Gembrong Goat Rescue from Extinction Through Directional Mating Program Based on 12-Microsatellite Markers

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ABSTRACT

Gembrong goat is endemic in Karangasem Regency. At the beginning of 2013, the current population in Karang asem regency were critical (only 20 goats), and if not treated seriously then it could become extinct. The decrease in number of population was caused by the government 'slack of attention, poor-quality feed and the limited use of science and technology, especially inthe field of genetic technology that could be utilized in Gembrong goat breeding programs. The initial step was to rescue and maintain the remaining 20-Gembrong goats in the breeding centers, Tumbu village, subdistrict of Karang asem, Bali. Several activities were conducted to increase population, including identifying characteristic phenotypic, functional genes analysis and description of morphology as basic considerations in determining the authenticity of Gembrong goat. Population development efforts were carried outby mating arrangements, feeding quality, improved reproductive management, disease control and recording system. To avoid mating between close relatives (inbreeding), directional mating based on DNA fingerprint analysis was developed in this study. Pedigree detection among 23Gembrong goats were analyzed using twelve (12) microsatellite markers(MAF035, ILSTS029, BMS1494, MCM527, BM1818, OARFCB20, OARAE54, ILSTS005, SRCRSP3, MAF70, ETH10, dan ILSTS11). The results showed DNA fingerprint of the *gembrong* goat population was created by genetic distance between individuals, and grouping in seven clades. To increase genetic diversity, mating arrangements between different clades was applied. Output of this study are increase in number of Gembrong goat population

Key Words: Gembrong goats, Endemic, Critical, Directional mating, Clade

INTRODUCTION

Gembrong goat is one of national assets, even world assets which is endemic in Bali island, located in Karangasem Regency. Gembrong goat has a unique characteristic which is long hair in face, neck and back legs.. Zein et al. (2012) reported that Gembrong goat was grouping in specific cluster, so that it was different with other local goats. The population of Gembrong goat was critical, if not treated seriously then it could become extinct and lost of priceless gentic resources. Current study in genetic marker based on DNA analysisis getting big attention in the world because it can be used in species identification including Gembrong goat based on DNA barcoding, DNA microsatellite, DNA mitochondrial and Y chromosome. Buchanan et al. (1994) reported that microsatellite marker is a major tool in genetic relationship evaluation among the breeds. In addition, analysis of fungtional genes that have a crucial role in controlling growth and reproduction is enable to use in accurate selection through marker assisted selection (MAS) approach (Hu, 2007). So that, mapping of major genes such as Pit-1 (*pituitary transcription factor*), GH (*growth hormone*), IGF-1 (*Insuline like growth factor*-1), MSTN (*myostatin*) and BMP (*bone morphogenetic protei*n) wereneeded for this study. This technology can accelerate the sustainability of animal conservation and utilization programs. Application of the technology can avoid the

apprehensive about contamination of Gembrong goat purity in consequence of unplanned crossbreeding with other goats such as Kacang and Peranakan Ettawa goats.This study aimed to rescue Gembrong goat from extinction through directional mating program based on 12 microsatellite markers.

MATERIALS AND METHOD

A total of 20 Gembrong goats (G01-G20) were collected from captivity, Tumbu village, two (2) Gembrong goats (G21-G22) were collected from Agung mountainside, and one (1) Gembrong goat (G23) was collected from BugBug village, Karangasem Regency, Bali.Each Gembrong goat individual was taken it's photo from the various sides. After that, phenotypics measuring based on Herrera et al., 1996 and morphplogy mesurements were conducted. Blood samples were collected from individual Gembrong goat about 1.0-1.5 ml for DNA analysis.DNA extraction was done using DNA extraction kit (DNeasy®Blood and Tissue Kit, Qiagen product).

Detection pedigree of fingerprint analysis using 12 microsatellite markers based on ISAG/FAO, 2004 (MAF035, ILSTS029, BMS1494, MCM527, BM1818, OARFCB20, OARAE54, ILSTS005, SRCRSP3, MAF70, ETH10, and ILSTS11) was performed to know the familiy relationship of Gembrong goat population. The pedigree detection classified the individual of Gembrong goats based on clades. Directional mating program was applied between different clades.Polymerase Chain Reaction(PCR)analysis which performed using multiplate/multiplex PCRtecnique. It consists of multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. Each PCR product had different fragment length and labeling dye marker (Pet, Ned, and Fam).

Analysis of fungtional genes which concerned with economic traits (growth and reproduction) was done toward 5 genes. There were *growth hormone* (GH), *Insulin like growth factor* I (IGF-I), *myostatin* (MSTN), *pituitary transcription factor-1 gene* (POU1F1) dan *bone morphogenetic protein* (BMP). After the optimation of fungtional genes through PCR analysis was succed, there was genotyping using PCR-RFLP technique using specific restriction enzymes (Dra I, Hae III, Hinf I dan Pst I) in each gene.

RESULTS AND DISCUSSION

Genetically, qualitative trait is controlled by 1 or 2 pairs of genes, non-additive gene action, not normal data distribution, less of environmental effect and not economic value. Result of qualitative traits analysis of Gembrong goat showed that: (1) hair colour of Gembrong goat was cream/white of 75% of the population and brown of 5% and mixing (white, brown/black) of 20%; (2) More than 95% was horned both male and female, only 5% non-horned; (3) Face shape of Gembrong goat was straight, it showed the differences between Gembrong goat with Kacang goat that has concave face shape and Etawa goat that has dome face shape; (4) The eras position was stand to the side (almost 100%); (5) The hair was straight especially the long hair in face that can cover it's face. Number of navel was 1 (95%), only 1 individual (5%) had 2 navels. Characteristic of qualitative traits of Gembrong goat from this study can be used as reference to determine the specific characteristic of Gembrong goat.

