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Genetic Relationship Between *Gembrong* Goat, *Kacang* Goat and *Kacang* X Etawah Crossbred (*PE*) Based on Their Mitochondrial DNA

I Gusti Lanang Oka[1], Wayan SayangYupardhi[1], Ida Bagus Mantra[1] , Nyoman Suyasa[2], Anak Agung Sagung Dewi[3]

[1] Faculty of Animal Science, Udayana University, Bukit Jimbaran, Bali
Phone/Fax: (0361)702771
[2] Institute for Studies on Agricultural Technology (BPTP), Denpasar, Bali,
Phone/Fax :(0361)720498, e-mail: bptpbali@yahoo.com
3 Disease Investigation Center (BBVet), Denpasar, Bali, Phone:(0361)720862,
e-mail: bbvet6@telkom.net

ABSTRACT

Gembrong goat is a specific type of goat which has long hair covers its whole body including its neck and face, originated from eastern part of Bali (Karangasem). A study of this type of goat and its relationship with other local goats (Kacang and Kacang x Etawah crossbred) was carried out at Sawe village, Jembrana, Bali.. A number of 12 gembrong goats, 3 kacang goats from Kubu village, Karangasem and 3 Kacang x Etawah crossbred goats from Denpasar were used in this study. Blood samples of all goats were collected for mitochondrial DNA (mtDNA) analysis. PCR amplification of D-loop mitochondrial DNA was carried out by using two primers i.e. CAP-F (5'-CGTGTATGCAAGTACATTAC -3') and CAP-R (5'-CTGATTAGTCATTAGTCCATC – 3') . Sequencing of 550 bp (base-pairs) of mitochondrial DNA (product of PCR) only found one polymorphic site at base number 231 with two haplotypes in gembrong goat only, while the other base-pairs were similar between the three goat types (Gembrong, Kacang and Kacang x Etawah crossbred) .The frequency of haplotype 1 was 83.3% and the frequency of haplotype 2 was 16.7%. It was concluded that based on their mitochondrial DNA sequences and "phylogenic analysis", the three types of goat (Gembrong,Kacang and Kacang xEtawah crossbred) had a very close genetic relationship (kinship).

Key words: Gembrong goat, mitochondrial DNA, genetic relationship

ABSTRAK

Kambing Gembrong merupakan jenis kambing yang khas yang memiliki bulu yang panjang menutupi seluruh bagian tubuhnya termasuk bagian leher dan mukanya, berasal dari Bali bagian timur yaitu Karangasem. Penelitian terhadap jenis kambing ini dan hubungannya dengan kambing lokal yang lain (kambing kacang dan persilangan kambing kacang dengan Etawah) dilaksanakan di dusun Sawe, Jembrana, Bali. Sejumlah 12 ekor kambing gembrong , 3 ekor kambing kacang dari desa Kubu, Karangasem dan 3 ekor kambing persilangan Kacang x Etawah dari Denpasar digunakan dalam penelitian ini.Pengambilan sampel darah dilakukan terhadap semua kambing (18 ekor) untuk analisa DNA mitokondrianya. PCR dilakukan untuk amplifikasi DNA pada bagian D-loopnya dengan menggunakan 2 (dua) primer yaitu; CAP-F(5'-CGTGTATGCAAGTACATTAC-3') dan CAP-R(5'-CTGATTAGTCATTAGT-CCATC-3'). Urutan (*sequence*) basa DNA mitokondria sepanjang 550 pasangan basa yang berhasil diamplifikasi dari ketiga jenis kambing ini menunjukkan hanya ada satu lokasi polimorfik yaitu pada basa nomor 231 dengan dua haplotipe pada kambing gembrong saja sedangkan pasangan basa yang lain semuanya sama antara ketiga jenis kambing tersebut. Frekuensi haplotipe 1 adalah 83,3% dan frekuensi haplotipe 2 sebanyak 16,7%. Dari sini dapat disimpulkan bahwa berdasarkan urutan DNA mitokondria dan analisis filogenik, ketiga jenis kambing tersebut (kambing Gembrong, kambing Kacang dan persilangan kambing Kacang x Etawah) memiliki hubungan genetic (kekerabatan) yang sangat dekat.

