with UPGMA method (10,000 × bootstrap replications)

According to Figure 3, the Gembrong goat is close to Y1A1 (Y1A) haplogroup goat populations that are mostly identified in Southeast Asian goat breeds [5]. However, no mutation sites were detected in the exonic region of *caprine* SRY gene. In contrast, two transition sites were found in the exonic region of SRY gene in the Lakor goat of Indonesia i.e. g.362A>G and g.410C>T [7]. Therefore, Vidal *et al.* [6] reported five variants of *caprine* SRY gene such as: Y1A, Y1B1, Y1B2, Y1C and Y2. The Y1A, Y1B and Y1C in the goats at central and eastern regions of Europe. The Y2 variant was observed in goat populations of southern Europe and Africa regions. Therefore, most of the goat breeds in the middle-east region were detected as Y1A variant. In addition, Masuko *et al.* [10] reported that the goats in Philippines (132 heads) were characterized as Y1AA (0.53), Y1B (0.05), Y2A (0.17) and Y2B (0.25) haplotypes. While, the goat breedss in South Sulawesi of Indonesia (56 heads) were characterized as Y1AA (0.57), Y2A (0.18) and Y2B (0,25) haplotypes.

The deletion mutation in the SRY gene of the Gembrong goats may be caused by the high inbreeding rate as reported by Ridho & Putra [4]. As a result, the mortality at post-weaned age in Gembrong goat was high (±30%) at the breeding center [11]. Hence, the breeding, selection and conservation programs of the Gembrong goats are essential in order to save the breed from extinction.

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Authors' contributions

DAW, WPBP and HH carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. NNR carried out the laboratory work. NAA and ILM participated in the manuscript evaluation. GAAP participated in the design of the study and performed the sample collection. NW and NPYM conceived of the study, and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of competing interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Statement of informed consent

The owner understood procedure and agreed that results related to investigation or treatment of their companion animals, could be published in Scientific Journal Acta Veterinaria-Beograd.

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KARAKTERIZACIJA GENSKIH SEKVENCI REGIONA Y (SRY) KOD UGROŽENE GEMBRONG KOZE (*CAPRA HIRCUS*) NA OSTRVU BALI, INDONEZIJA

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Gembrong koza je autohtona rasa sa Balija, Indonezija, koja je trenutno ugrožena visokim nivoom inbridinga zbog smanjenja veličine populacije. Genetska karakterizacija je važan pristup za podršku očuvanju rase i poboljšanja upravljanja uzgojem i merama selekcije. Cilj ove studije bio je da analizira region Y hromozoma koji određuje pol kod mužjaka rase Gembrong koza. Prikupljeni su uzorci krvi od deset mužjaka, a DNK je ekstrahovana i amplifikovana korišćenjem lančane reakcije polimeraze usmerene na fragment regiona Y hromozoma koji određuje pol. Amplifikovani proizvodi su sekvencionirani i analizirani kako bi se identifikovale genetske varijacije. Rezultati su otkrili dve mutacije insercijom-delecijom koje se nalaze u nekodirajućem regionu, što je dovelo do identifikacije tri genske varijante. Jedna varijanta je bila dominantna, dok su ostale bile prisutne u nižim frekvencijama. Pored toga, otkrivena je specifična tačkasta mutacija koja može da posluži kao potencijalni genetski marker specifičan za rasu. Filogenetska analiza je pokazala da Gembrong koze pripadaju posebnom genetskom klasteru u poređenju sa drugim rasama koza. Ovi nalazi pružaju vredne nove genetske informacije o rasi Gembrong koza, ističući njihovu jedinstvenost. Rezultati mogu doprineti razvoju odgovarajućih strategija uzgoja sa ciljem smanjenja inbridinga i podrške dugoročnim procesima očuvanja. Štaviše, ova studija naglašava važnost studija genetske karakterizacije u očuvanju ugroženih autohtonih rasa stoke.