Observation result of quantitative traits in Gembrong goat showed that body weight, body length, shoulder high, chest deep, chest wide and chest circumference were bigger in male than female.Genetically, quantitative trait is controlled by many genes (polygenes), additive gene action, normal data distribution, lot of environmental effect and high economic value.

Efforts of Gembrong goat development through directional mating program was sinergy with improving in maintanance system consists of feeding with high quality feed, improving in

reproduction management, disease control and recording system. Result of feed study showed that the grass, soybean waste and pollard have different nutrient component and it was fed depend on animal physiological status. Since there were application of disease control, improving of feed quality, improving of reproduction management, and recording system, there were no scabies goat, no died goat (mortality 0%) and increase in growth.

In this study, result of pedigree detection toward 23 individuals of Gembrong goat with DNA fingerprint analysis showed there were 7 clades (clade I-VII), whereas the clade VIII contained Gembrong goat (G21 and G22) was dissevered from the other clades (Figure 1 and 2), and this result was agreed to the our hypothesis. Result of morphology observation showed that G21 and G22 had dome face shape and it looks like Ettawa goat.Result of genetics analysis given evidence that Gembrong goat G21 and G22 had been contaminated by Ettawa blood. After the traceability, it could happen because there were breeders of Gembrong goat around them.

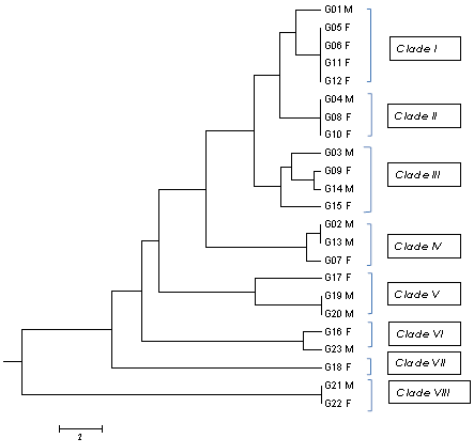

Figure 1. Result of pedegree detection DNA fingerprint analysis analysis.

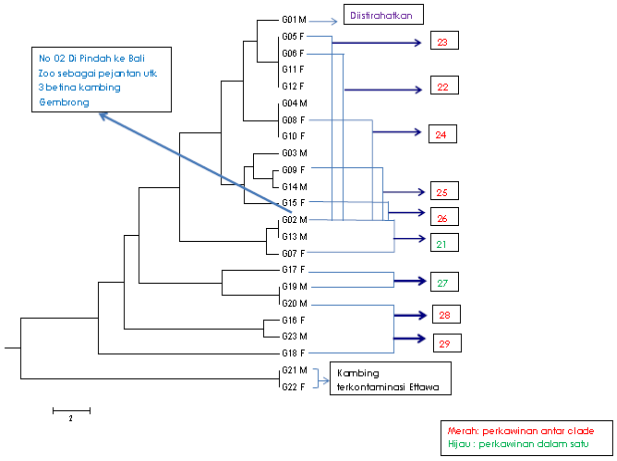

Figure 2. Result of pedigree detection between using female parental and the filial using fingerprint

The structure of family tree which showed in Figure 1 can be used as reference in mating program between male and female from different clade or between distant relatives. Mating program based on pedigree was done systematically and sustainability until the increase in number of population and forming a big gene pool. Li *et al.* (2008) reported that existence of a big gene pool was important to keep the sustainability of animal breeding and conservation.

Population development was occured, at the beginning of this study, the number of population only 20 goats, then the population increase to 29 goats, or increase about 45%. It came from eightGembrong female goat consisting of G07, G06, G09, G15, G08, G05, G18 and G17. Each female produced one kid except for G08 female which produced a twin kid. This female was mated by a male goat G20. (Figure 2).

Figure 2 showed even there was inter clade mating in captivity, Tumbu village,but the 5 Gembrong femalegoats (G06, G09, G15, G08, G05)that gave birth to a kid were mated by the same Gembrong male goat (G02). The G02 was very aggressive. It could happen because the other strong male goat(G01) was sick . Whereas the other male such as G13 and G14 were not mature yet and they were not ready to be mated, G23 was in BugBug village, G19 and G20 were in Taman Ujung. Before a pedigree detection result was ready, the mating which happened was not based on pedigree data. We also found some technical problem such as G02 was veryaggressive and difficult to be controlled. Mating in the same clade also

happened between G17 female with G19 male and G07 female with G02 male.At the end of october 2013, G02 was loaned by Bali Zoo Park to be mated with 3 female of Gembrong goat.

Result of fungtional genes analysis in Gembrong goat showed that GH and IGF1 locus were polymorphics. GH gene had 2 genotypes AB and BB with A and B allelic frequencies of 0.46 and 0.54, respectively, whereas IGF1 gene had 3 genotypes AA, AB and BB with A and B allelic frequencies of 0.59 and 0.41, respectively. MSTN, BMP and Pit-1 locus were monomorphics that had 1 genotype BB, AA and BB with A and B allelic frequencies of 0.00, 1.00, 1.00, 0.00, 0.00, 1.00, respectively. The less diversity of the 3 genes can caused by inbreeding, whereas the GH and IGF1 genes still had high diversity, even the GH gene only had 2 genotypes AB and BB with less proportion of BB.

CONCLUSION

Pedigree detection result classified Gembrong goat population into 7 clades and directional mating program was applied between different clades. Running the mating program is not as easy as we expected, because some technical problems occured in this study. In the first year of our study, the pedigree detection results from microsatellite analysis had not fully implemented in controlling the mating program of Gembrong goat in captivity.

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