Genetic Marker Approach for Confirming the Existing Twinning Trait in PO Cattle*

Endang T. Margawati, Paskah P. Agung, & Muhamad Ridwan*

Research Center for Biotechnology, the Indonesian Institute of Sciences (LIPI)
Jl. Raya Bogor KM. 46, Cibinong 16911. INDONESIA
*Tel/Fax. +62-21-8754587/875-4588; *E-mail: endangtri@hotmail.com*

Abstract

Up to present, Indonesia still depends on importing of the beef cattle from overseas both for slaughtering and feedlot needs. The Ongole ascendant (PO) cattle is one of Indonesian beef cattle representatives that has adapted very long in Indonesia and has a potential source of meat production. Almost 1% PO cows in central Java delivered twin calves both by AI and nature fertilization. This study was designed to confirm the existence of genetic marker associating with twinning trait in PO cattle. Amount of 35 DNA samples were collected from PO cows with twin birth experiences and twin calves. Amount of 12 DNA control samples was also involved. Three pairs of primers for microsatellite genetic markers of BMS-1216; BM-321and RM103 were used for amplification of all DNA samples. PCR products were visualized through 8% ND PAGE. The result showed that all fragments with twinning experience were on right sizes of BMS1216 (143-165bp); BM-321 (108-126bp) and RM 103 (114-144bp) of the Bovine chromosome 5. Even though negative samples delivered not clear bands but there were still in the right sizes of applied microsatellites. Microsatellite marker is only an early clue that obtained fragments might be bearing the putative locus associating with twin trait. Therefore in the coming study it needs to confirm the existence of candidate gene(s) associating with twinning traits by using SNP (Single Nucleotide Polymorphism) markers.

Keywords: genetic marker, twinning trait, PO cattle, confirmation

Introduction

Traditional genetic selection in livestock has been conducting up to present to select important traits which have economic values. This manner has contributed many advantages in livestock development this present. However, this traditional selection manner has been considered as not an effective way in breeding program since this selection is time consuming and involves many persons to measures the performance of desired traits.

In the past 20 years ago, there was a great changing in livestock breeding from quantitative genetics shifting to molecular genetics with emphasized in the identification of quantitative trait loci (QTL) and marker-assisted selection (MAS). In that past, advances in molecular genetics have lead to either the identification of multiple genes or genetic markers or single gene associated with specific traits (Dekkers, 2004). In addition, those genetic markers could identify QTL or genomic regions that affect quantitative traits.

Genetic selection in cattle for some difficult traits or the trait with low heritability, such as reproduction, it has traditionally had very little success (Allan *et al*., 2009). The advances in DNA technologies, genetic selection of traits with low heritability could improve reproductive efficiency more rapidly in cattle. One important trait of reproductive traits is twinning birth in industry of cattle producers. This twinning trait is shown very small percentage in cattle since cattle is a uniporous cattle with a single delivered calf in each birth. As reported byKomisarek and Dorynek (2002), the accidence of twinning birth is smaller (about 1%) in beef cattle and more than 4% in dairy cattle then increased in more age in cows.

The QTL study associated with twinning trait was reported in chromosome 5 of bovine by using genome-wide linkage analysis(Lien *et al*., 2000).Microsatellite markers have been used in initial genome mapping (De Atley *et al*., 2008) and also used to identify the location of Quantitative Trait Location (QTL). Even though today it most efforts involved SNP (Single Nucleotide Polymorphism) marker in genome mapping. Microsatellites have been identified in both coding and non-coding regions of the genome and have been utilized to detect QTL (Sellner, *et al*., 2007).

Our observation of twinning birth in Ongole ascendant (PO) at one district of Grobogan regency in central Java, it was often occurred and delivered every year. Owners or stakeholders of twinning birth have fertilized their cows by using either bull (naturally) or AI (Artificial Insemination), (unreported data).Based on that information, this study was designed to confirm those cows whether the twinning trait affected genetically or by chance. All DNA samples of cows delivering twin birth were used for confirming the candidate gene (s) associated with twinning birth in PO cattle by application of microsatellite markers.

Materials and Methods

DNA Genome Samples

Samples of DNA were collected from fresh blood of 35 cows delivering twinning birth at some districts under Grobogan regency of Central Java Province in 2011. As 12 DNA control samples (PO without twin background) were also included. A modified method of high salt (Montgomery and Sise, 1990) was used to extract the DNA samples.

