Allele frequency estimation of BLAD (Bovine Leukocyte Adhesion Deficiency) in dairy cattle in Enrekang regency South Sulawesi Indonesia

M I A Dagong[1] , L Rahim[1], R R S R Aprilita Bugiwati[1] and Nurmulyaningsih[2]

[1] Animal Breeding and Genetic Laboratory, Faculty of Animal Science, Hasanuddin University

[2] Animal Production Study Program, Faculty of Animal Science, Hasanuddin University

E-mail: ihsandagong@gmail.com

Abstract. Bovine leukocyte adhesion deficiency (BLAD) is a genetic disorder in dairy cows that are generally lethal or premature in cattle that can have an impact on productivity and result in losses for farmers. The purpose of this study is to identify the distribution of the BLAD recessive allele in dairy cows raised in Enrekang dairy cattle development centers. Identification of recessive BLAD allele using the PCR-RFLP method. A total of 80 DNA samples in a collection of blood samples from FH dairy cows from Enrekang regency (50 heads from Cendana and 30 heads from Anggeraja district) were extracted and amplified by PCR, then the PCR product was cut with the T*aq*1 restriction enzymes. The identification of the BLAD carrier alleles is calculated according to the frequency of genotypes and alleles. The results obtained from this study is (+/-) heterozygous cows (BLAD carrier) found 1 head (with a frequency of the allele (-) (BLAD recessive) of 0.01) in the Cendana district of 50 samples, while the Bovine population in the district Anggeraja did not found any BLAD recessive allele. The results of this study concluded that there is a normal heterozygous dairy cows (BLAD carrier) in the dairy cows population in the Enrekang regency, although the proportion / frequency of alleles is very low (0.625%). This research can be used as a reference in selecting dairy cattle, especially those related to the spread of lethal alleles in dairy cattle development areas in South Sulawesi.

1. Introduction

Dairy cows have spread to several regions of Indonesia and, in general, farmers raise them on a small and large scale. Dairy cows have considerable potential to develop because, in addition to producing milk, they also produce meat. Genetic factors are one of the main factors that contribute to increase the productivity of dairy cattle. However, there are several genetic disorders that are lethal and often found in dairy cows. One genetic disorder in dairy cows that often occurs is bovine leukocyte adhesion deficiency (BLAD).

Bovine leukocyte adhesion deficiency (BLAD) or granulocytopathic syndrome is a genetic disease in cattle caused by the appearance of point mutations in exon 2 of the CD18 gene, this mutation causes β2 heterodimer molecules not expressed in the surface of neutrophils. In homozygous recessive conditions, this mutation causes lethal or premature death in cattle [1]. Bovine leukocyte adhesion

deficiency (BLAD) is generally lethal in dairy cattle before reaching adulthood, thus affecting productivity and causing losses to farmers.

BLAD genetic disorders were first identified in North America [1] which then spread to various countries such as India [2], Turkey [3], Iran [4], Japan [5], Romania [6], Pakistan [7] and the Czech Republic [8].

Enrekang Regency is a dairy cattle development center in South Sulawesi. The development of dairy cattle population in this area is supported by the development of Dangke dairy products, a type of soft cheese made from milk [9]. There are two region for dairy cattle development in Enrekang, namely Cendana and Anggeraja districts. The purpose of this study was to identify the presence of recessive alleles from bovine leukocyte adhesion deficiency (BLAD) in dairy cows. With the knowledge of livestock carrying recessive alleles, breeding strategies can be carried out to reduce or eliminate BLAD recessive aleles.

2. Method

2.1. Blood sample collection

This study used about 80 DNA samples collected from blood samples from Fries Holland dairy cows on smallholder farms in Enrekang regency (50 heads from Cendana district and 30 heads from Anggeraja district). About 5 ml of blood sample were collected from the jugular vein of each dairy cow in a vacutainer tubes containing EDTA.