Key words: Gembrong goat, mitochondrial DNA, genetic relationship

INTRODUCTION

Gembrong goats were reported as a specific type of goat differ from the indigenous breed (*Kacang* goats) and their Etawah-crossbreds (*PE*), which are found in Karangasem regency, eastern part of Bali Island (Matram *et al*., 1993). This type of goat is called *Gembrong* goats because of their long hair cover their body, neck and up to their face particularly in buck (male goat). The word *gembrong* come from Balinese word which means long hair. Unfortunately, the population of this type of goats is decreasing from year to year because of the extensive management. With the extensive farming, *Gembrong* goats were kept in the field together with the other local goats. In such condition these type of goats were reported by farmers as the easiest breed to be killed by wild dogs. It is difficult to find gembrong goats nowadays in this region. In order to maintain their genetic resources, Institute for Studies on Agricultural Technology (*Balai Pengkajian Teknologi Pertanian-BPTP)* of Bali tried to breed them intensively at a particular area in Sawe village, Jembrana.

There are some other breeds of goat that have long hair such as Angora goats originated in central China and develop extensively in Turkey and South Africa, and Kashmiri originated in India and also found in Tibet (Devendra and McLeroy, 1982). Angora breed has long ringlets or curly hair which is called mohair while the Kashmiri has fine silky undercoat hair called cashmere and sometimes referred to as pashmina especially in India (Shelton, 1992). Australian researchers have started a selection program on their feral goats for cashmere (fine silky hair) production since 1979 (Restall and Pattie, 1991). Therefore, breeding of *Gembrong* goats in Bali seemed to have a good commercial future.

Genotyping is becoming widely practiced in animal breeding with applications using both genetic marker loci and known gene loci (Kinghorn, 2002). Puja and Sulabda (2009) studied DNA genome from hair of *Gembrong* goats. It was reported that 9 out of 10 markers used in their study were successfully amplified and the number of alleles ranged from 1 to 4 per locus of micro-satellite in *Gembrong* goat. Since it is still very little information could be got about *Gembrong* goat up to this year, research team of The Faculty of Animal Science in collaboration with *BPTP* Bali carried out a study on *Gembrong* goats including relationship between *Gembrong* goat and local goats in Bali (*Kacang* goat and their Etawah crossbreds/*PE*) and their other performances such as hematology, length of their hair, body weights, body dimensions, and some reproductive aspects. However, because of lack of the animals (*Gembrong* goats) number, this paper was limited to the relationship between *Gembrong* goats and other local goats (*Kacang* and *Kacang x Etawah* crossbred goats) based on their mitochondrial DNA sequences and “phylogenic analysis” of their haplotypes.

RESEARCH METHODS

This study was carried out at The Institute for Studies on Agricultural Technology *(Balai Pengkajian Teknologi Pertanian -BPTP*) farm in Sawe village, Jembrana, Bali since May until October 2009. A total of 18 goats consisted of 12 *Gembrong* goats (7 males and 5 females) belong to *BPTP*, while 3 *Kacang* goats belong to a farmer in Kubu village, Karangasem (East of Bali) and 3 *Kacang* x *Etawah* crossbreds (*PE*) belong to a goat collector (merchant) in Denpasar, were used in this study. Four ml of blood sample was collected from jugular vein of each goat for DNA analysis. Amplification of mitochondrial DNA (mtDNA) was carried out at the Medical and Molecular Biology Laboratory, Faculty of Veterinary Science, Udayana University using Polymerase Change Reaction (PCR) technique with two primers which are specific for goats (Luikart *et al*., 2001) i.e. CAP-F primer (5’-CGTGTATGCAAGTACATTAC -3’) and CAP-R primer (5’-CTGATTAGTCATTAGTCCATC-3’).

Each PCR product was analyzed using single–strand sequencing reaction with an automated DNA sequencer at Eijkman Institute, Jakarta. DNA sequence was aligned using MEGA 4 program (Tamura *et al*., 2007). Mitochondrial DNA sequences were also compared with DNA sequence of goats which were down- loaded from Gene Bank. The genetic variations for each individual within a species which is called as haplotype will be grouped based on their nucleotide sequences. “Phylogenic tree” of the haplotypes was reconstructed according to neighbour-joining method described by Saitou and Nei (1987) and Tamura *et al.,* (2004). Variation of each sequence was determined by DNAsp 4.10 (Rozas *et al*., 2003).

