The Genetic Variation of Bali Cattle (*Bos javanicus*) Based on Sex Related Y Chromosome Gene

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Abstract. Bali cattle is very popular Indonesian local beef related to their status in community living process of farmers in Indonesia, especially as providers of meat and exotic animal. Bali cattle were able to adapt the limited environment and becoming local livestock that existed until recently. In our early study by microsatellites showed that Bali cattle have specific allele. In this study we analyzed the variance of partly sex related Y (SRY) gene sequence in Bali cattle bull as a source of cement for Artificial Insemination (AI). Blood from 17 two location of AI center, Singosari, Malang and Baturiti, Bali was collected and then extracted to get the DNA genome. PCR reaction was done to amplify partially of SRY gene segment and followed by sequencing PCR products to get the DNA sequence of SRY gene. The SRY gene sequence was used to determine the genetic variation and phylogenetic relationship. We found that Bali cattle bull from Singosari has relatively closed genetic relationship with Baturiti. It is also supported that in early data some Bali bulls of Singosari were came from Baturiti. It has been known that Baturiti is the one source of Bali cattle bull with promising genetic potential. While, in general that Bali bull where came from two areas were not different on reproductive performances. It is important to understand about the genetic variation of Bali cattle in molecular level related to conservation effort and maintaining the genetic characters of the local cattle. So, it will not become extinct or even decreased the genetic quality of Indonesian indigenous cattle.

Key Words : Bali cattle, SRY gene, artificial insemination, phylogenetic, allele

Introduction

Among the Indonesian local cattle, Bali cattle have the huge genetic potential, primarily as meat animal. However, the existence of local cattle in Indonesia in recent decades got serious attention of government and society. In fact, local cattle like Bali cattle has been proven are better able to survive and adapt to local environmental conditions as well as socially and culturally has long interacted with the human. The efforts to protect the genetic diversity of local cattle should be maintained and even improved, especially for the purpose of selection and utilization of specific genes that are often not owned by the modern breed which came from tight selection.

Studies on the SRY gene, a specific gene for the male sex is rarely in Indonesia, especially on local cattle. The benefits of using of SRY gene marker which obtained from the earlier study in sheep have been found an *A-oY1* allele on wild big horn sheep (*Ovis canadensis*), two subspecies of thin tail sheep (*Ovis dalli*), European Mouflon sheep (*Ovis musimon*) and Barbary sheep (*Ammontragis lervia*) (Meadows et al*.,* 2006). Also on the human SRY gene and Y chromosome STR DNA were used to study the origins of race in the world (Thomas et al*.,* 1999; Ruiz-Linares et al*.,* 1999; Gusmáo et al*.,* 2001; Bortolini et al*.,* 2002 and Kurihara et al*.,* 2004), study on differentiation between Chinesse indigenous buffalo and introduced river buffalo (Zhang et al*.,* 2006), and for sex

determination in bovine embryo (Lu et al*.,* 2006), then finding that any protein encoded by SRY showed sequence-specific DNA binding activity, which was absent or reduced in SRY from certain XY females with gonadal dysgenesis (Harley et al*.,* 1992; Nasrin et al*.,* 1991; Tohonen et al*.,* 2005).

The important finding that the *SRY* coding region among primates and rodents suggests that this gene is rapidly evolving (Tucker et al*.,* 1993; Whitfield et al*.,* 1993). Contrary, results from wallaby and domestic ruminants appear to indicate that sequence evolution of the *SRY* gene is less rapid (O'Neill et al*.,* 1997; Payen et al*.,* 1994). It remains a challenge how the amazingly complex sex determination pathways evolve in various animal systems, especially in the *Bovidae* family (Cheng et al*.,* 2001).

This study was designed to document the variation of the SRY gene haplotypes in Bali cattle bull in hopes identified the specific marker or unique to the local cattle of Indonesia. So, it will produce more basic data of DNA markers and in further can contribute information to complete the cattle genome map that already exist. This is important because it can be used as conservation of existing local cattle germplasm.

Materials and Methods

DNA samples preparation

The blood samples as source of DNA genome were isolated from male Bali cattle that were determined from two Center of Artificial Insemination (AI) Institution, that are BBIB Singosari, Malang, East Java Province and BIBD Baturiti, Tabanan, Bali Province. About 17 heads of Bali cattle bulls were choosing as source of blood samples. Whole blood cells (5-10 mL) were collected from jugular vein of cattle and preserved in vaccutainer tubes containing EDTA as anticoagulant. DNA extracted with based on phenol-chloroform standart protocol according to Sambrook et al*.*, (1989). After genomic DNA from isolation purified and measured the concentration, then prepared for a template in PCR reaction.