PCR Optimization and Amplification

In prior to DNA amplification, it was performed optimization of PCR in order to get suitable annealing temperatures of three pairs of microsatellite primers using a PCR gradient (Techne, UK). The work of PCR was conducted based on the suitable annealing temperatures. Three pairs of primers for microsatellite genetic markers were BMS-1216 (Accession No. G18633); BM-321 (Accession No. G18515) and RM103 (Accession No.U10391) drawn from NCBI, see Table 1. Those primers were used for amplification of all DNA samples. The PCR reagent consisted of 2 µl DNA template; 4 µl of 10 ρm primer; 2.5 µl of buffer for Taq polymerase; 2 µl of 25mM $MgCl_2$, 2 µl of 2.5 mM dNTP, 0.3 µl Taq Polymerase (1 unit per 1 µl) , and added dd-H_2O up to 25 µl of total volume. A program of PCR was performed as following procedure: 2 min at 94 ºC as initial denaturation, followed by 30 cycles of 30 sec at 94 ºC, 45 sec at 54/59 ºC, and 30 sec at 72 ºC, with a subsequent 5 min final extension at 72 ºC.

Table 1. Nucleotides of the Used Primers

Primer	F/R	Primer Sequens	Acc No.	Annealing (°C)
BMS1216	F	GCCTGCATGTGTCTGTGG	G18678	59
	R	TCTGTGTCGGAATACCCTCC		
RM103	F	TCTGTGCACTTTACATTTAACAGA	U10391	54
	R	GTGGTCTATTGAACTTTTGTTCAGA		
BM321	F	AAGGGTCAGACAAAACTTAGCA	G18515	54
	R	ATCCTTGCCCTAATTCTCATTC		

Sources: Ihara *et al.* (2004).

Visualization of Targeted Fragment

PCR products were visualized through 8% ND-PAGE (Non-Denaturing Poly-Acrylamide Gel Electrophoresis). Targeted fragments were detected by confirming the emerged bands with the 100bpDNA ladder. The right sizes of band performance

indicated the candidate locus of the candidate of desired gene encoding the twin birth trait.

Results and Discussion

Samples of DNA collected for this study were drawn from all cows delivering twin calves and some of twin calves. Total of DNA samples was 35 and stored at -20°C. Optimization of PCR for confirming the closely suitable annealing temperatures was conducted to all three primer pairs of chosen microsatellite genetic markers by using a gradient PCR. The obtained annealing temperatures were 54 °C (RM 103; BM 321) and 59 °C (BMS 1216).

Based on those annealing temperatures, each of DNA samples (35) were amplified with the mentioned above of PCR program and PCR reaction using 3 pairs of genetic microsatellite primers. Therefore, it was found 105 fragments bearing candidate locus that expected containing genes encoding twinning trait in PO cattle, see Figure 1.

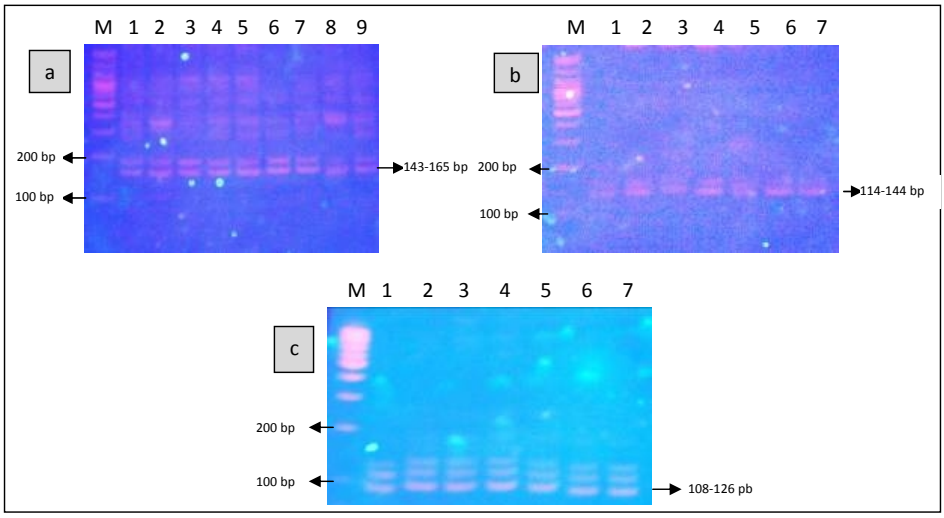

Figure 1. Amplification results on 8% ND-PAGE using primers of BMS 1216(a), RM 103 (b), BM 321 (c). M: 100bp DNALadder; No. 1 to 7; 1 to 9: DNAsamples of PO cattle.

