Variation of Interleukin-8 (IL-8) Receptor Gene Based on Single Strand Conformation Polymorphism (SSCP) in Indonesian Friesian Holstein Dairy Cattle

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Abstract. Interleukin-8 (IL-8) receptor gene is response to bacterial invasion, endothelial and epithelial cells by release the chemokine interleukin-8 (IL-8). IL-8 mediates neutrophil function, allowing neutrophils to resolve bacterial infections by migrating through blood vessel walls and to the site of infection. IL-8 also impacts neutrophil killing and survival ability during the inflammatory response. Genes that associated with neutrophil function also has potency as a genetic marker for mastitis disease, the migration of neutrophil from blood to infections side is essentially to detect the mastitis pathogen in general. It has been known that mastitis disease was the major problem in dairy cattle industry since influencing the milk quality, especially in bacterial content. The aim of this research is to detect the variation of IL-8 receptor gene in Indonesian Frisien Holstein (FH) dairy cattle which keep by farmer at Pujon District of Malang Regency. DNA genom was extracted from whole blood cells by standart phenol-chloroform methods. PCR reaction was done by using genomic DNA as a template while primer was the set of nucleotide that flanking IL-8 receptor gene. Polymorphism analysis of IL-8 receptor gene was based on number and frequency of SSCP (*Single Strand Conformation Polymorphism*) pattern of PCR products. From research result showed that was found five patterns of SCCP from IL-8 receptor gene of Indonesian FH which came from Pujon District, Malang Regency. This is also illustrated that of IL-8 receptor gene of Indonesian FH dairy cattle was enough polymorphism. The number pattern of SSCP-1 and SSCP-2 were found higher than others. Also, those patterns less than 50 % of samples were suspected as sub clinical mastitis. It means that this preliminary study showed that SSCP-1 and SSCP-2 patterns could be as a candidate marker for mastitis resistance of Indonesian FH dairy cattle. While others patterns could be used in determination of IL-8 receptor gene polymorphism of Indonesian FH dairy cattle based on SCCP patterns. This is important because we need this data for completing the genetic variation of Indonesian cattle data base.

Key Words : IL-8 gene, mastitis, SSCP, chemokine, Friesian Holstein

Introduction

Mastitis is the one of dairy cattle disease that was attack about 60-90% of Indonesian dairy cattle. This disease caused high lost for dairy farmer since it has impact in milk production and quality. It was happen without unrealized by farmer and by unexpectedly dairy cattle was fall in worst health condition. Generally, antibiotic was applied to cure mastitis by *intra mammae*, but the using of antibiotic is difficult to control and not safety for health because control over the use of antibiotics is difficult. For the security interests of consumers of dairy products, hence the use of antibiotic is avoided (Nurdin and Mihrani, 2006).

The improvement of dairy cattle managements have been done for long ago in an effort to increase milk production. However, the genetic traits that associated with disease resistance are not addressed. The intensity of selection for production traits in dairy cows is also causing an increase in the incidence of diseases such as mastitis (Van Dorp et al., 1999), which is a very important disease in dairy cattle that resulting in a high prevalence and causes economic loss is also quite high (Owen et al., 2000). Losses due to mastitis include milk rejection, additional costs for health care and drugs, increase management costs and decreased in the quantity and quality of milk, also milk processing industry production decline (DeGraves and Fetrow,

1993). Approximately 75% of revenue was decreased as a result of sub clinical mastitis due to reduced milk production (Colleu and Le excess-Duval, 1995).

Mastitis can be one of the prominent factors that caused declining in quality of milk. This disease causes inflammation of the udder and the number of cases were occupy most in the entire world. Mastitis is different from other animal diseases in which several different types of bacteria that can infect the udder, where the symptoms that appear included inflammantory the udder. These pathogens invade the udder, and then multiply in the udder, and produce a toxic compound that causes inflammation of the udder. Also resulting in lower milk production and alter the quality of milk. Because mastitis is caused by multiple pathogens, it is quite difficult to handle, while the economic losses due to mastitis is big enough. In the United States estimated losses due to mastitis for dairy producers exposed to more than two million billion dollars per year. Thus, mastitis continues to be one or a limiting factor for the dairy industry in America and around the world (Oliver, 2009).

