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Short Communication

SRY Gene Based Kinship Relationship of Limousin and Madrasin Cattle

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Key words
Limousin, Madrasin, Genetic, SRY, Gene

ABSTRACT

This study aimed to determine the kinship and genetic diversity between Limousin and Madrasin cattle based on the sex determining region-Y (*SRY*) gene. *SRY* gene is used in the analysis of genetic diversity for sex determination based on the paternal pathway (Y chromosome). The DNA samples used for this study were 10 frozen straw of Limousin cattle and 10 samples of Madrasin cattle. The method used in this research was a descriptive analysis by duplex polymerase chain reaction and analyzing the results of the *SRY* gene sequencing of limousine cattle and Madrasin cattle. In the present study, we showed that the *SRY* gene of Madrasin cattle and Limousine cattle could be amplified with SRY F and SRY R primers with a PCR product length of 318 bp. Based on these results, Madura, Limousin, and Madrasin cattle had similarities based on the *SRY* gene (paternal pathway).

Genetic diversity in a population is a fundamental capital for application of breeding technology in of animals (Casarini and Crepieux, 2019). The genetic diversity of a population described in the diversity of animal appearances is a reflection of the genetic information it possesses. As an illustration, Bali cattle living on Bali Island have different gene constructions from the Madura cattle population in Madura Island. These differences can be expressed in adaptability, body size, and disease resistance. This component greatly affects the ability to adapt (adaptation) to environmental changes such as the degradation of environmental quality as a medium for animal growth. This difference in appearance is caused during domestication, the types or breeds of animals are genetically separated due to adjustments to each local environment and the needs of the local community so that different nations are produced (Utomo *et al*., 2020). Animal adaptability exists because animals can produce a variance of physical morphologies, behavior, and physiological condition as a reaction to environmental changes (regulation of gene expression) (Ahmad *et al*., 2020).

One sex determination gene used in the analysis of animals' genetic diversity is the sex determining region-Y (*SRY*) gene (Chang *et al*., 2013). The position of the *SRY* gene is on the Y chromosome and stimulates male sex differentiation so it can be used based on the paternal pathway (Cheng *et al*., 2016). The study of genetic diversity by the *SRY* gene has been conducted in several commodities in cattle. Regional *SRY* is a small gene of 600 base pairs (bp) and encodes a protein with 78 amino acids through the HMG (high mobility group) box region (Law *et al*., 2017; Kurtz *et al*., 2021). Y-chromosomal DNA markers are expected to have another geographical distribution and paternally inherited markers would reveal male introgression via breeding management with natural matting and artificial insemination as well (Amin, 2021).

For sex determination in mammals XY chromosome

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system is used, where the X chromosome is for the female sex (XX) and the Y chromosome is for the male (XY). The genetic marker on the Y chromosome was used in this study because it has less variation that occurs in it (Siswijono *et al*., 2017). Two genetic markers were used: ubiquitously transcribed Y chromosome Tetratricopeptide (*UTY*) was used to determine sex and sex-determining region Y (*SRY*) was used to confirm the outcome of the *UTY* gene. The *UTY* gene is important in male reproduction, while SRY is a gene that is responsible for the formation of the gland (prostate) and external genital organs (Weller *et al*., 2016). The *SRY* gene contains a determinant factor of testes which is located on the segment of 35-kb on sleeve short-chromosome Y. If there is a development of the functional *SRY* gene, the gonad bipotential will undergo determination into the testes and regression of Müllerian duct (Leroy *et al*., 2018).

This study used the *SRY* gene to obtain data on genetic diversity and kinship between Limousin and Madrasin cattle. This research is conducted to provide information on the genetic variances of Limousin and Madrasin cattle based on SRY gene. This finding of the of the study can be used as additional information of the promoter region of SRY gene of local breed in Indonesia.

