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SOMATIC CELL COUNT, TEAT CHARACTERISTICS, MILK COMPOSITION AND SINGLE NUCLEOTIDE POLYMORPHISMS IN CANDIDATE GENES FOR MASTITIS IN FRIESIAN AND JERSEY COWS

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By

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SOMATIC CELL COUNT, TEAT CHARACTERISTICS, MILK COMPOSITION AND SINGLE NUCLEOTIDE POLYMORPHISMS IN CANDIDATE GENES FOR MASTITIS IN FRIESIAN AND JERSEY COWS

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December 2017

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Mastitis is a clinical condition of dairy animals characterized by inflammation of the mammary gland. It is classified as either clinical or subclinical, in which the former is associated with physical, chemical and bacteriological changes in milk as well as changes in udder morphological characteristics in dairy cows. Somatic cell count (SCC) is an important indicator of udder health and mastitis. Two types of immunity, natural or innate and acquired immunity, act together to protect the animals' mammary gland especially at early stage of infection. The bovine toll-like receptor 4 gene (*TLR4*), mannose-binding lectin 1 gene (*MBL1*) and peptidoglycan recognition protein 2 gene (*PGLYRP2*) have been identified to be responsible for these immune responses. There is limited information on the single nucleotide polymorphisms **(**SNPs) in these genes in the Malaysian dairy cattle populations, and reports on their association with mastitis resistance or susceptibility is lacking. This study was undertaken to investigate the udder mastitis status, milk somatic cell score (SCS), teat characteristics, some milk composition traits and SNPs in three candidate genes for bovine mastitis resistance in Friesian and Jersey cows, in order to identify potential genetic markers that may be used in marker assisted selection.

The Friesian and Jersey lactating cows in a commercial dairy farm were used in this research. Milk somatic cell count (SCC) was estimated using a somatic cell counter and converted to SCS, and mastitis status was established using California mastitis test (CMT). Teat lengths were measured, their functioning tested, and the orifices observed and scored. Milk composition traits were determined by infrared spectroscopy. DNA was extracted from the milk of 40 infected and 37 healthy cows, and the individual DNA samples were screened and genotyped for five SNP loci in the exons and introns of *TLR4*, *MBL1* and *PGLYRP2* using the PCR-restriction fragment polymorphism (PCR-RFLP) technique.

There was no significant ($P>0.05$) differences in the numbers of infected (based on California mastitis test) and functional teats between Friesian and Jersey cows reared under common management. There was also no significant differences in SCS between the breeds nor between the lactations. However, the late lactation stage milk had the highest SCS ($P <0.05$). Udder quarters, lactation stage and lactation number had a significant ($P<0.05$) effect on the teat length. The mean rear quarter teat length was significantly ($P<0.05$) lower than the front quarter teats. Significant relationship between teat length and quarter mastitis status was observed. The shorter the teat length, the higher was the tendency for the udder quarter to be infected. Teat-end hyperkeratosis score showed a significant differences between breeds and udder quarters. Lactation number, lactation stage, and breed had a significant ($P<0.05$) effects on milk lactose, but not on solid non-fat. Lactation number and lactation stage had significant ($P<0.05$) effects on milk fat and protein. There was no significant influence of the genotypes at the investigated loci of the TLR4, MBL 1 and PGLYRP-2 genes on SCS, milk fat, protein and lactose. There was a significant ($P<0.05$) association between teat condition and mastitis status. Therefore, udder morphological traits should be included in selection and breeding for mastitis resistance. A larger herd size, evaluation of more SNP loci, and analysis of data from complete lactation cycles of each animal would provide a more valid information on the effects of various influencing factors and informative markers for SCS and milk composition traits for the Friesian and Jersey dairy cattle in Malaysian.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KIRAAN SE SOMATIC, CIRI TEAT, KOMPOSISI SUSU DAN POLIMORFISME NUKLEOTIDA TUNGGAL DALAM GEN CALON UNTUK MASTITIS DI LEMBU FRIESIAN DAN JERSEY