RESULTS AND DISCUSSION

Twelve fragments of *Gembrong* goat mitochondrial DNA with 550 base pairs (bp) length were successfully amplified using the PCR technique. Analysis of mitochondrial DNA sequences of the 12 *Gembrong* goats, 3 *Kacang* goats and 3 *Kacang x Etawah* crossbreds using DNAsp 4.10 found one polymorphic site at base number 231 with two haplotypes (Figure 1). Male *Gembrong* goats number 2 and 3 (GJ2 and GJ3) had Thymine at base number 231 while the other *Gembrong* goats, *kacang* goats (KC) and *Kacang* x Etawah crossbreds (PE) had Cytosine instead of Thymine. All the other bases along the 550 bp were similar in all goats studied. Polymorphic in D-loop location of mitochondrial DNA did not change the phenotype of Gembrong goats because this location is non coding area which is not expressed. Boore (1999) reported that this location take an important role only in regulation and initiation of replication and transcription of mitochondrial DNA.

Relationship between *Gembrong* goat (KG), *Kacang* goat (KC) and *Kacang x Etawah* crossbred (*PE*) is shown in “phylogenic tree” (Figure 2). Two haplotypes were found in *Gembrong* goats. It appeared that *Gembrong* goat particularly the haplotype 1 had a close relationship with *Kacang* and *PE* goats. Frequency of haplotype 1 was found 83.3 % (10 out of 12) and haplotype 2 was 16.7 % (2 out of 12). Genetic distance between *Gembrong* goat (haplotype 1) and *Kacang* goat and *Kacang x Etawah* crossbred were found 0.000, while the genetic distance between haplotype 1 and haplotype 2 of *Gembrong* goats were only 0.014. Animals which were inherited from the same maternal breed will have similar type of mitochondrial DNA (Ratnayani *et al*. 2007). If *Gembrong* goat was compared to the other breed of goats from other countries, the haplotype 1 had a close relationship with *Capra hircus* SN31 from China while the haplotype 2 had a close relationship with *C. hircus* HV1 from east Asia. Genetic variation of *Gembrong* goats in this study was very low. This low variation might because of the small number of goats found in this area and inbreeding probably the main factor since Frankham (2005) described that the number of animals in a population influences its genetic variation. It is important to develop this goat breed urgently otherwise it will be extinct from Bali. *Gembrong* goat should be conserved in Bali because this animal is the only specific breed which is found in Indonesia. As a specific local indigenous species, its genetic resources should be conserved and developed as a pure breed animal in Bali. According to Wibowo (2001), the genetic variations in a particular population may be resulted because of several factors such as natural selection, environmental factor, mutation or breeding. The long hair of *Gembrong* goats might be as a result of mutation

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GJ1 ATA TAG TAC ATT AAA CGA TTT TCC ACA TGC ATA TTA AGC ACG TAT ATC [240]
#GJ2 ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... [240]
#GJ3 ... ... ... ... ... ... ... ... ... ... ... ... ..T ... ... ... [240]
#GJ4 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GJ5 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GJ6 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GJ7 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GB1 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GB2 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GB4 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#GB5 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#KC1 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#KC2 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#KC3 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#PE1 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#PE2 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
#PE3 ... ... ... ... ... ... ... ... ... ... ... ... ..C ... ... ... [240]
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Figure 2. Sequences of mitochondria DNA *Gembrong* goats (*GJ* and *GB*), *Kacang* goats (*KC*) and *Kacang x Etawah* crossbreds (*PE*)

