Identification of Bovine Citrullinaemia (BC) disease-carrying alleles in Dairy cattle from Enrekang regency

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Abstract. Bovine Citrullinaemia (BC) disease is a genetic disorder that causes increased ammonia in the blood circulation and lethal. In order not to spread the disease, it is advisable to avoid the spread of Bovine Citrullinaemia (BC) disease-carrying alleles in the dairy cow population. This study aims to identify the distribution of allele carriers of Bovine Citrullinaemia (BC) in dairy cows in the Enrekang regency using PCR-RFLP method. A total of 80 DNA samples originating from the dairy development center in Enrekang (50 heads from Cendana and 30 heads from Angeraja district). DNA samples were amplified by PCR, PCR products were then cut using AvaII restriction enzymes. Identification of BC alleles carriers were calculated based on genotype and allele frequencies. These research found that about 0.6% of Bovine Citrullinaemia recessive allele frequencies in Enrekang and still relatively very low. Although the frequency of BC alleles identified is quite low (0.6), the spread of these alleles needs to be anticipated because they can influence the development of dairy cattle populations in the future. It is necessary to identify local cows in South Sulawesi to prevent the spread of BC genetic disorders in the population thereby reducing losses to farmers.

1. Introduction
The system used to increase the population of dairy cattle in Indonesia is generally carried out by a mating system with artificial insemination technology (AI). Artificial insemination is widely used in breeding dairy cows, but it also can possibly bring negative impacts such as the spread of genetic diseases such as genetic disorders that are lethal in the breeding population of dairy cows. One of the genetic disorders reported in many dairy cow populations is Bovine Citrullinaemia (BC) disease [1].

Bovine Citrullinaemia is a genetic disorder that causes an increase in ammonia in the blood circulation [2], this disease is caused by an error in the metabolic system that causes reduced activity of the urea cycle enzyme, namely the enzyme argininosuccinate synthase (ASS) [3]. This disease is lethal in FH dairy calves and causes FH calves to only be able to last for approximately 3 weeks [4]. So that this disease does not spread, it is advisable to avoid spreading alleles that carry Bovine Citrullinaemia (BC) in dairy cattle populations. One effort to avoid spreading these alleles is by identifying the Bovine Citrullinaemia (BC) carrier alleles in the dairy cow population in the development center area.

Several genetic disorders caused by genetics are reported to be quite high in the dairy cow population [5]. Specifically in Enrekang District, it has been reported the distribution of lethal alleles in dairy cattle populations such as BLAD [6] and FXID [7], so that the distribution of other alleles such as BC needs
to be identified how much the frequency of these lethal gene alleles in dairy cattle populations, especially in Enrekang. The population of dairy cows in Enrekang is one of the largest dairy development centers outside Java. At present the population of dairy cows in this area is recorded as 1,500 heads and generally the milk production produced is sold in the form of processed milk namely Dangke a type of soft cheese traditionally made [8].

This study aims to identify the distribution of alleles from Bovine Citrullinaemia (BC) in dairy cows in Enrekang district which decreases the genetic traits of offspring. By knowing the distribution of lethal alleles, selection can be done to reduce the proportion of alleles that have a negative impact on the productivity of dairy cows.

2. Methods

2.1. Samples collection
A total of 80 blood samples were collected from two regional development centers for dairy cattle in Enrekang District, 50 samples from Cendana sub-district and 30 samples from Anggeraja sub-district. The blood sample is then continued for DNA extraction using the Geneaid DNA extraction kit by following the extraction instructions provided.

2.2. BC alleles identification
In order to detect BC alleles a pair of primers amplifying the BC gene with nucleotide base sequences as follows forward 5’-GGCCAGGGACCGTGTTCATTGAGGACATC-3’ and reverse 5’-TTTCCTGGGACCCCGTGACACATACTTG-3’ [4] are used to multiply the target DNA. DNA templates from dairy cow samples were then PCR with an anning temperature at 57°C using the standard PCR protocol. The PCR product is then cut using the AvaII restriction enzyme to determine the BC genotype or allele.

2.3. Data analysis
Data in the form of allele frequencies and genotype frequencies were calculated using Popgene software [9].

3. Results and discussion

3.1. Amplification of the Bovine Citrullinaemia gene
Throughout 198 bp the Bovine Citrullinaemia gene target in the exon 5 region was successfully amplified in this study. The results of the BC gene amplification can be seen in figure 1 as follows:

![Figure 1. Bovine gene amplification results visualized on 1.5% Agarose gel, M: Marker (100 bp); 1-7: Dairy cow samples from Enrekang regency; bp: basepair.](image-url)
The length of the Bovine Citrullinaemia exon 5 gene fragment in the resulting study is 198 bp, the length of the product is in accordance with previous studies reported by [4] and [10] that the length of the PCR product for the Bovine Citrullinaemia gene is 198 bp. While those produced by [1,11,12] with amplicon length are 185 bp, while [13,14] amplify the BC gene with a target length of 177 bp. The result of gene amplification at the PCR stage is to find out the length of the gene fragment to be investigated but it is not yet known whether the PCR product carries a normal, heterozygous or recessive allele, so it is necessary to proceed to the RFLP stage, the genotyping stage by using restriction enzymes to identify alleles in the sample studied.

