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by S Baba

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Genetic polymorphism of MHC DRB3 (Major Histocompatibility Complex) of Bali cattle at Maiwa Breeding Center, South Sulawesi Indonesia

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Abstract. One of the obstacles in the development of Bali cattle is a common disease such as Jembrana, Brucellosis or Anthrax. Breeding programs for disease resistance are an effort to produce superior livestock resistant to disease. The Major Histocompatibility Complex (MHC) is a group of loci that consists of a collection of important (major) genes that control the immune response. It is known that alleles variations of the MHC genes have a relationship with natural immunity to several diseases in cattle. The aim of this study was to identify the diversity of MHC genes in the Bali cattle population at the Maiwa Breeding Center (MBC). A total of 629 heads of Bali cattle were used as samples in this study. The MHC DRB3 gene was amplified with primers flanking the region of exon 2 using a PCR machine. The product of amplification is 284 bp in length. Genotyping and identification of the MHC alleles were carried out by the PCR-RFLP method using *Pst*I restriction enzymes. The results showed that the alleles variation of the MHC gene showed that P allele frequency were 0.856 and 0.938, while the p allele were 0.145 and 0.062 in Barru and Enrekang population, respectively. Observed heterozygosity values obtained in 0.144 showed that the genetic diversity of the MHC genes in the Bali cattle population at MBC was low. The chi-square values ($p < 0.05$) showed that the MHC gene of the Bali cattle at MBC was not in the Hardy Weinberg equilibrium and indicated the possibility of a strong selection of the MHC gene. MHC gene diversity data can be used as one of the references to develop Bali cattle at the MBC.

1. Introduction

Indonesia is a country with high biodiversity, namely Bali cattle. As an Indonesian native cattle, Bali cattle have spread throughout the Indonesian territory and are kept generally on smallholder farms. Bali cattle have high adaptability to harsh environments, however, these cattle have a susceptibility to several types of diseases, including Jembrana, Anthrax, and Brucellosis. These diseases, which often infect Bali cattle in small farmers including breeding centers managed in partnership with communities such as the Maiwa Breeding Center, in Barru and Enrekang regency in South Sulawesi province, Indonesia.



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The effort to improve the genetic quality of Bali cattle is conducting selection to produce superior Bali cattle stock seeds that are resistant to disease. Genotypic detection of bovine lymphocyte antigens is one way to reveal problems with the immunogenetic concept. The genetic approach concept controlling the difference in reactivity of the immune response and the susceptibility of the body to disease. Lymphocyte antigen in cattle as Major Histocompatibility Complex (MHC) or BoLA (Bovine Lymphocyte Antigen), is one of the surface antigens found in nucleated cells, especially lymphocyte cells, which were more immunogenic compared to other surface antigens. MHC is a glycoprotein consisting of a collection of important (major) genes, which were divided into three classes, namely MHC class I, class II and class III [1, 2]. MHC in each species has a high polymorphism.

The polymorphism of the MHC gene can cause changes in genetic characters that are different from their parents. Genetic diversity in a Bali cattle population will result differences immune activity each individual in a population. This will affects the resistance and susceptibility of individuals to disease. The purpose of this study was to identify the diversity of MHC gene alleles in the Bali cattle population in the Maiwa Breeding Center, through the characterization of the MHC gene it is expected to be one of the information that can provide an overview of the genetic quality of Bali cattle produced in a breeding program managed by Maiwa Breeding Center (MBC).

2. Method

2.1. Blood samples collection and DNA extraction

A total of 629 Bali cattle from MBC part farmers spread across two regencies (Barru = 475 heads and Enrekang = 154 heads) were sampled in this study. Blood samples of those cattle were collected from the jugular vein using vacuteiner plus EDTA tube contain which was then stored at -20 °C before DNA extraction. DNA was extracted using the Geneaid DNA extraction kit by following the protocol provided.