PCR reactions and sequencing analysis

Primer pair of partly SRY gene that using in this study are by the primer sequences SRY-4 : 5’ – GCC TGG ACT TTC TTG TGC TTA – 3’ and SRY-5 : 5’ – ACA GTG GGA ACA AAA GAC TAT – 3’. Amplified products were sequenced in both directions with ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction v.3.1 (Applied Bio-systems, Foster City CA, USA). Sequencing was carried out using an ABI PRISMR 3100-Avant Genetic Analyzer (Applera, Foster City, CA). Data were collected using the ABI PRISM Data collection software and analysed with DNA Sequencing Analysis software v.3.7 (Applied Biosystems, USA). The sequence nucleotides data were used to identify variations of SRY gene among cattle and to construct the phylogenetic tree of Bali cattle bull.

Results and Discussion

Variation of SRY gene in Bali cattle bull

SRY has been identified on the Y chromosome in many placental mammals. In marsupials an SRY orthologue was shown to map to the Y chromosome (Foster et al*.,* 1992), confirming a probable ancestral role for it in mammalian sex determination. Aasen and Medrano (1990) studied with samples in humans, cattle, sheep and goats were proving that the SRY gene has role on the male sex determining gene, instead of ZFY and ZFX genes, where there is one band in males and no DNA bands in females. Also, in Amelogenin gene which showed that the female sex chromosome (X) there is a 6 bp deletion in intron compared with male sex (Y), which produces 106 bp PCR product for the female sex, and 112 bp for the male sex (Sullivan et al*.,* 1993).

Studies on Y chromosome are of particular interest in livestock species because in common breeding strategies only a few males contribute genetically to the next generation (Lindgren et al*.,* 2004). The mammalian Y chromosome has two components, a pseudoautosomal region which frequently recombines with the X chromosome and a male-specific region (MSY). 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). Molecular variation in the Y chromosome provides information about genetic diversity, since it reveals the pattern of distribution of paternal lineages. For instance, it may indicate stocks upgrading, which is often performed by using sires from breeds with the desired properties. Up to date, however, few phylogenetic surveys involving the Y chromosome have been reported in domestic species due to a lack of MSY variation. Indeed, very low rates of nucleotide diversity have been reported within the MSY of horse (Lindgren et al., 2004), cattle (Hellborg and Ellegren, 2004), sheep (Meadows et al*.,* 2006; Marzanov et al*.,* 2006) and goat (Luikart et al., 2001). Analysis of mtDNA, Y-chromosomal DNA and microsatellites indicated a purely Banteng origin of Indonesian Bali cattle. However, mtDNA and nuclear DNA in a Bali cattle population kept in Malaysia was of mixed zebu-banteng origin (Nijman et al*.,* 2003).

In setting performance bull as a source of semen, it must be considered the genotypic performance, especially on the ability of reproduction. It is intended that the male sperm sources should have a maximum reproductive character as a semen provider. So the bull will be efficient, because by having a maximum fertility rate it is also the offspring will have a maximum level of productivity as derived from the male quality. Here, we need the right set of genetic indicators to predict the ability of reproduction and sperm production capability of the male (bull) that will used as a source of cement. In the relation of SRY gene, Bali cattle bull need to confirm about the genetic potential based on this gene. Because, it will be influenced the sperm quality of Bali cattle bull, especially for AI needs.

From sequence nucleotides of partly SRY gene we can obtain many data about percent of similarity and variation of SRY gene from two populations which presented in Tabel 1.

From Table 1 showed that by overall we found the highest similarity is 31.6% of Bali cattle bull population compare to other *Bos javanicus* (Gen Bank accses number AY079146.2). Consequently, we found the highest variant is 76.1%. It is indicates that Bali cattle from two populations were high enough variation in SRY gene sequence. So, it needs further study where can analyze that we can identified the relation between reproduction performance and genetically variation of SRY gene. Because in many studies showed that the action of SRY gene will influence on sex development. Like Sinclair et al*.,* (1990) reported that the Y chromosome-linked SRY gene is responsible for male sex determination in mammals. Mutation in the SRY gene can result in male-to-female sex reversal (Berta et al*.,* 1990; Jäger et al*.,* 1990).