2.2. DNA extraction and BLAD allele identification

DNA was extracted and purified using DNA extraction kit by following the extraction protocol standards provided. The results of DNA extraction then continued for the PCR reaction process for the determination of BLAD alele. All procedur were carried out at the Integrated Biotechnology Laboratory of Animal Science Faculty, Hasanuddin University.

The PCR amplification was carried out in a total volume of 25 μL consisting of DNA template (~100 ng), 0.25 mM each BLAD forward and reverse primer (Table 1), 150 μM dNTP, 2.5 mM Mg2+, 1x buffer and 1 U/ μL Taq polymerase. The PCR condition including initial denaturing step of 94 ^{o}C for 4 min, followed by 34 cycles of 94 ^{o}C for 45 s, primer annealing at 64 ^{o}C for 45 s, and 72 ^{o}C for 1 min, with a final cycle at 72 ^{o}C for 5 min. The PCR products of BLAD gene was digested with T*aq*1 restriction endonuclease enzym according from manufacturer (Fermentas). Digested PCR products were electrophoresed on 2% agarose gel and then visualized under UV-transiluminator (gel documentation system).

Table 1. Primer sequence and restriction endonuclease enzyme.

Gene	Primer	Restriction enzyme	PCR size	Reference
BLAD	F: 5'- GAATAGGCATCCTGCATCATATC CACCA-3'	T*aq*1	357 bp	[10]
	R: 5'- CTTGGGGTTTCAGGGGAAGATGGA GTAG-3'			

Note : F = Forward, R = Reverse

2.3. Data analysis

Allele and genotype frequencies were calculated using PopGene program [11].

3. Results and Discussion

The amplified PCR product of the BLAD gene produced 357 bp fragment of amplicon. This result is in accordance with that reported by [10, 12]. Determination of the genotype of the BLAD gene in dairy cattle in this study using the PCR-RFLP (polymerase chain reaction restriction fragment length polymorphism) method with T*aq*1 as an enzyme restriction. The T*aq*1 enzyme recognizes the point

mutation that changes adenine by guanine (A → G).

This is consistent with the report of [13] that the results of sequencing mutations of the BLAD allele known in base 383 in the CD18 gene contained a point mutation of adenine to guanine, which changes the function of the CD18 gene so that a genetic disorder known as BLAD appears. The DNA sequences in the BLAD gene based on the primer used and the T*aq*1 restriction sites shown on Figure 1.

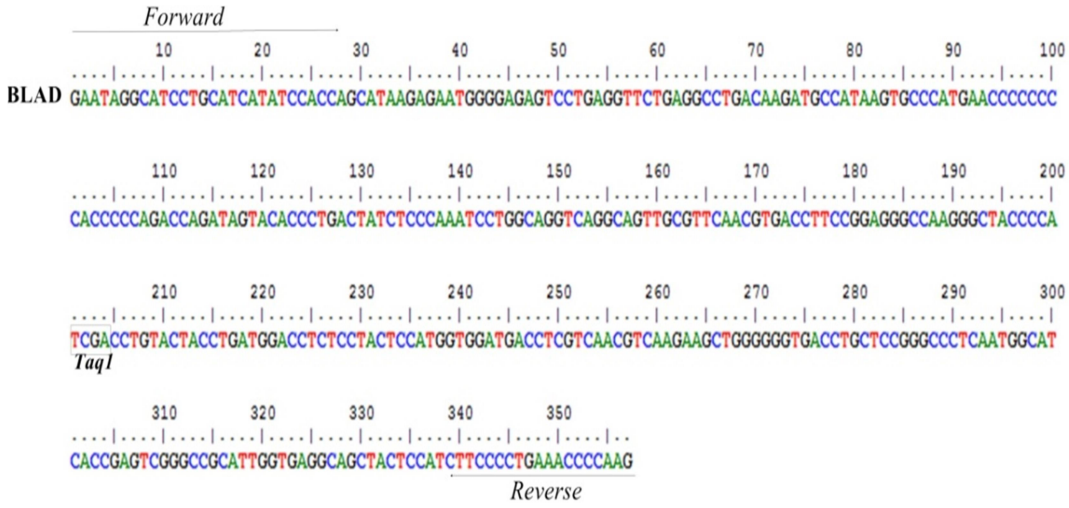

Figure 1. BLAD gene DNA sequences and the T*aq*1 enzyme restriction site.

