

Prevalence of *Mycoplasma bovis* and Other Contagious Bovine Mastitis Pathogens in Bulk Tank Milk of Dairy Cattle Herds in Khon Kaen Province, Thailand

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Abstract

Mastitis is a most frequent and costly disease of dairy cattle worldwide. All three contagious mastitis pathogens, *Mycoplasma bovis*, *Streptococcus agalactiae* and *Staphylococcus aureus* were investigated in 55 bulk tank milk samples from dairy cattle herds in Khon Kaen Province Thailand, by nested PCR and/or conventional bacterial culture. Bulk milk somatic cell count (BMSCC) was used as indicator for mastitis problem; i.e. $> 5 \times 10^5$ somatic cells/ml of milk. The prevalence of *Mycoplasma bovis*, *Streptococcus agalactiae* and *Staphylococcus aureus* were 1.8%, 21.8% and 7.3%, respectively. Results from BMSCC indicated mastitis problem in 47 herds. However, 34 high-BMSCC with negative-contagious pathogen identification samples suggested the uncovered problem of udder health in the studied group.

Keywords : Bulk tank milk, mastitis, *Mycoplasma bovis*, somatic cell count, *Staphylococcus aureus*, *Streptococcus agalactiae*

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บทคัดย่อ

ความชุกของเชื้อ *Mycoplasma bovis* และเชื้อก่อโรคเต้านมอักเสบติดต่อชนิดอื่นในน้ำนมถักรวม ฟาร์มโคนมในเขตจังหวัดขอนแก่น ประเทศไทย

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เต้านมอักเสบเป็นปัญหาที่พบบ่อยและมีค่าใช้จ่ายสูงในการเลี้ยงโคนมทั่วโลก เชื้อก่อโรคเต้านมอักเสบติดต่อมี 3 ชนิดที่สำคัญคือ *Mycoplasma bovis*, *Streptococcus agalactiae* และ *Staphylococcus aureus* ซึ่งได้ถูกศึกษาในงานวิจัยนี้ในตัวอย่างน้ำนมถักรวมจากฟาร์มโคนมจำนวน 55 ฟาร์ม ในเขตจังหวัดขอนแก่น โดยเชื้อทั้งสามถูกวินิจฉัยโดยวิธีการ nested PCR ร่วมกับการเลี้ยงเชื้อและวินิจฉัยเชื้อทั่วไป ตัวอย่างน้ำนมจะถูกตรวจหาเซลล์โซมาติกเพื่อเป็นตัวบ่งชี้ปัญหาเต้านมอักเสบในฟาร์ม นมถักรวมฟาร์มมีค่าเซลล์โซมาติก สูงกว่า 5×10^5 เซลล์/นม 1 มล. จะถูกคัดลินให้เป็นนมจากฟาร์มที่มีปัญหาเต้านมอักเสบ จากการศึกษานี้พบความชุกของ *Mycoplasma bovis*, *Streptococcus agalactiae* และ *Staphylococcus aureus* เป็น 1.8%, 21.8% และ 7.3% ตามลำดับ และมี 47 ฟาร์มที่มี BMSCC ในระดับสูง อย่างไรก็ตามมีฟาร์มที่มีเซลล์โซมาติกสูงจำนวน 34 ฟาร์มที่ตรวจไม่พบเชื้อเต้านมอักเสบติดต่อทั้งสามชนิด ดังนั้นยังมีภาวะอื่นที่ก่อให้เกิดเต้านมอักเสบและควรต้องมีการศึกษาต่อไป

คำสำคัญ : น้ำนมถักรวม เต้านมอักเสบ *Mycoplasma bovis* เซลล์โซมาติก *Staphylococcus aureus* *Streptococcus agalactiae*

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Introduction

Mastitis, an inflammation of mammary glands, is the most frequent and costly disease of dairy cattle worldwide. In most of the cases, mastitis is caused by infection of microorganism. The pathogens cause an inflammation of the mammary glands, which then increase number of somatic cell in the produced milk. Thus the bulk milk somatic cell count (BMSCC) is used as a mastitis indicator in dairy herd (Jayarao and Wolfgang, 2003; Jayarao et al, 2004). The contagious mastitis pathogens comprise of *Streptococcus agalactiae* (*Str. agalactiae*), *Staphylococcus aureus* (*Sta. aureus*) and mycoplasma (Jayarao and Wolfgang, 2003).

Mycoplasmas are highly contagious and can be an economically important cause of milk loss and increased culling of infected cows. The infected cow produces a low quality and quantity of milk, served as a source of the infection and would be culled from herd. Among the different species of mycoplasma that infect cattle, *Mycoplasma bovis* (*M. bovis*) is the most pathogenic and common cause of mastitis (Jasper, 1977). It is considered an important agent of clinical mastitis in the US, Australia and Europe (Jasper, 1977; Pfutzner and Sachse, 1996).