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Mastitis adalah penyakit haiwan tenusu yang dicirikan oleh inflamasi kelenjar mamari. Ia diklasifikasikan sebagai klinikal atau subklinikal, di mana yang pertama dikaitkan dengan perubahan fizikal, kimia dan bakteriologi dalam susu serta perubahan dalam ciri morfologi udder dalam lembu tenusu. Bilangan sel somatik (SCC) adalah penunjuk penting dalam kesihatan udder dan mastitis. Dua jenis keimunan, semula jadi atau innat dan imuniti yang diperolehi, bertindak bersama untuk melindungi kelenjar mamari mamalia terutamanya pada peringkat awal jangkitan. Gen *bovine toll-like receptor 4* (*TLR4*), gen mannose-binding lectin 1 (*MBL1*) dan gen peptidoglycan recognition protein 2 (*PGLYRP2*) telah dikenal pasti bertanggungjawab terhadap tindak balas imun ini. Terdapat maklumat terhad mengenai *single nucleotide polymorphism* (SNP) dalam gen ini dalam populasi lembu tenusu di Malaysia, dan laporan mengenai perkaitan mereka dengan resistan terhadap mastitis atau rentan adalah kurang. Kajian ini dijalankan untuk menyelidik status mastitis udder, skor sel somatik susu (SCS), ciri puting, beberapa sifat komposisi susu dan SNPs dalam tiga gen calon untuk mastitis bovine pada lembu betina Friesian dan Jersey, supaya dapat mengenal pasti penanda genetic yang berpotensi yang dapat diguna dalam *marker assisted selection*.

Lembu Friesian dan Jersey yang berlaktasi di sebuah ladang tenusu komersil digunakan dalam kajian ini. Kiraan sel somatik susu (SCC) dianggarkan menggunakan pengira sel somatik dan ditukar kepada SCS, dan status mastitis ditentukan menggunakan ujian California mastitis (CMT). Panjang puting diukur, fungsinya diuji, dan pembukaan diperhatikan dan dicatat. Ciri komposisi susu ditentukan oleh spektroskopi inframerah. DNA diekstrak daripada susu 40 ekor lembu betina yang dijangkiti dan 37 ekor yang sihat, dan sampel DNA individu telah ditapis dan digenotipkan untuk lima loki SNP dalam exon dan intron *TLR4*, *MBL1* dan *PGLYRP2* menggunakan teknik PCR-restriction fragment polymorphism (PCR-RFLP).

Tidak terdapat perbezaan signifikan ($P<0.05$) dalam jumlah puting yang dijangkiti (berdasarkan ujian mastitis California) dan berfungsi di antara lembu Friesian dan Jersey yang dipelihara di bawah pengurusan yang sama. Juga tidak terdapat perbezaan yang signifikan pada SCS antara baka atau antara laktasi. Walau bagaimanapun, susu peringkat laktasi lewat mempunyai SCS tertinggi ($P <0.05$). Sukuan udder, peringkat laktasi dan numbor laktasi mempunyai kesan yang signifikan ($P <0.05$) ke atas panjang puting. Min panjang puting bahagian belakang adalah lebih rendah secara signifikan ($P <0.05$) daripada sukuan udder depan. Perkaitan yang signifikan antara panjang puting dan status mastitis sukuan diperhatikan. Semakin panjang puting, semakin tinggi prevalensi mastitis. Skor hiperkeratosis hujung puting menunjukkan perbezaan yang signifikan antara baka dan kuar udang. Nombor laktasi, peringkat laktasi dan baka mempunyai kesan yang signifikan ($P <0.05$) terhadap laktosa susu, tetapi bukan terhadap lemak tidak padat. Nombor laktasi dan peringkat laktasi mempunyai kesan yang signifikan($P <0.05$) terhadap lemak dan protein susu. Tidak terdapat perkaitan yang signifikan SCS, lemak, protein dan laktos susu dengan genotip di loki yang disiasat bagi gen TLR4, MBL 1 dan PGLYRP-2, walaupun beberapa genotip telah mempunyai SCS yang rendah berbanding dengan yang lain. Terdapat perkaitan signifikan ($P <0.05$) antara keadaan puting dan status mastitis. Oleh itu, sifat morfologi udder perlu dimasukkan dalam pemilihan dan pembiakbakaan untuk resistan terhadap mastitis. Saiz gerompok yang lebih besar, penilaian lebih banyak loki SNP, dan analisis data dari kitaran laktasi yang lengkap setiap haiwan akan memberikan maklumat yang lebih sah mengenai kesan pelbagai faktor yang mempengaruhi dan penanda bermaklumat bagi SCS dan ciri komposisi susu untuk lembu tenusu Friesian dan Jersey di Malaysia.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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LIST OF ABREVIATIONS

CMT	California mastitis test
bp	base pair
DNA	deoxyribonucleic acid
E. coli	*Escherichia coli*
FAO	Food and Agricultural Organisation
DVS	Department of Veterinary Service
IDF	International Dairy Federation
IMI	Intramammary infection
kb	kilobase
MBL	mannose binding lectin
M	molar
NMC	National Mastitis Council
PGLYRP	peptidoglycan recognition proteins
PMN	polymorphonuclear neutrophils
SCC	somatic cell count
SCS	somatic cell score
TLR	toll like receptor
RE	restriction enzyme

CHAPTER 1

INTRODUCTION

The demand for milk and other dairy product is on the increase globally in which Malaysia is not an exception. Milk is a complex mixture secreted from the udder of mammals which comprises of lactose, proteins, fat, and vitamins among other components. The bulk of milk used worldwide is from cows although milk from buffalo, sheep, goat, as well as camel, are of commercial importance (FAO, 2007). Milk is an ideal food for the young mammals, human and animals (Vaclavik and Christian, 2014). Milk from normal healthy Holstein - Friesian dairy cows comprises 87% water, 3.8% fat, 3.4% protein, 4.5% lactose in addition to other components such as 1.3% minerals and a trace of epithelial cells (Ruegg, 2001).