Materials and methods

The study was approved by Animal Care and Use Committee of Veterinary of Medicine Faculty, Universitas Airlangga (reference number: 1.KE.200.03.2021). This study was conducted from December 2021 to June 2022 at Faculty of Veterinary Medicine, Universitas Airlangga, Surabaya, Indonesia and National Research and Innovation Agency (BRIN), Bogor, Indonesia.

The DNA sampel used for this study were 10 frozen straw of Limousin cattle and 10 samples of Madrasin cattle. Two pairs of primers, forward and reverse were used for gene amplification, F 5-AAG GGG AGA ACA GTT AGG GAG A-3' and R 5-ATC GGG TTG CAT AGT ATT GAA G-3'. For duplex polymerase chain reaction, 25 µl of PCR amplification reaction mixture contained 12.5 µl GoTag Green Master Mix (Promega), 2 µl DNA samples, 1 µl primer F (10 pmol), 1 µl primer R (10 pmol), and 8.5 µl nuclease-free water (Promega). The Duplex PCR program consisted of pre denaturation at 94º C for 5 min, denaturation at 94ºC for 30 sec, annealing at 60ºC for 30 sec, and extension at 72ºC for 30 sec with 45 repetitions of the cycle. After that, the cycle ends with the final extension of 72ºC for 10 min, followed by a hold time at a temperature of 4ºC.

The analyzed PCR products were conducted by electrophoresis on 1.5% agarose gel using SYBr dye (Invitrogen S7563, North America). Molecular markers (Ladder, Invitrogen) measuring 100 bp were electrophoresed together to determine the size of the PCR product. Furthermore, the gel electrophoresis results were observed using the gel documentation system and the size of the amplicon was determined using the software provided in the gel doc. Results can be seen at 318 bp. Determination of nucleotide sequences is carried out using DNA sequencing, which is the final step to obtain nucleotide sequence data from fragments resulting from the multiplication of DNA fragments. Single bands on an agarose gel as a PCR product were used as templates in the sequencing reaction using forward and reverse primers during amplification.

Analysis of the results of this sequencing was carried out by comparing the nucleotide base sequence of the sample with the nucleotide sequence of Madura cattle as the main reference using the Molecular Evolutionary Genetics Analysis (MEGA) 7 program.

Results and discussion

Figure 1 shows the genetic variation found among Madrasin cattle, Limousin cattle, and Madura cattle.

The nucleotide frequency of SRY gene sequences for Madrasin, Limousin, and Madura cattle is shown in Table I.

Fig. 1. Alignment results in the nucleotide bases of Madrasin Cattle, Limousin Cattle, and Madura Cattle (MD1S SRY= Madrasin Cattle SRY Gene 1; MD2S SRY = Madrasin Cattle SRY Gene 2; MD3S SRY= Madrasin Cattle SRY Gene 3; L2S SRY= Limousin Cattle SRY Gene 2; L1S SRY= Limousin Cattle SRY Gene 1; M2S SRY= Madura cattle SRY gene).

The construction of the phylogeny tree was used to see the kinship between Madrasin cattle, Limousin cattle, and Madura cattle. The results of the phylogenetic tree are presented in Figure 2 and the genetic distance matrix is shown in Table II.

Table I. Nucleotide frequency *SRY* genes in Madrasin, Limousin, and Madura cattle.

Sample	Nucleotide frequency (%)				Total
	T	C	A	G	
MD1S SRY	18.5	24.6	33.0	23.9	276
MD2S SRY	18.5	24.6	33.0	23.9	276
MD3S SRY	18.5	24.6	33.0	23.9	276
L2S SRY	18.5	24.6	33.0	23.9	276
L1S SRY	18.5	24.6	33.0	23.9	276
M2S SRY	18.5	24.6	33.3	23.6	276
Avg.	18.5	24.6	33.0	23.9	276

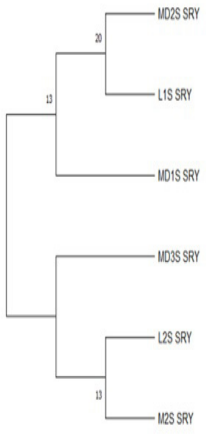

Fig. 2. Phylogenetic tree of Madrasin, Limousin and Madura cattle based on *SRY* gene.