Figure 1 showed representative fragments of the PO DNA twin samples. The results of DNA amplification showed that all fragments or bands were on right sizes of BMS1216 (143-165bp); BM-321 (108-126bp) and RM 103 (114-144bp) on the Bovine chromosome 5. This finding indicates that the all DNA samples might bear the putative locus of twinning trait that encoded by twinning gene(s). The whole fragments showed at the same pattern of monomorphyc double bands.

Therefore, allele frequency of the three microsatellite markers is a hundred percent of monomorphyc. In this study, there was not found specific allele(s) for twinning trait since the twinning trait is influence by multi genes (Komisarek and Dorynek, 2002). Twinning trait therefore could be associated with either high ovulation rate (Meuwissen *et al.*, 2002; Allan *et al*., 2009) or higher milk production (Fricke and Wiltbank, 1999; Wiltbank *et al*., 2000; Weller *et.al*., 2008;) or could be associated with growth trait.

As comparation, negative samples of PO DNA or without twinning birth experience were also amplified with those three microsatellites. However the result seemed to be a weak approval of either bearing or without bearing the putative locus of twinning traits (Figure 2). Compared to the twinning sample, 12 DNA samples without twinning background did not show a significant difference between twinning and negative (control) samples.

This finding is still a rough report as an initial clue that the whole DNA samples derived from all cows and calves showed as early proof for twinning birth traits. This finding might be supported by the source of collected DNA samples were derived from cows with twinning history in their live and twin calves.

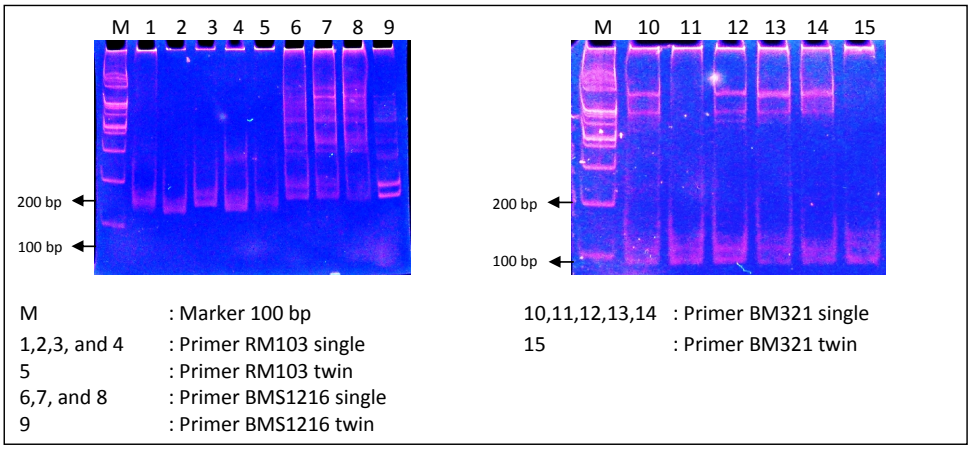

Figure 2. Negative (control) samples amplification results on 8% ND-PAGE using primers of BMS1216, RM103, and BM321.

Identified locus in this study was still a wider location of desired gene of twinning birth. The incidence of twinning birth is associated with the high of ovulation rates in cattle (Allan *et al*., 2009). Genomic scans for ovulation rates have been studied in selection of cattle population to increase the incidence of twinning rate (Kappes *et al*., 2000).Twinning trait is multiple genes that can be traced by implementation of molecular genetics of microsatellite. Advances in molecular genetics could identify multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or ge-

nomic regions that affect quantitative traits (Dekkers, 2004). In addition, this molecular genetics has advantage in enhancing the selection response of particular traits that are difficult to improve by conventional selection such as traits with a lower heritability or traits for which the measurement of phenotype is difficult, expensive and only possible late in life, or not possible on selection candidates.