3.2. Identification of Bovine Citrullinaemia gene by PCR-RFLP method

Determination of the genotype of the Bovine Citrullinaemia exon 5 gene in dairy cows in this study using the PCR-RFLP method using the AvaII restriction enzyme. The AvaII enzyme recognizes the cutting site (TGA). Visualization results using 8% polyacrylamide gel by looking at the length of the Bovine Citrullinaemia gene fragment resulting from enzyme cutting showed that the fragments obtained were 198 bp, 109 bp, 89 bp. The genotype that has been identified can be seen in figure 2.

![Figure 2. Visualization of PCR-RFLP Bovine Citrullinaemia gene, M: marker 100 bp, 1, 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13 genotype homozygous (Normal BC) ++/+ , and line 8 genotip heterozygous (BC carrier) +/- .](image)

The genotype obtained was differentiated based on the number of bands that appeared in 8% polyacrylamide gel, namely homozygous ++/+ genotype produced by two fragments (89 bp and 109 bp) and heterozygous +/- genotype produced three fragments (89 bp, 109 bp and 198 bp). This is consistent with studies [4] and [10] that the results of PCR products of BC genes that have been cut using the AvaII enzyme produce two fragments namely 89 bp and 109 bp for normal alleles and the career ones produce three fragments namely 89 bp, 109 bp and 198 bp.

Normal alleles in BC genes that are cut with the AvaII enzyme and visualized using polyacrylamide gel or agarose gel produce two fragments, this indicates that the DNA sequence in the allele is normal because no mutation points are found. Heterozygous genotypes that carry normal alleles and bovine citrullinaemia recessive alleles will produce three fragments, whereas the recessive homozygous genotype only produces one fragment because a mutation point is found that changes the composition of DNA so that it is not recognized by the AvaII enzyme. According to [4,15] the point of the BC mutation is a change from C (cytosine) to T (thymine) so that the formation of the CGA codon that encodes arginine changes to a stop codon (TGA) in codon 86 that encodes the argininosuccinate synthetase (ASS) gene causing an urea cycle disorder.
3.3. Genotype and allele frequencies

The results of the analysis of genotype and allele frequencies in BC gene fragments in dairy cows can be seen in table 2.

| Dairy Population          | Genotype Freq. | Allele Freq. |
|---------------------------|----------------|--------------|
|                           | +/+            | +/−          | +            | −             |
| Cendana subdistrict       | 100            | 0            | 100          | 0             |
| Anggeraja subdistrict     | 96.7           | 3.3          | 98.3         | 1.7           |
| Total                     | 98.75          | 1.25         | 99.4         | 0.6           |

Based on the data in table 2 it can be seen that the frequency of homozygous +/+ alleles in the Cendana district is 100% and heterozygous alleles are not found. In Anggeraja sub-district the frequency of homozygous +/+ alleles was 98.3% and heterozygous alleles +/− 1.7% were found because 1 heterozygous sample was found in the population that was detected by visualizing the results of RFLP PCR on polyacrylamide gel. The total normal +/+ allele frequency is 99.4% and the heterozygous +/− frequency is 0.6%.

The number of allele frequencies in this study is different from previous studies by Perwitasari et al [1] who found heterozygous (carrier) allele frequencies in smallholder farms in Indonesia by 0.14%, while [16] found heterozygous allele frequencies in Shandong province of China which were quite high at 1.55%, whereas Li et al [14] only gained 0.16%. In India it was reported by [17] that the frequency was also quite high at 1.67%. The highest allele frequency was found in Australia as much as 50% of Australian FH cows and 30% of males at AI centers, this is because most dairy cattle breeds in Australia are derived from the Linmack Kriss King (LMKK) [18]. In the USA and Germany it was reported by [4] that the frequency of citrullinaemia was still very low, whereas in Turkey and Iran no heterozygous alleles were found in the population [18,19].

Although the results of this study indicate that the frequency of heterozygous BC alleles in the population appears to be very low, if there is no follow-up to eliminate this trait, the mutant alleles will spread to their offspring. As revealed by [19] normal cows mated with heterozygous cows will produce 50% homozygous and 50% heterozygous offspring, when heterozygous cows are mated with heterozygotes, then the potential for offspring is 25% normal, 50% heterozygous and 25% lethal.