2.2. MHC allele identification

To identify MHC gene alleles, a pair of primers [3] was used to verify the ex 2 MHC gene segment. Prediction of the target length of an amplicon was 284 bp. PCR reactions were performed in 25 μ l aliquot containing 100 ng DNA templates, 0.25 mM forward and reverse primer, 150 μ M dNTP, 2.5 mM Mg²⁺ and 1 Unit of Taq DNA polymerase. The PCR protocol was used as follows: 2 minutes initial denaturation at 94 °C, then continued with 35 cycles of denaturation at 95 °C for 45 seconds, annealing temperature at 64 °C for 60 seconds and extension at 72 °C for 60 seconds. Then continued with a final extension at 72 °C for 5 minutes. PCR products then were visualized on 1.5% agarose gel. MHC alleles were identified with PCR-RFLP method. The PCR product were digested with PstI enzyme restriction in 7 μ l aliquots. Restriction fragments were subjected to electrophoresis in 1.5 % agarose gel with ethidium bromide in 1x TBE buffer. Alleles were identified based on the separation rate of the restriction DNA bands.

2.3. Statistical analysis

All genetic diversity data were analyzed by using Popgene32 software version 1.31 [4] to calculate allele frequencies, Hardy-Weinberg equilibrium, observed and expected heterozygosity.

3. Results and discussion

Based on the research, it was found that the MHC DRB3 exon 2 gene was successfully amplified using the SensoQuest Germany thermocycler machine, with annealing temperature of 64 °C. The results of gene segment amplification can be visualized in the 1.5% agarose gel presented in figure 1. The product length of the MHC gene amplification DRB3 exon 2 was 284 bp. This was consistent with the research produced by [5, 6] who reported the length of PCR products (284 bp) for MHC (BoLA). After DNA extraction process and amplification, it proceed to the genotyping stage using the

*Pst*I restriction enzyme. Based on the genotyping results, heterozygosity data included observed and expected heterozygosity, alleles and genotypes frequencies and H-W equilibrium for these genes in the population were obtained.

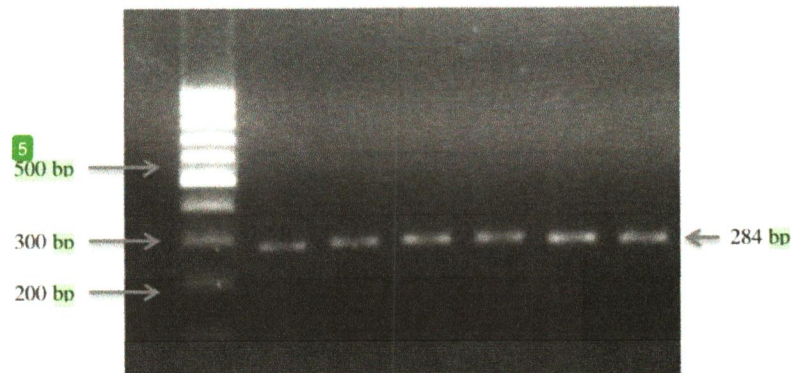


Figure 1. PCR amplification of MHC DRB3 gene exon 2 region in Bali cattle population

The results of the MHC RFLP gene are presented in figure 2. The PCR-RFLP result showed that two types of alleles were obtained with different fragment lengths, namely the P allele with a fragment length of 226, 44 and 15 bp, and p allele with 270 and 15 bp, respectively.