Therefore, this result needs to be further studied with emphasizes on using SNP markers. Nowadays, most efforts involve a Single Nucleotide Polymorphism (SNP) marker. The SNP marker could detect mutation of by either insertion of deletion of single nucleotide.

Conclusion

Based on the applying of three microsatellite markers in the confirming of twinning traits in PO cattle it showed that all DNA samples emerged the right size of targeted fragments in the same pattern of monomorphyc bands. This is an early indication that the obtained fragments might be bearing putative locus encoding twinning trait. Further study in Single Nucleotide Polymorphism (SNP) might be necessary to confirm the existence of the twin gene(s) in PO cattle.

Acknowledgement

This research was funded by Thematic DIPA-LIPI grant 2011. Authors acknowledged to people who helped in blood collection and staff at the livestock services of Grobogan regency.

References

Allan, M. F., L.A. Kuehn, R. A. Cushman, W. M. Snelling, S. E. Echternkamp, and R. M. Thallman. 2009. Confirmation of quantitative trait loci using a low-density single nucleotide polymorphism map for twinning and ovulation rate on bovine chromosome 5. J.Anim.Sci. 87:46–56.

De Atley, K. L., G. Rincon, C. R. Farber, J. F. Medrano, R. M. Enns, G. A. Silver, M. G. Thomas. 2008. Association of microsatellite ETH10 genotypes with growth and carcass trait levels in Brangus cattle. Proceedings, Western Section, American Society of Animal Science Vol. 59: 69-71.

Dekkers, J.C.M. 2004. Commercial application of marker-and gene-assisted selection in livestock: strategies and lessons. J. Anim. Sci. 82(E. Suppl.):E313–E328.

Fricke, P. M. and M. C. Wiltbank. 1999. Effect of milk production on the incidence of double ovulation in dairy cows. Therio genology 52: 1133-1143

Ihara, N., A. Takasuga, K. Mizoshita, H. Takeda, M. Sugimoto, Y. Mizoguchi, T.

Hirano, T. Itoh, T. Watanabe, K.M. Reed, W.M. Snelling, S.M. Kappes, C.W. Beattie, G.L. Bennett and Y. Sugimoto. 2004. A Comprehensive genetic map of the cattle genome based on 3802 microsatellites. Genome Research 14: 1987-1998.

Kappes, S. M., G. L. Bennett, J. W. Keele, S. E. Echternkamp, K. E.Gregory, and R. M. Thallman. 2000. Initial results of genomic scans for ovulation rate in a cattle population selected for increased twinning rate. J. Anim. Sci. 78:3053–3059.

Komisarek, J. and Z. Dorynek. 2002. Genetics aspects of twinning in cattle. J. Appl. Genet. 43(1): 55-68.

Lien, S., A. Karlsen, G. Klemetsdal, D.I Vage and I. Olsaker. 2000. A Primary screen of the bovine genome for quantitative trait loci affecting twinning rate. Mamm. Genome 11: 877-882.

Meuwissen, T.H.E., A. Karlsen, S. Lien, I. Olsaker and M. E. Goddard. 2002. Fine mapping of a quantitative trait locus for twinning rate using combined linkage and linkage disequilibrium mapping. Genetics161: 373-379.

Montgomery, G.W and J. A. Sise. 1990. Extraction of DNA from sheep white blood cells. New Zealand J Agric.Res. 33: 437-441.

Sellner, E. M., J. W. Kim, M. C. Mc Clure, K. H. Taylor, R. D. Schnabel, and J. F. Taylor. 2007. Board invited review: Applications of genomic information in livestock. J. Anim. Sci. 85:3148-3158.

Weller, J.I., M. Golik, M. E. Seroussi, M. Ron and E. Ezra. 2008. Detection of Quantitative Trait Loci Affecting Twinning Rate in Israeli Holsteins by the Daughter Design. J. Dairy Sci. 91(6): 2469 - 2474

Wiltbank, M. C., P. M. Fricke, S.Songsritavong, R. Sartoi and O. J. Ginther.2000. Mechanisms that prevent and produce double ovulations in dairy cattle. J. Dairy Sci.83: 2998-3007.