Dairy cattle enterprises on a small scale associated with the meaning of the provision of resources for poor farmers, in example education or family income for school children and fulfil other basic needs of family life. On the other hand, of dairy cattle in the suburbs is one of the different strategies in an effort to meet family needs, such as contributions to food security, incomes and family labor, savings and insurance (Guendel, 2001). However, increasing the productivity and sustainability in general is limited by several factors, including poor management, insufficient resources both in quantity and quality of feed, lack of genetic improvement of livestock, reproduction that is not maximal as well as animal health controls are lacking. Improve the genetic quality of livestock in one case a final effort in research and breeding, but the prominent thing that must be emphasized is the improvement of livestock management systems existing resources, through the achievement of the optimum balance between genotype and climatic conditions and management and feeding (Jonsson et al., 1993).

Transmitting mastitis pathogens generally caused by *Streptococcus agalactiae* and *Staphylococcus aureus*. *Mycoplasma bovis* and other Mycoplasma species reportedly found an increase in transmitting mastitis pathogens. The main source of organism transmission is infected udder and then transmitting mastitis pathogens spread from infected cattle to uninfected cattle, especially when milking. Some characteristics of female dairy cattle that has problem of transmitting mastitis including: (1) the prevalence of intramammary infection (IMI) high during the lactation period, (2) total number of Somatic Cell Count (TSCC) high, (3) the period of infection in a long time, and (4) the proportion is lower for infections that cause clinical mastitis (infection generally sub-clinical mastitis), and the prevalence of infection is low enough during the dry period (Oliver, 1988).

Several last researched identification that several areas of QTL (quantitative traits loci) associated with functional quantitative traits in beef cattle, including mastitis (Schwerin et al., 2003). QTLs found in most regions of chromosomes that are spread from 10 to 40 cM (Stella and Boettcher, 2004), including hundreds of thousands genes in it. The main objective of QTL analysis is identification of a gene that causes the expression of quantitative characters, including the properly QTL mapping that associated with mastitis, where it can be used as Marker Assisted Selection (MAS) that enables and facilitates the identification of genes and alleles for a trait of resistance gene (Reinard and Riollet 2005). A large number of genetic polymorphism across regions of genes or genetic character of which is associated with the nature of mastitis has been identified in some cattle. Through the latest molecular technologies such as DNA microarrays offer the possibility to observe several different studies of gene expression in simultaneous, including response to disease and also the resistance or susceptibility to mastitis (Silveri et al. 2006; Vanselow et al., 2006).

Genes associated with immune response in mammary gland is the potential for mastitis character. Several studies conducted have been able to identify gene polymorphisms in the gene complex histocompatability major complex (MHC), cytokine and cytokine

receptors and the natural resistance-associated macrophage protein 1 (NRAMP-1) (Dietz et al., 1997; Grosse et al., 1999; Ables et al., 2002). However, few studies have shown an association between MHC genes on DRB3.2 alleles *3, *11, *16 and *23 with occurrence of mastitis or immune function (Dietz et al., 1997; Kelm et al., 1997; Sharif et al., 1998 and 2000; Park et al., 2004).

Genes that associated with neutrophil function is also a potential for the genetic character of mastitis, as known to the migration of neutrophils from the blood into the area of infection is essential to detect most cases of mastitis pathogens (Paape et al., 2000). The ability of neutrophil migration into infected tissue areas depends on the introduction of inflammatory mediators by neutrophils cytokine, chemokine, and the complement receptor (Burvenich et al., 1994). Two chemokine receptors found on the surface of neutrophils, namely CXCR1 and CXCR2 is necessary to maximize the function of neutrophils during infection (Murphy and Tiffany, 1991). Introduction by CXCR1 and CXCR2 chemokine induces neutrophil activity, chemotaxis, and the incidence of these pathogens phagocytosis (Peveri et al., 1988; Podolin et al., 2002).