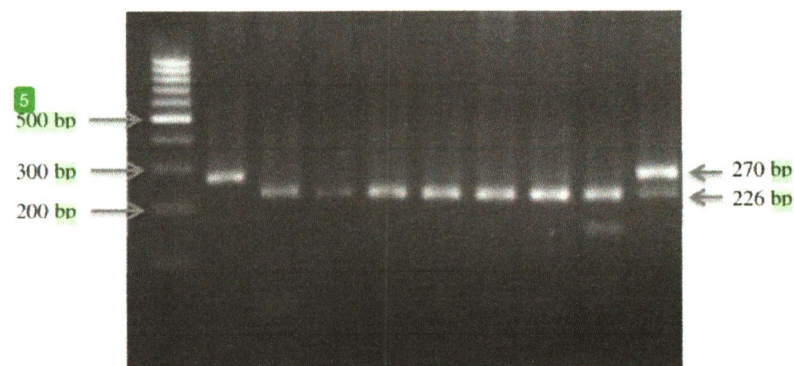


Figure 2. PCR-RFLP of MHC DRB3 exon 2 genotyping with *Pst*I enzyme restriction.

The results show that predominant MHC alleles found in Bali cattle populations at MBC were P alleles with frequency of 0.856 and 0.938 at the Barru and Enrekang population, respectively. These results was similar to the result reported by [7] who identified MHC gene variations in the Bali cattle population in South Sulawesi rural areas.

The observed and expected heterozygosity of MHC DRB3 gene in both population presented in table 2. Result showed that, The MHC gene heterozygosity in both populations were low, This indicated that the genetic diversity of the MHC gene, especially the locus of *Pst*I in the population of Bali cattle was low. [3] reported that the genetic diversity of the MHC gene at the *Hae*III locus in the Bali cattle population at South Sulawesi was quite high with the discovery of at least 5 alleles variation. MHC DRB3 locus is one of the most polymorphic locus in class II in cattle. Approximately 64 MHC DRB3 exon 2 alleles have been identified by the PCR-RFLP method [8, 9]. And by sequencing of cloned genomic DNA were identified approximately 89 alleles of MHC gene [10, 11, 12, 13, 14, 15, 16, 17].

Table 1. Allele and genotype frequencies of MHC DRB3 gene in Bali cattle at Maiwa Breeding Center.

MBC Bali Cattle Population (n)	Genotype Frequency			Allele Frequency	
	PP	Pp	pp	P	p
Barru Regency (475)	0.77	0.17	0.06	0.856	0.144
Enrekang Regency (154)	0.90	0.08	0.02	0.938	0.062

Note : n = number of samples (heads)

The chi-square values ($p < 0.05$) showed that the MHC gene in the Bali cattle population at Maiwa Breeding Center was not in the Hardy Weinberg equilibrium. Indicated that there is strong possibility selection of the MHC gene at Maiwa Breeding Center. MHC gene diversity data can be used as one of the references to develop Bali cattle at Maiwa Breeding Center.

Table 2. Observed and expected heterozygosity of MHC DRB3 gene in Bali cattle at Maiwa Breeding Center

MBC Bali Cattle Population (n)	Heterozygosity			(X^2)
	Obs (Ho)	Exp (He)	Average	
Barru Regency (475)	0.166	0.247	0.207	51.08
Enrekang Regency (154)	0.04	0.116	0.115	12.09

Note : n=number of samples (heads); Ho= observed heterozygosity; He= expected heterozygosity; X^2 =chi-square

4. Conclusion

The genetic diversity of the MHC gene of Bali cattle population at MBC is an important information that can be used as a reference in Bali cattle breeding programs for disease resistance.

5. Acknowledgement

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References

- [1] Davies C J, Andersson L, Ellis S A, Hensen E J, Lewin H A, Mikko S, Muggli-Cockett N E, Van der Poel J J and Russel G C 1997 *Anim. Genet.* **28** p 159-168
- [2] Bastos-Silveira C, Luis C, Ginja C, Gama L T and Oom MM 2008 *Anim. Genet.* **40** p 101-105.
- [3] Ningtiyas W D, Dagong M I A, Rahim L, Bugiwaty S R A and Ishak A B L 2014. Allelic variation of MHC DRB3 gene in Bali and crossbred cattle from South Sulawesi, Indonesia. Proceedings of the 16th AAAP Animal Science Congress Vol. II. Yogyakarta, 10-14 November