Mastitis is defined as an inflammatory response of the mammary gland (Internal Dairy Federation, 1987). Contagious and environmental pathogens usually cause it. The pathogen, usually bacteria, enters the mammary gland through the teat canal. When they succeed in overcoming the innate defense mechanism of the animal, they localized in the udder and multiply. Here, the microbes produce toxins that destroy cell membranes and damage milk-producing tissues (Hillerton & Berry, 2005; Zhao & Lacasse, 2008). Damage to the mammary gland epithelial tissues is triggered not only by the bacteria but also by the host immune action. When this happens, the alveoli will become fragile, with resultant permeability to other extracellular fluid which mix with the milk and result in visible change in the milk and the udder (Zhao & Lacasse, 2008). Mastitis is classified as clinical mastitis, the form associated with inflammation of the mammary gland (visible swelling and reddening), wateriness of the milk, sore teat, presence of pus, flakes, and blood in the milk, and higher somatic cell count (SCC) (Zhao & Lacasse, 2008; Fedota *et al.,* 2015), or as sub-clinical mastitis, where the infected animals show no physical signs of the disease, the milk appear normal but milk yield is reduced and SCC elevated (DeGraves & Fetrow, 1993; Shitandi & Kihumbu, 2004).

Milk somatic cells are milk epithelial cells, usually shed from the inner lining of the mammary glands, and leukocytes which originated from the blood cells. Somatic cells composed of 75% leucocytes (which comprises neutrophils, macrophages, lymphocytes, erythrocytes) and 25% epithelial cells. (Sharma *et al.* 2011; Zhao & Lacasse, 2008). When there is a presence of infectious bacteria, injury in the mammary gland tissues and stress, there will be influx somatic cells notably polymorphonuclear neutrophils (PMN) to neutralize the effect of the invading pathogen. Therefore, SCC is an important indicator of udder health and mastitis (Pantoja *et al.*, 2009).

Selection for mastitis resistance/resilience or susceptibility mainly focuses on SCC or somatic cell score (SCS), the transformed values of SCC, as an indicator of udder health and mastitis. Accuracy of this selection may be improved if optimum udder health index, udder and teat morphometric are added to SCC in breeding programs (Singh & Bansal, 2013; Dube *et al.*, 2008). However, mastitis is a polygenic trait. The greatest challenge faced by molecular geneticists is the identification of markers for genes that control the phenotypic variation in mastitis resistance/ resilience (Sender *et al*., 2013; Ogorevc *et al*., 2009).

A number of candidate genes for mastitis resistance have been reported, which have the potential of reducing mastitis susceptibility in dairy herds (Turner, 2003; Garred *et al*., 2006; Tydell *et al*., 2006; Dziarski & Gupta, 2010; Kumar & Yadav, 2012; Gupta *et al.*, 2015;). However, application of marker assisted selection strategy to control mastitis requires understanding of the genetic variations in the local herds, and identifying informative single nucleotide polymorphisms (SNPs) and genotypes associated with mastitis.

1.1 Research Problem

There are limited information on the SNPs related to mastitis resistance or susceptibility in the Malaysian dairy cattle herds. In addition there is a lack of reports on udder morphological characteristics in local dairy cattle populations. There are also no published works investigating the association of SNPs in the candidate genes with SCS, udder or teat characteristics or milk composition traits.

1.2 Objectives

The general objective of the study was to evaluate the udder mastitis status, milk SCS, teat characteristics, some milk composition traits and SNPs in candidate genes for bovine mastitis resistance in Friesian and Jersey cows in one dairy cattle farm in Malaysia, in order to identify potential genetic markers that may be used in marker assisted selection.

The specific objectives were:

1. To estimate the milk SCC and SCS of Friesian and Jersey cows at different lactations and lactation stages in a dairy cattle herd.
2. To evaluate the functioning of udders, teat characteristics and milk composition traits in the above dairy cows.
3. To identify single nucleotide polymorphisms in three candidate genes for bovine mastitis.
4. To determine the association of SCC, SCS, udder morphological characteristics and milk composition traits with the SNPs and genotypes at the three candidate genes for mastitis resistance in the Friesian and Jersey cows.

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LIST OF PUBLICATIONS

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