Some species are known to have two neutrophils CXCR1 and CXCR2 receptor for IL-8 gene, or also known as IL8 and IL8-Ra-Rb. (Holmes et al., 2001). The two receptors are very important in controlling the activation of neutrophils by chemokines; they can also be distinguished from the end of the chain 5', which refers to the end of the chain of N' terminal. (Catusse et al., 2002). CXCR1 showed high affinity binding to IL-8, whereas CXCR2 was not only binds to both IL-8 but also with others such as the derivates chemokine epithelial cells of the neutrophil attractant-78 and associated with cell growth Oncogene (Wuyts et al., 1998). Therefore, this receptor has the ability to bind to the same chemokine, the action of the two receptors are mutually supportive at the time of the response to inflammantory (swelling).

Interleukin-8 receptor gene (IL-8) is one candidate gene for resistance to mastitis. Interleukin-8 receptor is essential for the process of neutrophil migration into the mammary gland. Receptor gene locus IL-8 gene has been mapped and correlated with the loci that code for disease resistance genes (Blackwell et al., 2000; Grosse et al., 1999).

Materials and Methods

Collection of DNA Samples

DNA was extracted from total blood-cells which were collected from cattle as many as 10 ml per sample of 25 heads dairy cattle and preserved using EDTA 10%. The area where the samples came from dairy cattle farmers at Pujon Sub District, Malang Regency, East Java Province.

DNA Amplification and IL 8-SSCP Detection

DNA extraction using phenol-chloroform standard method (Sambrook et al., 1989) was then preserved in TE buffer. Locus of IL-8 receptor gene was amplified by oligonucleotide primers IL8Rec-SSCP Forward (5'-CCTCCGTGAGGCCTATCAAC-3'), and IL8Rec-SSCP Reverse (5'-AGGTCTCAGCAATCACATGG-3') with the 311-basepair product of PCR.

PCR reaction performed with total volume 25µl of the mixture solution consisting of Taq DNA polymerase and 10 X buffer Taq polymerase (100 mM Tris-Cl, pH 3.8, 500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin), dNTPs'S mix (dGTP, dATP, dTTP and dCTP) (Pharmacia) and sterile dH_2O. While conditions for the PCR reaction in the thermocycler machines are designed with pre-denaturation temperature 94^oC for 5 minutes, denaturation 94^oC in 45 second, annealing 60^oC in 30 seconds, 72^oC in 30 seconds and post-extension PCR 4^oC. For multiplication, the cycle is repeated 40 times.

Molecular patterns of genetic variation of alleles of IL-8R gene from PCR products obtained by treated PCR products with a temperature of about 100^0C in order to create a single strain of DNA, then cooled suddenly at a temperature of 4^0C to get back conformation of single DNA strains. The result of a single conformation of DNA then separated by polyacrylamide gel electrophoresis and followed by silver staining. SSCP band patterns that appeared on polyacrylamide gel with staining by silver in each allele was assumed as a variation of the IL-8R gene. Diversity or

variation of IL-8R gene was determined from the differences of SSCP pattern of each individual sample.

Results and Discussion

The SSCP pattern which results from visualization on the polyacrylamide gel bands have found five different patterns of SSCP, two of the SSCP banding pattern represents 66.6% (n = 48) of the sample population (Figure 2). Preliminary analysis samples of milk from dairy cows sample showed that more than 75% cows had mastitis and was associated with IL-8 pattern of the SSCP. While, according to Youngerman et al. (2004) study was found that pattern of SSCP-1 has higher frequency (24.5%) compared to other patterns. However, in this study, the dairy cattle in the area Pujon sub district has frequency two more of SSCP-2 pattern (45.8%) than other SSCP patterns and in this study SSCP-3 pattern was not found in the research of Youngerman et al. (2004).