The genotype of the BLAD gene was obtained by measuring the length of the BLAD gene fragment from the cut with the T*aq*1 enzyme. The visualization results show that the length of the fragments obtained is 357, 201 and 156 bp. The genotype found in dairy cattle can be seen in Figure 2.

The results show that each genotype can be distinguished based on the number of DNA bands that appear. Normal BLAD homozygous (+/+) were produced two fragments (156 and 201 bp), while heterozygous or BLAD carriers (+/-) with three fragments (156, 201 and 357 bp). This condition is in accordance with the research conducted by [10], who reported that the PCR product of BLAD gene which was restricted by T*aq*1 enzyme would distinguish normal alleles with two fragments (156 and 201 bp). Whereas BLAD carriers will produce three fragments 156, 201 and 357 bp.

Based on Table 2, it is known that the frequency of recessive BLAD genes in Enrekang Regency is very low (0.006), and is only found in Cendana district. Although only in a very small percentage, the presence of this recessive allele needs to be watched carefully considering the largest population of dairy cows in Enrekang Regency is in Cendana district. The possibility of the spread of this recessive alele comes from the origin of these dairy cattle which are mostly imported from Java, and the possibility of spread through artificial insemination. As is known the proportion of recessive alleles in Java is quite large as reported by [14], that the recessive BLAD frequency in dairy cows in Baturaden (Central Java) and Lembang (West Java) were 2.4 %.

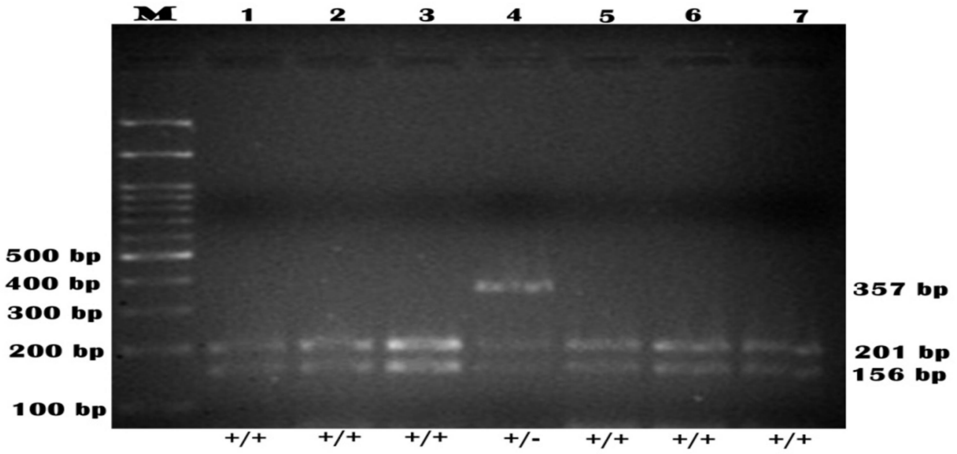

Figure 2. Visualization of BLAD gene PCR-RFLP, M: marker DNA ladder 100 bp, Line 1, 2, 3, 5, 6, 7 were normal BLAD homozygous (+/+), and Line 4 was heterozygous carrier (+/-) .

The results of genotype and allele frequencies analysis in BLAD gene from two dairy populations can be seen in Table 2.

Table 2. Alleles and genotype frequencies of BLAD gene

Dairy Population	Genotype Frequency		Allele Frequency	
	+/+ (Normal)	+/- (Carrier)	+	-
Cendana	0.98	0.02	0.99	0.01
Angeraja	1	0	1	0
Total	0.987	0.013	0.994	0.006

The frequency of heterozygous dairy cattle (BLAD carriers) in some countries is quite high. [3] reported that in Turkey FH dairy cows found 8.4% heterozygous BLAD, whereas [10] reported that 3.5%. While in India (3.23%) [2], Iran (3.33%) [4] and Jepang (8.79%) [5].