2014. Yogyakarta (Indonesia): Ministry of Agriculture, Indonesian Society of Animal Sciences, Gadjah Mada University. p. 97-100
- [4] Yeh F C, Yang R C and Boyle T 1999 *POPGENE version 1.31 : Microsoft Window-based Freeware for population genetic analysis*. (Edmonton, AB, Canada : University of Alberta Canada)
- [5] Wu X X, Yang Z P, Wang X L, Mao Y J, Li S C, Shi X K, Chen Y 2010 *J. Biomed. Sci. and Eng.* **3** p 221 – 225
- [6] Chu M X, Ye S C, Qiao L, Wang J X, Feng T, Huang D W, Cao G L, Di R, Fang L, Chen G H 2012 *Mol. Biol. Rep.* **39** p 2909 – 2914
- [7] Ishak A B L, Dagong M I A and Nurul P 2016. Polymorphisms of Bovine Lymphocyte Antigen (BoLA DRB 3.2 locus *Pst*I) gene in Bali cattle population from South Sulawesi Province. *Proc. Intsem LPVT* p. 19 – 23.
- [8] Van Eijk M J T, Stewart-Haynes J A and Lewin H A 1992 *Anim. Genet.* **23** p 483-496.
- [9] Gelhaus A, Schnittger L, Mehlitz D, Horstmann R D and Meyer C G 1995 *Anim. Genet.* **26** p 147-153
- [10] Ammer H, Schwaiger F W, Kammerbauer C, Gomolka M, Arriens A, Lazary S and Epplen J T 1992 *Immunogenetics* **35** p 332 – 340
- [11] Aida Y, Niimi M, Asahina M, Okada K, Nakai Y and Ogimoto K 1995 *Biochem. Biophys. Res. Commun.* **209** p 981-988
- [12] Russell G C, Davies C J, Andersson L, Ellis S A, Hensen E J, Lewin H A, Mikko S, Muggli-Cockett N E and van der Poel J J 1997 *Anim. Genet.* **28** p 169 – 180
- [13] Russell G C, Fraser D C, Craigmile S, Oliver R A, Dutia B M and Glass E J 2000 *Anim. Genet.* **31** p 219 – 222
- [14] Maillard J C, Renard C, Chardon P, Chantal I and Bensaid A 1999 *Anim. Genet.* **30** p 200 – 203
- [15] Maillard J C, Chantal I and Berthier D 2001 *Anim. Genet.* **32** p 44 – 46
- [16] Takeshima S, Ikegami M, Morita M, Nakai Y and Aida Y 2001 *Immunogenetics* **53** p 74 – 81
- [17] Takeshima S, Nakai Y, Ohta M and Aida Y 2002 *J. Dairy Sci.* **85** p 1630 – 1632

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"Characterization of bovine MHC DRB3 diversity in global cattle breeds, with a focus on cattle in Myanmar", Research Square, 2020
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7	krishikosh.egranth.ac.in Internet Source	% 1
8	www.researchgate.net Internet Source	% 1
9	Bhushan, Bharat, B. N. Patra, Chandan Paswan, Umang, Pushpendra Kumar, Triveni Dutt, P. J. Das, Arjava Sharma, and S. P.S. Ahlawat. "DNA Polymorphism of BoLA-DRB3.2 Gene in Tharparkar Cattle (<i>Bos indicus</i>) by PCR-RFLP", Journal of Applied Animal Research, 2007. Publication	% 1
10	www.lrrd.org Internet Source	% 1
11	www.ajol.info Internet Source	<% 1
12	S. A. Ledwidge. "Multi-primer target PCR for rapid identification of bovine DRB3 alleles", Animal Genetics, 8/7/2001 Publication	<% 1
13	F. D. Sheikh. "DRB3.2 gene polymorphism and its association with pashmina production in Changthangi goat", International Journal of Immunogenetics, 8/2006 Publication	<% 1

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