Among the 48 samples of dairy cattle that were observed, there are about 50% of cattle samples that sub clinical mastitis (SCM) has a pattern-SSCP-1 and SSCP-2. This means that as an early indication if the pattern of SSCP-1 and SSCP-1-2 in cattle Pujon more resistant to mastitis, and other suspect less resistant to mastitis. While this is a preliminary study but the receptor gene polymorphisms on IL-8 of Indonesia FH in Pujon supports that the specification of the polymorphism of IL-8 is suspected associated with mastitis resistance. Although the samples that observed in Indonesian FH from Pujon sub distrcit almost 100% indication of mastitis (clinical and sub clinical), but this preliminary study indicates a

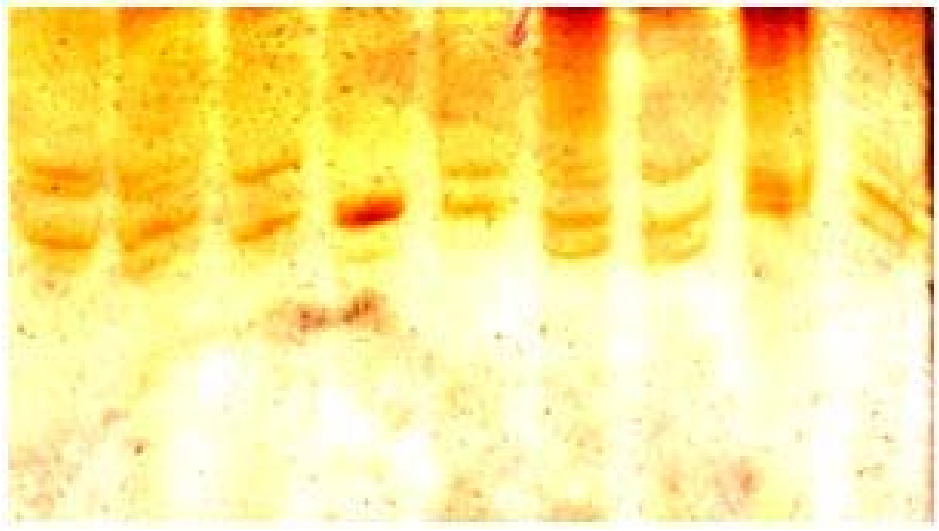

Figure 1. Sampel of IL 8-SSCP patterns from Indonesian FH of Pujon. Number 1 – 6 are SSCP patterns.

Pattern	1	2	3	4	5
Sample (n)	10	22	7	3	6
Percentage (%)	20.8	45.8	14.5	6.25	12.5

Figure 2. Number and percentage of each SSCP pattern of Indonesian FH

pattern of SSCP-SSCP-1 and 2 of the IL-8R gene receptor on some sample that sub clinical mastitis, the expected haplotype can be used as characterized for the early detection of mastitis resistance. But for future study is needed more number of samples and can also be followed by sequencing of SSCP patterns specific IL-8R that suspected as a candidate for resistance to mastitis. Study should be followed data analysis of microbes contained in the udder and milk to supplement the data analysis of Interleukin-8 receptor usage as a reliable marker in detecting cases of disease resistant or susceptible to mastitis.

Conclusions

The result showed that there are at least five types of SSCP patterns of Interleukin-8 receptor gene (IL-8R) in Indonesian FH from Pujon district, Malang regency. This is also illustrates that the polymorphisms of IL-8R gene is high enough to dairy cattle that keep by farmers. From the five patterns obtained, the pattern of SSCP-1 and 2 were the most in number compared with other patterns. Similarly at the second SSCP patterns were also found nearly 50% of individuals suspected of having a sub sample of clinical mastitis. So, based on this preliminary study, the two SSCP patterns can be used as candidate markers to predict or characterized the resistance to mastitis in Indonesia FH. While, other SSCP patterns although fewer in number than the pattern of SSCP-1 and 2, but can we use as basis in determining polymorphism SSCP pattern of IL-8 in Indonesia FH. It is important to determine the genetic diversity of Indonesia FH dairy cattle based on IL-R gene polymorphism SSCP because the use of genetic markers is expected to improve the management of dairy cattle rearing, especially those associated with prevention of disease.

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