M. bovis causes substantial economic losses to the dairy industry primarily through causation of an intractable, untreatable mastitis (Brown et al., 1990; Gonzalez et al., 1992). Moreover, mycoplasma intramammary infection of dairy cattle is serious condition that can result in milk loss and elimination of infected animals from a herd because of the difficulty in treatment (Ayling et al., 2004; Brown et al., 1990; Byrne et al., 2005; Jasper, 1982; Kirk and Lauerman, 1994). In most of mastitis studies report prevalence of *Str. agalactiae* and *Sta. aureus* because it can be identified by a conventional bacterial identification method whilst *M. bovis* need a more complicated step for the identification. Thus the true prevalence of *M. bovis* is probably underestimated.

Up to December 2007, there were 511 dairy herds in Khon Kaen province of Thailand. Most of the herds were small to medium size, i.e. 3-20 lactating cows/herds that produce milk on average 12 kg/cow/day. Farmer usually use milking machine, mostly are locally produced, to collect milk from their cows. Milk in the bucket is then transferred into herd's bulk tank before send for sell at the nearby local co-operative milk collection centre (MC) twice a day. At the MC the quality of milk from each farm will

collected. The milk samples will be examined, i.e. bulk milk somatic cell count (BMSCC) and total bacterial count, once a month at the Veterinary Research and Development centre, Khon Kaen. Result of the milk quality testing is used to set price of buying. In 1999, the Department of Livestock Development of Thailand announced a dairy herd standard which stated that the BMSCC should not exceed 5×10^5 cells/ml of raw milk. However, approximate 40% of the Thai dairy herds had BMSCC greater than the standard (Bureau of Quality Control of Livestock Products, 2009). Moreover, mastitis still be the most concerned problem of Thai dairy farmers. Many studies had been done on identification of the pathogenic organisms of mastitis in Thailand; however, mycoplasma mastitis had not been examined.

The purpose of this study was to determine the herd prevalence of *M. bovis* and other contagious mastitis pathogens in bulk milk from dairy cattle herds in Khon Kaen province and determine the association between presences of the identified pathogen and mean BMSCC.

Materials and Methods

Bulk tank milk samples: A survey study was carried out in October 2008 in a MC in Mueng District of Khon Kaen province. By monthly records from July to September 2008, the average BMSCC of about 200 dairy herds in MC was 1.21×10^6 cells/ml. There were 52 bulk tank milk (BTM) samples had bulk tank milk somatic cell count (BMSCC) greater than 1.00×10^6 cells/ml for at least once during the time. In the group, 46 herds sent their milk to the MC on the sampling day and were then BTM collected. Nine herds that had BTM SCC less than 0.50×10^6 cells/ml from July–September, were selected as a free-mastitis group in the study. All 55 samples were collected 60 ml. aseptically and were kept at 4°C until analyzed.

Bacterial identification: All 55 BTMs were carried at 4°C to isolate the pathogens of interest at the Faculty of Veterinary Medicine, Khon Kaen University on the day.

Mycoplasma bovis: To minimize the false negative results, identification of *M. bovis* was done on filtrated, cultivated milk samples. Firstly, 0.5 ml of milk were mixed with 2 ml of modified Heyflick's broth (Difco™ PPLO Broth plus 30% of Difco™ PPLO Supplement) before were filtrated through 0.45 micron What Man® filter. The filtrated milk dilution was then incubated

at 37°C for 8 days (Hogan et al., 1999). DNAeasy® Blood and Tissue kits (Qiagen®, Germany) was used to extract bacterial DNA from the cultured broth; all the procedures were followed the manufacturer's instruction. A commercially available nested polymerase chain reaction (nPCR) kits for detection of *Mycoplasma bovis* (Genekam™, Germany) was used according to the manufactured suggestions; positive and negative controls, which provided with the kits, were analyzed in every steps of the nPCR.

Streptococcus agalactiae and Staphylococcus aureus: The organisms were cultivated primarily from a 50 microlitre of vortex-mixed milk onto a sheep blood agar. The culture plate was then incubated in 37°C incubator overnight before were examined for the specific bacterial colonies of the pathogens. *Streptococcus agalactiae* was identified by a hemolysis pinpoint colony on blood agar, Gram's stain, negative-catalase test, negative-oxidase test, negative-all four sugar utilizations (manitol, sorbital, raffinose and inulin), negative-bile esculin test, negative cultivation in 6.5% NaCl and positive CAMP test. *Staphylococcus aureus* was identified by α - and β -hemolysis colony, Gram's stain, a positive-catalase test, negative-oxidase test, a positive-tube coagulase test, oxidation of manitol and yellowish colony on purple base agar (National Mastitis Council, 1987; Quinn et al., 1994).

Bulk tank milk somatic cell count: The milk samples were analyzed the somatic cell numbers by using Fossomatic 5000 Basic (Foss Electric, Denmark) at the Veterinary Research and Development centre, Khon Kaen.

Statistical analysis: A Student's t-test was used to test if the BMSCC was different between pathogen negative and positive BTM. The analysis was performed using statistic software, Stata version 8.2 (Stata Corporation, College Station, Texas US).