The phylogenetic of SRY gene in Bali cattle bull

We also construct the phylogenetic tree from the partly segment of SRY gene that presented in Fig. 1. In general, we can concluded that two population of Bali cattle were close enough in genetic relationship based on SRY gene and the shortest distance was found between Bali cattle of BBIB Singosari 4 with Bali cattle from BIBD Baturiti 7. This is consistent with previous information that Bali cattle bulls that any bull that rearing in BBIB Singosari came from BIBD Baturiti (Herliantin, 2010; personal communication), so might it be close in genetic relationship. This is important

Tabel 1. Percentage of similarity and variant of nucleotides of Bali cattle SRY gene compare to *Bos javanicus* (Gen Bank accses number AY079146.2)

Code of Sample	Total nucleotide sequence (bp)	Number of Similarity	Percent of Similarity *(%)*	Number of Variant	Percent of Variant (%)
Singosari 1	190	55	28.9	135	71.1
Singosari 2	190	60	31.6	130	68.4
Singosari 4	190	51	26.8	139	73.2
Singosari 6	190	54	28.4	136	71.6
Singosari 7	190	53	27.9	137	72.1
Singosari 9	190	59	31.1	131	68.9
Baturiti 1	190	47	24.7	143	75.3
Baturiti 2	190	53	27.9	137	72.1
Baturiti 4	190	45	23.7	145	76.1
Baturiti 5	190	48	25.3	142	74.7
Baturiti 6	190	50	26.3	140	73.7
Baturiti 7	190	49	25.8	141	74.2
Baturiti 8	190	48	25.3	142	74.7
Baturiti 9	190	51	26.8	139	73.2
Baturiti 10	190	54	28.4	136	71..6
Baturiti 12	190	51	26.8	139	73.2

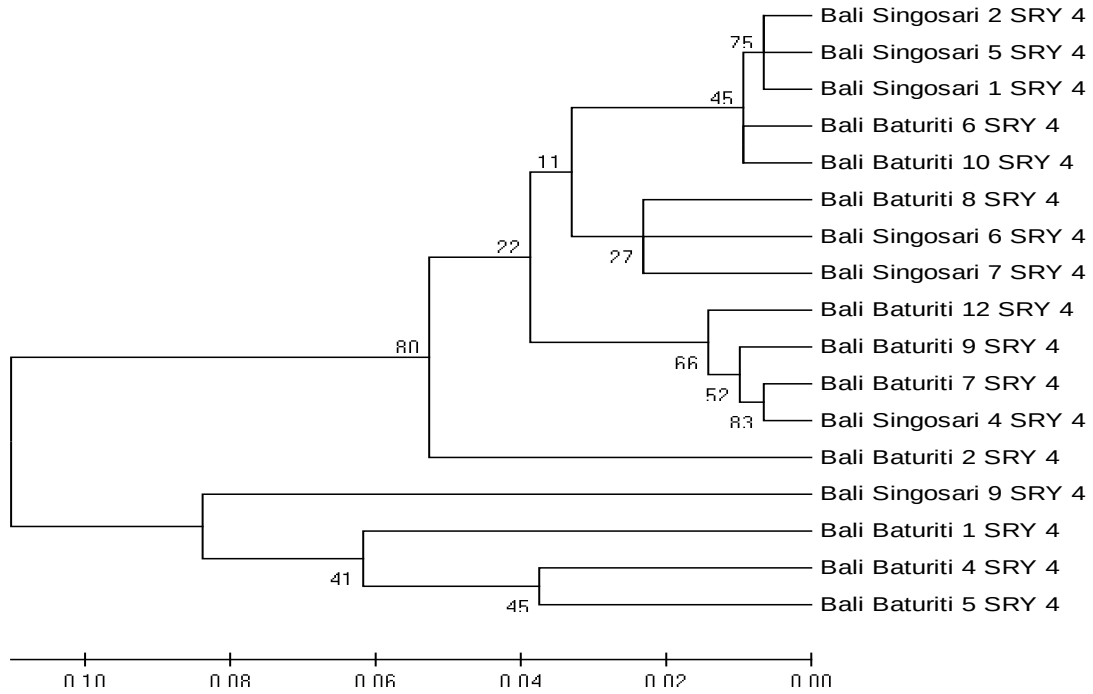

Figure 1. The phylogenetic relation between Bali cattle from Singosari, Malang and Baturiti, Bali

that good semen is needed for the AI program and Baturiti is the one source of Bali cattle genetic has been kwon have good genetic source of Bali cattle.

Winaya (2010) reported that based on Y-chromosome microsatellite, Bali cattle and Madura cattle are most closely distance compare to others in genetic relationship. This is also supporting the argument before where presumably domestic cattle in Southeast Asia and Indonesia are suggested to be of hybrid origin via crossing of zebu with Bali cattle, which is a domestic form of the Banteng (Groeneveld et al*.,* 2009). Indeed, Kikkawa et al.

(2003) and Mohamad et al. (2009) found Banteng mtDNA in Indonesian zebus, most notably in the Madura (56%) and Galekan (94%) breeds. The mixed species origin of Indonesian zebus was confirmed by microsatellite analysis (Mohamad et al., 2009).

Conclusions

In general that Bali bull where came from two areas were not different on reproductive performances. It is important to understand about the genetic variation of Bali cattle in molecular level related to conservation effort and maintaining the genetic characters of the local cattle.

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