4. Conclusion

It was found 0.6% Bovine Citrullinaemia heterozygous allele frequency in Enrekang regency and was still classified as very low. It is necessary to identify local cows in South Sulawesi to prevent the spread of BC genetic disorders in the population, thereby reducing losses to farmers.

Aknowledgements

The author would like to thank you very much for the assistance and cooperation provided by the dairy cattle groups in the district of Cendana and in the district of Enrekang.

References

[1] Perwitasari D, Anggraeni A Tiesnamurti B Khabibah N and Mahfud K 2009 Identifikasi molecular beberapa kelainan genetik pada sapi perah (Bogor: Institut Pertanian Bogor)
[2] Healy P J, Harper P A and Dennis J A 1990 Bovine citrullinaemia: a clinical, pathological, biochemical and genetic study. *Aust. Vet. J.* 67 255–8
[3] Şahin E, Karsli T Galiç A and Balcioğlu M Ş 2013 Identification of bovine leukocyte adhesion deficiency (BLAD) and bovine citrullinaemia (BC) alleles in Holstein cows reared in Antalya region *J. Appl. Anim. Res.* 41 56–60
[4] Grupe S, Dietl G and Schwerin M 1996 Population survey of citrullinemia on German Holsteins Livest. Prod. Sci. 45 35–8
[5] Siswanti S W, Sumantri C and Jakarta 2014 Detection of factor XI deficiency (FXID) and complex vertebral malformation (CVM) in Bali cattle Media Peternak. 37 143–50
[6] Dagong M I A, Rahim L Aprilita Bugiwati R R S R and Nurmulyaningsih 2018 Allele frequency estimation of BLAD (Bovine Leukocyte Adhesion Deficiency) in dairy cattle in Enrekang regency South Sulawesi Indonesia IOP Conf. Ser. Earth Environ. Sci. 207 012031
[7] Mutmainnah, Rahim L Bugiwati S R A and Dagong M I A 2019 Factor XI Deficiency (FXID) alleles distribution in dairy cow population in Enrekang regency, South Sulawesi IOP Conf. Ser. Earth Environ. Sci. 343 012036
[8] Baba S, Muktiyan A, Ako A and Dagong M I A 2011 Variety and need of feed technology of small-scale dairy farmer in Enrekang regency Media Peternak. 34 146–54
[9] Yeh C F, Yang C R and Boyle T 1999 POPGENE version 1.31 : Microsoft Window-based freeware for population genetic analysis (Edmonton: University of Alberta Canada)
[10] Meydan H, Yildiz M A and Agerholm J S 2010 Screening for bovine leukocyte adhesion deficiency, deficiency of uridine monophosphate synthase, complex vertebral malformation, bovine citrullinemia, and factor XI deficiency in Holstein cows reared in Turkey Acta Vet. Scand. 52
[11] Kotikalapudi R, Patel R K Kushwah R S and Sunkara P S S 2014 Identification of citrullinemia carrier and detection of a new silent mutation at 240bp position in Ass1 gene of normal holstein cattle Genetika 46 515–20
[12] Oner Y, Keskina A and Elmaci C 2010 Identification of BLAD, DUMPS, citrullinemia and factor XI deficiency in Holstein cattle in Turkey Asian J. Anim. Vet. Adv. 5 60–5
[13] Vatasescu R, Georgescu S E Kevorkian S Manea M A Rebedea M Dinischiotu A Tesio C D and Costache M 2006 Citrullinemia diagnostication on cattle breed Zoot. Bioteh 39 127–30
[14] Li J, Wang H Zhang Y Hou M Zhang J and Zhang Y 2011 Identification of BLAD and citrullinemia in Chinese Holstein cattle Anim. Sci. Pap. Reports 29 37–42
[15] Patel R K, Singh K M Soni K J Chauhan J B and Sambasiva Rao K R S 2006 Lack of carriers of citrullinemia and DUMPS in Indian Holstein cattle J. Appl. Genet. 47 239–42
[16] Wang H, Li J Hou M Zhang X Liu W and Zhong J 2009 Development and application of PCR-RFLP for detecting bovine citrullinemia and deficiency of uridine monophosphate synthase. Chinese J. Vet. Sci. 29 661–4
[17] Robinson J L, Burns J L Magura C E and Shanks R D 1993 Low Incidence of Citrullinemia Carriers Among Dairy Cattle of the United States J. Dairy Sci. 76 853–8
[18] Meydan H, Ugurlu M and Yildiz M A 2012 Monitoring of BLAD, DUMPS, CVM, BC and FXID in Turkish Native Cattle Breeds Tarım Bilim. Dergisi-Journal Agric. Sci. 18 239–45
[19] Cyrus E, Cyrus A Naser E K Mohammad C Jamal F and Hamid R S 2012 Study of citrullinemia disorder in Khuzestan Holstein cattle population of Iran African J. Biotechnol. 11 2587–90