Factor XI Deficiency (FXID) alleles distribution in dairy cow population in Enrekang regency, South Sulawesi

Mutmainnah¹, L Rahim², S R A Bugiwati² and M I A Dagong³,⁴
¹Animal Husbandry Study Program, Faculty of Animal Science, Universitas Hasanuddin, Makassar
²Animal Breeding and Genetic Laboratory, Faculty of Animal Science, Universitas Hasanuddin, Makassar
³Integrated Biotechnology Laboratory, Faculty of Animal Science, Universitas Hasanuddin, Makassar
E-mail: ihsandagong@gmail.com

Abstract. Factor XI Deficiency (FXID) is a genetic defect due to an autosomal recessive allele in dairy cows, thus cause a large economic and productivity loss in livestock. The purpose of this study was to identify the distribution of recessive alleles of FXID in dairy cows in Enrekang regency using the PCR method. A total of 80 DNA samples were isolated from the FH dairy cow blood sample collected from the Enrekang dairy farms (50 samples from Cendana district and 30 samples from Anggeraja District). All DNA samples were amplified by the PCR technique. The identification of the FXID carrier allele was calculated based on the genotype and allele frequencies. Results showed that 1 of 50 samples from Cendana district was FXID heterozygous while none of 30 samples from Anggeraja district showed FXID alleles. This study concluded that there was a normal heterozygous (Ff) FXID carrier in dairy cow population in Enrekang Regency with the allele frequency was 1.25%.

1. Introduction
Dairy cows in Indonesia are generally farmed by the community both in large and small scale farm. Those livestock businesses are potential to be developed. One important aspect that should be concerned in cattle breeding is free of genetic defects. According to [1], genetic defects are highly undesirable in a breeding program since it may cause a decrease in production and reproduction, anatomy abnormality, and in some cases cause mortality if the animals have a lethal mutation gene.

One genetic defect detected in dairy cows is Factor XI Deficiency (FXID) which is a genetic defect due to autosomal recessive alleles. FXID was first identified in humans and dairy cows in Ohio, USA. According to [2,3], this genetic defect has spread to several countries due to the use of FXID allele-carrying males in artificial insemination programs such as in the United States [4], Japan [5], The Czech Republic [6], India [7], Turkey [8], and Poland [9]. Factor XI deficiency causes a disruption in the process of blood clotting. Cows affected by this condition also experience difficulty in breeding, becoming more susceptible to diseases such as pneumonia and mastitis, the occurrence of repeated mating frequencies, and smaller follicular diameters [10].
In animal husbandry studies, FXID causes huge economic and productivity losses in livestock. The production and distribution of frozen semen of Friesian-Holstein (FH) dairy cows which are commonly used in artificial insemination programs in Enrekang Regency came from males imported from several countries. Thus, the possibility of spread of FXID genetic defects can occur in dairy farms in Enrekang Regency. According to [11], the number of incidental diseases and calf deaths were still quite high in Enrekang Regency. The case of calf death is caused by several factors, such as management, feed (nutrition), disease, and genetic defects. There are several genetic defects found in dairy cows such as BLAD, FXID, BC [1]. Specifically in the dairy cattle population in Enrekang, [12] reported recessive BLAD alleles in a fairly small frequency (0.006) which indicates the possibility of other recessive gene alleles in dairy cattle populations such as FXID. This finding causes the need for research to identify the FXID carrier alleles by using the molecular Polymerase Chain Reaction (PCR) technique in the dairy cattle population in Enrekang Regency. The study will provide information for FH cattle breeders and animal husbandry services in the selection program of the negative allele.

2. Methods

2.1. Samples collection and DNA isolation
A total of 80 whole blood samples were obtained from dairy farms in Enrekang Regency, i.e., 50 samples from Cendana District and 30 samples from Anggeraja District. The blood sample was collected aseptically from the jugular vein with a volume of 5 ml. The DNA then isolated using a DNA extraction kit according to the protocol provided.

2.2. FXID alleles identification
The identification of the FXID allele was done by amplification of DNA template using the primer flanking the FXID gene fragment in the forward 5'-CCCACTGGCTAGGAATCGTT-3' and reverse 5'-CAAGGCAATGTCATATCCAC-3' region [4]. The expected amplicons lengths were 320 bp (mutant FXID) and 244 bp (normal FXID).

The PCR total reaction mixture was 25 µl consisting of 1 µl (~ 100 ng) DNA template, 0.25 mM of each FXID primer, Mg$^{2+}$, 1× buffer, and 1 U/µl Taq polymerase. The PCR conditions were as follow: initial denaturation at 94°C for 4 minutes, 30 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 45 seconds, and extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes. The PCR product then electrophoresed and visualized using 1% agarose by UV transilluminator.