BLAD is a genetic disorder that affects the low level of productivity of dairy cows that can cause premature death. Therefore, it is necessary to control the spread of BLAD cases by identifying heterozygous dairy cows (BLAD carriers). This is in accordance with research conducted by [15] that BLAD can cause large losses, namely if there is a mating between individual carriers who have 25% chance of having a premature death. According to [14] that in a program to reduce the negative effects of BLAD, what can be done is to identify heterozygous cows and prevent them from mating with a heterozygous parent.

4. Conclusion

There is a relatively small proportion of BLAD recessive alleles in Enrekang regency (0.006). Although in a small percentage, the existence of this allele needs to be watched out because it is in the center of dairy cattle development in Cendana district.

5. Acknowledgment

This research was supported by Institute of Research and Community Service of Hasanuddin University through the Internal Competitive Research Grant (Hibah Kompetitif Internal BOPTN 2015). We would also like to show our gratitude to the Enrekang dairy farmers in Cendana and Anggeraja district for cooperation in the implementation of this research.

References

[1] Shuster D E, Kehrli M E, Ackermann M R and Gilbert R O 1992 *Proc. Natl. Acad. Sci. USA.* **89** p 9225 - 9229
[2] Patel R K, Singh K M, Soni K J and Rao K R 2007 *J. Appl. Genet.* **48** p 153 - 155
[3] Akyus B and Ertugrul O 2006 *Acta Vet. Hung* **54** p 173 - 178
[4] Norouzy A, Nassiry M R, Shahrody F E, Javadmanesh A, Abadi M R M and Sulimova G E 2005 *Genetika* **41** p 1697 - 1701
[5] Nagahata H, Miura T, Tagaki K, Ohtake M, Noda H, Yasuda T and Nioka K 1997 *J. Vet. Med. Sci.* **59** p 233 - 238
[6] Vatasescu-Balcan R, Georgescu E S, Adina M M, Mariana R, Anca D and Marieta C 2006 *Roumanian Biotechnological Letters.* **11** p 2881 - 2884
[7] Nasreen F, Malik N A, Riaz M N and Qureshi J A 2009 *Hereditas*. **146** p 74 - 78
[8] Citek J, Rehout V, Hajkova J, and Pahkova J 2006 *Veterinarni Medicina* **51** p 333 - 339
[9] Baba S, Muktiani A, Ako A and Dagong M I A 2011 *Media Peternakan* **34** p 146 - 154
[10] Meydan H, Yidiz M A and Agerholm J S 2010 *Acta Veterinaria Scandinavica.* **52**:56
[11] Yeh F C, Yang R C and Boyle T 1999 POPGENE version 1.31 : Microsoft Window-based freeware for population genetic analysis. University of Alberta Canada. Edmonton, AB
[12] Meydan H, Ugurlu M and Yildiz M A 2012 *Tarim Bilimleri Dergisi–Journal of Agricultural Sciences*. **18** p 239 -245
[13] Kriegesmann B, Jansen S, Baumgartner B G and Brenig B 1997 *J Dairy Sci.* **80** p 2547 - 2549
[14] Farajallah A, Sumantri C and Muttaqin W N 2007 *Identifikasi alel pembawa bovine leucocyte adhesion deficiency (BLAD) pada sapi perah Friesian Holstein di Indonesia.* Seminar Nasional Teknologi Pertanian dan Veteriner. Departemen Biologi FMIPA Institut Pertanian Bogor.
[15] Herodita L U 2009. *Identifikasi bovine leukocyte adhesion deficiency (BLAD) pada peternakan sapi Friesian-Holstein di Jawa - Bali.* Departemen Biologi Fakultas Matematika dan Ilmu Pengetahuan Alam, Institut Pertanian Bogor, Bogor.