***.** *(dot) in the same column is base code similar to the top base code A, G, T or C*

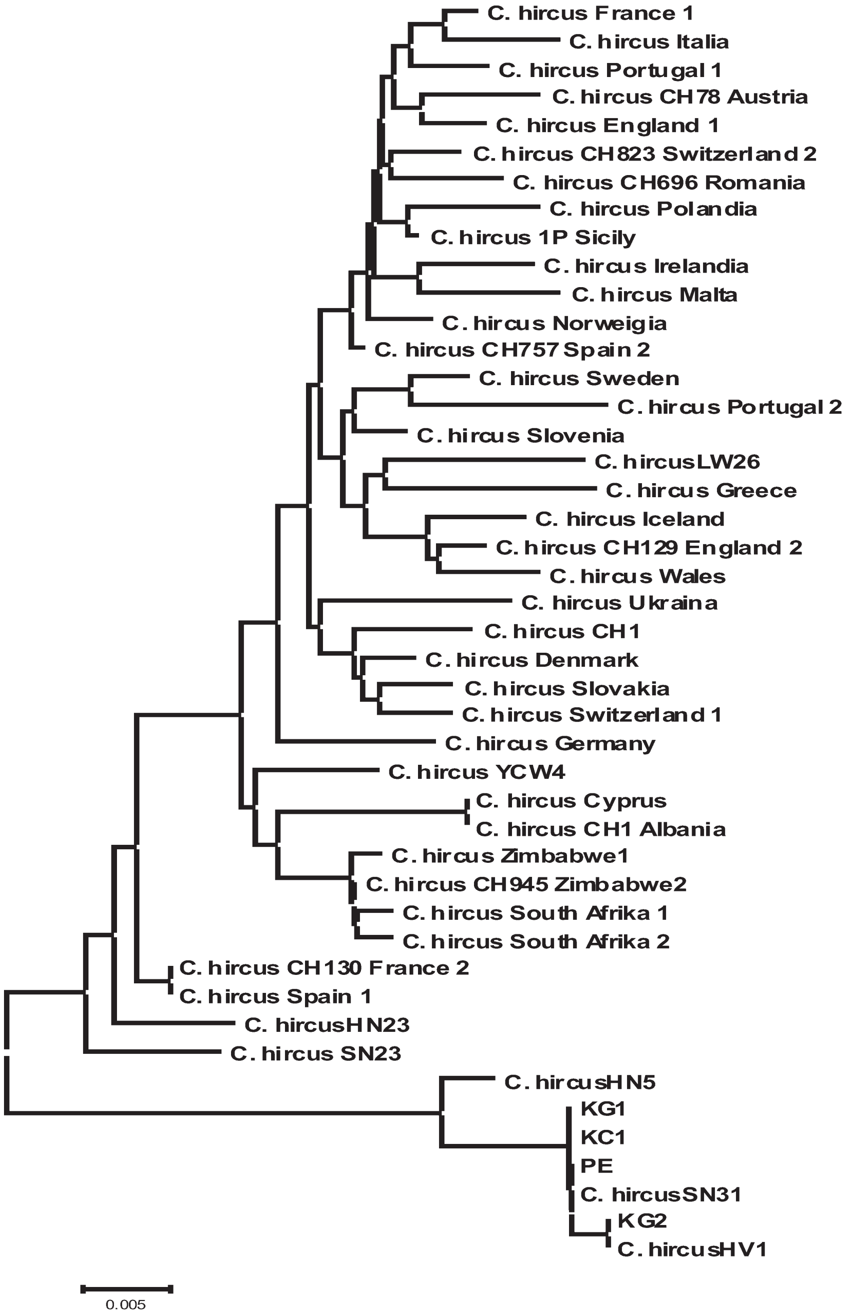

Figure 2. Phylogenic tree of *Gembrong* goat (*KG*), *Kacang* goat (*KC*), *Kacang x Etawah* crossbred (*PE*) and several goats in the world.

of gene which is responsible for the hair length of the local (indigenous) goats in Bali. However, more intensive studies are needed in order to get an accurate conclusion.

CONCLUSIONS

Based on the mitochondrial DNA analysis of the three types of goat (*Gembrong, Kacang* and *Kacang x Etawah* crossbred) and analysis of their phylogenic tree, it could be concluded that *Gembrong* goat had a close genetic relationship (kinship) with the indigenous breed (*Kacang*) and *Kacang x Etawah* crossbred (*PE*) in Bali.

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