2.3. Statistical data analysis
Allele distribution data and genotype frequency were calculated using PopGene software [13].

3. Results and Discussion

3.1. FXID gene amplification
The FXID gene genotypes of dairy cow DNA samples were obtained by measuring the length of the FXID gene fragment using the PCR method. The amplification of the FXID gene in this study was successfully carried out with the annealing temperature of 55°C. Research conducted by [5,7,9] amplified the FXID gene of dairy cows with similar PCR condition. The annealing temperature is crucial since the optimal annealing temperature determines the attachment ability of the primers to obtain a specific band of the target gene [14]. The FXID primers used successfully amplified the DNA sample by producing fragment lengths of 244 bp and 320 bp. Visualization on agarose gel showed the amplicon of 244 bp and 320 bp. The genotype found in dairy cows was presented in figure 1.
Figure 1. The PCR Visualization of FXID gene, M: marker 100 bp, column 1, 2, 3, 5, 6, and 7 were homozygous genotype (normal FXID) (FF), and column 4 was heterozygous genotype (carrier FXID) (Ff).

Alleles with a fragment length of 244 bp are homozygous (normal) genotypes, whereas alleles with fragments of 244 bp and 320 bp are heterozygous genotypes (carrier FXID). The result was following research conducted by [4], that normal cows had 244 bp fragment lengths, FXID carrier cows had 244 bp and 320 bp fragment lengths, while mutant cows had 320 bp fragment lengths. Other research conducted by [5, 9] also produced 244 bp fragment lengths in normal dairy cows and 244 bp and 320 bp in FXID carrier dairy cows.

According to [15], FXID in recessive homozygous cows could cause abortion, the calves died after 48 hours and had severe bleeding in the lungs after parturition. The homozygous calves experienced bleeding in the brain and spine. In this study, there was no recessive homozygous genotype because the DNA samples were obtained from live (normal and carrier) cows.

3.2. Distribution of genotype and allele frequency of FXID gene

The results of the analysis of genotype and allele frequencies in the FXID gene fragment in dairy cows were presented in Table 1.

Table 1. FXID allele and genotype frequencies

| No. | Location (n)       | Genotype Frequency | Allele Frequency (%) |
|-----|--------------------|--------------------|----------------------|
|     |                    | FF  | Ff  | F  | f  |
| 1.  | Cendana district   | 49  | 1   | 0.99 | 0.01 |
| 2.  | Anggeraja district | 30  | 0   | 1   | 0   |
|     | Total              | 79  | 1   | 0.994 | 0.006 |
Table 1 showed that out of 80 DNA samples analyzed, only one sample was individual FXID carrier (the genotype Ff = 1.25%; the frequency of the FXID carrier allele (f) = 0.625%). The results obtained differed from the study by [16] that out of the 676 samples examined, five were FXID carriers (FXID carrier frequencies (Ff) genotype were 0.74% and FXID carrier allele frequencies (f) were 0.004%. Research by [8] reported that the frequency of FXID-carrying alleles in Holstein cattle in Turkey was 0.9%. The results obtained in this study were different because the number of samples examined was smaller than the number of samples studied by [1,8,16]. The FXID carrier events in FH cattle have been reported in several countries with different frequencies, as shown in table 2.

**Table 2.** Research data and identification of FXID genetic defects in several countries.

| Countries                  | Sample size | FXID carrier frequency | References |
|----------------------------|-------------|------------------------|------------|
| US                         | 419         | 5                      | 1.20%      | [4]        |
| Japan                      | 40          | 1                      | 2.50%      | [5]        |
| India                      | 330         | 2                      | 0.60%      | [7]        |
| Czech republic             | 279         | 1                      | 0.36%      | [6]        |
| Poland                     | 103         | 3                      | 2.91%      | [9]        |
| Turkey                     | 350         | 4                      | 1.20%      | [1]        |
| Indonesia (Enrekang Regency)| 80          | 1                      | 1.25%      | This study |

Table 2 showed that the FXID genetic defects of dairy cows in several countries occur in a very small frequency, which ranges from 0.36 to 2.91%. The highest incidence of FXID in Poland was caused by a superior male, detected to had FXID, which had produced 8,960 doses of semen and had been inseminated to 1,576 females [9].

FXID genetic defects in dairy cows are very important to be identified. Bulls which used to produce frozen semen must be free of genetic defects because the frozen semen will be used for artificial insemination to productive cows throughout Indonesia. Also, cattle detected carrying FXID (carrier) alleles should be removed from the population to avoid spreading FXID alleles.

### 3.3 DNA sequence analysis of FXID gene

The DNA sequencing was carried out to detect the presence of FXID recessive alleles in dairy cows. In this study, of all samples analyzed, one individual carrier was obtained. In the mutant FXID allele, nucleotide insertion was found in the DNA sequences (figure 2).