The results of this study have been conducted against the gene of sex determining region Y (*SRY*) Madura cattle, and Limousin cattle and obtained their Madrasin genetic variation. The sex determining Region Y gene variation was obtained from the similarity of the nucleotide sequences among the 5 cattle samples used in this study. The difference in the nucleotide sequence was only found in one base location of the Madura cattle sequence. The difference in base sequences between Madura and Madrasin cattle was obtained at location 225 where the base sequence A was only found in Madura cattle and G in Madrasin and Limousin cattle. The existence of Y chromosomes determines typically the sex in mammals. Male sex would be determined by the normal function of the Y chromosome gene or Sex-detremining region Y (Hartatik *et al*., 2018). Sex chromosomes (X and Y on mammals) are widely discussed as proof of genetic evolution. It could be seen from the similarity of the promoter of the SRY gene from different animal species (Ellergen, 2011). SRY gene polymorphism also been identified on Madura cattle, Friesian Holstein cattle, Sahiwal cattle, Bali cattle and Buffalo (Arslan *et al*., 2017). The polymorphism showed specific alleles type which can be used as an effective genetic marker to detect crossbreeding between livestock based on the inherited mutation (polymorphism) similarity (Bai *et al*., 2010).

The *SRY* gene is a marker gene inherited from the male side (bull) and it is expected to be used as a marker to monitor the crossbreeding. The monitoring of those cattle is an initial effort to increase the genetic variation and enhance the genetic qualities without threatening the germplasm purity (Ciptadi *et al*., 2021; Leven, 2019). The results of the phylogenetic tree of Madrasin, Limousin and Madura cattle based on SRY gene showed that it can be seen that Madrasin cattle have the same or almost the same genetics as Limousin cattle based on the SRY gene (the genetic distance between Madrasin and Limousin cattle is 0.000 while the genetic distance between Madrasin and Madura cattle is 0.003) but very far from *Bos taurus* (genetic distance 0.052) and *Bos indicus*. The genetic distance between the 5 research samples for Madrasin cattle codes 1 and 3 compared to Madura cattle is 0.003, while for Madrasin and Limousin cattle, the distance is very close or the same based on the *SRY* gene. The study of genetic variation in an organism, especially in livestock between types of organisms is very important because it relates to variations in phenotype. The phenotypic variation can be due to genetic variation or environmental variation or environmental and genetic variations. Knowledge of genetic variation has many

Table II. Genetic distance matrix of Madrasin, Limousin and Madura cattle based on *SRY* gene.

	MD1S_SRY	MD3S_SRY	L2S_SRY	L1S_SRY
MD1S_SRY				
MD3S_SRY	0.0000000000			
L2S_SRY	0.0000000000	0.0000000000		
L1S_SRY	0.0000000000	0.0000000000	0.0000000000	
M2S_SRY	0.0036335924	0.0036335924	0.0036335924	0.0036335924

useful applications. The application of this genetic variation, for example, to identify animals and find their origin, determine kinship and gene mapping (Utomo *et al*., 2021; Raschia *et al*., 2018).

To our knowledge, the current study genetic of variation can also be used as a basis for species conservation. Conservation of animal species is very important because many types of animals are currently threatened with extinction and the genes they carry may be useful in the future (Mohammad *et al*., 2009). Based on these study, it is assumed that there are the SRY genes of Madrasin cattle and Limousine cattle could be amplified with SRY F and SRY R primers with a PCR product length of 318 bp. Madura, Limousin, and Madrasin cattle have similarities based on the SRY gene (paternal pathway). The limitation of this study was taken during pandemic time. Thus, further detailed investigations of are necessary to research on associated genes that more comprehensive results can be collated in local Indonesian cattle.

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Statement of conflict of interest

The authors have declared no conflict of interest.

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