**Figure 2.** The DNA sequences alignment of FXID normal and mutant alleles
FXID occurs because of the insertion of 76 nucleotide bases in exon 12, whereas in normal cows there was no nucleotide base insertion in the DNA sequences (figure 2). A study conducted in the US reported that the FXID in dairy cows occurred due to mutations in the exon 12 Factor XI gene on chromosome 27 with the insertion of 76 nucleotide bases [4]. This insertion consists of imperfect polyadenine sequences followed by repeating 14 base pairs in the normal sequence at the end of insertion in FXID individuals (GAA ATA ATA ATT CA). Another study also reported sequencing results for FXID carrier alleles in dairy cows found the insertion of 76 bases pairs containing polyadenine sequences with stop codons (TAA) [8].

4. Conclusion
In the population of dairy cows in Enrekang, heterozygous (FXID carrier) dairy cows were found in normal phenotype conditions with the allele frequency was 1.25%. The FXID carrier allele identification technique can be used to prevent the spread of genetic defects in a farm. Cattle identified as FXID alleles carrier should be excluded from the population.

5. Acknowledgment
This research was supported by LPPM Universitas Hasanuddin through an internal competitive grant in 2015. The authors also thanked for the support and facilities provided by the group of dairy farmers and field technical personnel of the Enrekang Livestock and Fisheries Service.

References
[1] 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 citrullinaemia, and factor XI deficiency in Holstein cows reared in Turkey Acta Vet. Scand. 52 56–63
[2] Gentry P A 1984 The relationship between factor XI coagulant and factor XI antigenic activity in cattle. Can. J. Comp. Med. 48 58–62
[3] Perwitasari D, Anggraeni A, Tiesnamurti B, Khabibah N and Mahfud K 2009 Identifikasi Molekular Beberapa Kelainan Genetik pada Sapi Perah Laporan Kerjasama Kemitraan Penelitian Pertanian dengan Perguruan Tinggi (KKP3T) (Bogor: Institut Pertanian Bogor)
[4] Marron B M, Robinson J L, Gentry P A and Beever J E 2004 Identification of a mutation associated with factor XI deficiency in Holstein cattle Anim. Genet. 35 454–6
[5] Ghanem M E, Nishibori M, Nakao T, Nakatani K and Akita M 2005 Factor XI deficiency in a Holstein cow with repeat breeding in Japan J. Vet. Med. Sci. 67 713–5
[6] Citek J, Rehout V, Hajkova J and Pavkova J 2006 Monitoring of the genetic health of cattle in the Czech Republic Vet Med 51 333–9
[7] Patel R K, Soni K J, Chauhan J B, Singh K M and Sambasiva Rao K R S 2007 Factor XI deficiency in Indian Bos taurus, Bos indicus, Bos taurus x Bos indicus crossbreds and Bubalus bubalis Genet. Mol. Biol. 30 580–3
[8] Meydan H, Yildiz M A, Özdil F, Gedik Y and Özboyaz C 2009 Identification of factor XI deficiency in Holstein cow in Turkey Acta Vet. Scand. 51 5
[9] Gurgul A, Rubiś D and Slota E 2009 Identification of carriers of the mutation causing coagulation factor XI deficiency in Polish Holstein-Friesian cattle J. Appl. Genet. 50 149–52
[10] Liptrap R M, Gentry P A, Ross M L and Cummings E 1995 Preliminary findings of altered follicular activity in Holstein cows with coagulation factor XI deficiency Vet. Res. Commun. 19 463–71
[11] Sirajuddin S N, Siregar H, Juanda B and Dharmawan A H 2011 Perbedaan kebijakan pemerintah daerah terkait usaha sapi perah di propinsi Sulawesi Selatan J. Aktual. 6 1–12
[12] Dagong M I A, Rahim L, Bugiwati R R S R A 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 12031
[13] Yeh F C, Yang R C and Boyle T 1999 *POPGENE Version 1.31. Microsoft Window-based Freeware for Population Genetic Analysis* (Edmonton, Canada: University of Alberta)

[14] Siswanti S W and Sumantri C 2014 Detection of Factor XI Deficiency (FXID) and Complex Vertebral Malformation (CVM) in Bali cattle *Media Peternak.* 37 143–50

[15] Gentry P A and Ross M L 1994 Coagulation factor XI deficiency in Holstein cattle: expression and distribution of factor XI activity. *Can. J. Vet. Res.* 58 242

[16] Mahfud K 2009 *Deteksi Dini Kelainan Genetik Complex Vertebral Malformation dan Factor XI Deficiency pada Sapi Perah Friesian-Holstein* (Bogor: Institut Pertanian Bogor)