Results and Discussion

The contagious mastitis pathogens were identified in 14 out of the overall 55 BTM (25.5%). *M. bovis*, *Str. agalactiae* and *Sta. aureus* was isolated from 1 (1.8%), 12 (21.8%) and 4 (7.3%) herds, respectively.

Studies of bovine mastitis in Thailand, since the 1990s, however, never been done on the presence of *M. bovis* even the mycoplasma mastitis has been reported

in many geographical locations that contain intensive dairy productions (Fox et al., 2005; Ghadersohi et al., 1999; Kirk et al., 1997; Olde Riekerink et al., 2006; ter Laak et al., 1992). The prevalence of *M. bovis* in BTM in Khon Kaen province did not differ from other reports. Recent studies, suggested that 1% to 6% of the dairy herds had at least 1 cow with mycoplasma-induced mastitis (Fox et al., 2003; Jasper et al., 1979; Kirk et al., 1997; Kirk and Lauerman, 1994; Olde Riekerink et al., 2006). Shedding patterns, minimum level of detection and dilution by milk from other members and, in this situation, the transportation of milk from herd to MC, may influence the true detectable prevalence (Kirk and Lauerman, 1994). Other pathogenic mycoplasmas, however, were not examined because of the most frequently identified and highly pathogenic mycoplasmas is the *M. bovis* (Ayling et al., 2004; Gonzalez and Wilson, 2003) thus we aimed only at the species. *M. bovis* may be found also in cattle that have joint and/or lung infections but the presence in bulk milk that had very high SCC strongly indicated the source of the pathogen.

Str. agalactiae and *Sta. aureus* was found in 21.8% and 7.3% of 55 BTM, respectively. The prevalence was lower than the study in 2002 by Sukolapong et al. The lower prevalence may result from improve milking practice of farmers during the time period and, probably, sample selection. Nature of shedding the organisms in milk may affect the true prevalence. *Str. agalactiae* is shed in a larger number thus it can be easily cultured from BTM. On the other hand, *Sta. aureus* is shed infrequently and with a very low numbers and, as a result, few *Sta. aureus* present in BTM (Jayarao and Wolfgang, 2003). Thus the true prevalence of *Sta. aureus* infection in individual probably was higher than the detected prevalence in BTM.

On average, the BMSCC of the 55 herds was 1.08×10^6 cells/ml of milk (range 0.00 – 7.65×10^6 ; $sd = 0.17 \times 10^6$). In the low BMSCC group (9 herds), 8 herds had continuing low BMSCC in October, had not found any mastitis pathogens and its mean BMSCC was 0.18×10^6 cells/ml. Another herd raised BMSCC to 0.65×10^6 cells/ml but none of the contagious pathogen was identified. The association of the BMSCC with presence of all three pathogens is showed in Table 1. Herd that had BTM sample positive for any of the contagious

pathogens had a mean BMSCC that was 0.72×10^6 cells/ml higher than the counts from herds that had no pathogens isolated ($p = 0.03$).

The mean BMSCC of *Str. agalactiae*-positive herds was 1.77×10^6 cells/ml. which higher than that of negative herds ($p = 0.01$). Eleven herds had BMSCC greater than 0.50×10^6 cells/ml. Fenlon et al. (1995) also reported a good correlation between the identification of streptococci in bulk tank milk and BMSCC. One *S. agalactiae*-positive herd, however, had a rather low BMSCC (0.25×10^6 cells/ml) in the sampling time but had very high level on the earlier 3-months (mean BMSCC was 0.99×10^6 cells/ml). The recently low BMSCC probably resulted from incomplete treatment or the re-infection in a small proportion of lactating cows.

M. bovis and *Sta. aureus* were identified in herds that had BMSCC greater than 0.50×10^6 cells/ml. Mean 3-months BMSCC, from July to September, of the *M. bovis* positive-herd was 3.01×10^6 cells/ml. Because *M. bovis* had identified only in one herd thus we could not determined the effect of the pathogen on BMSCC. Moreover, in a study by Fox et al. (2003) on BMSCC and presence of mycoplasma, report none significantly difference between the positive and negative-mycoplasma herds. Explanations could be that the isolation of *Mycoplasma* spp. in bulk tank milk is not related the number of the shedding cows (Gonzalez et al., 1986).

Fourty-seven herds had high BMSCC ($>0.50 \times 10^6$ cells/ml) but 34 of them (72.3%) had none of the contagious pathogens in BTM. Beside udder infection with contagious mastitis pathogens, variation in BMSCC is also influenced by the stage of lactation, season of the year, and individual cow responses to infection (Harmon, 1998). Thus, the high BMSCC in the free-pathogen herds probably resulted from (1) the infections of other mastitis pathogens, i.e. *Str. dysgalactiae*, *Str. bovis*, Coagulase-negative staphylococci and other *Mycoplasma* spp., (2) stage of lactation and (3) aseptic mastitis and (4) low sensitivity of the BTM culture. Study in the same population by Sukolapong et al. (2002) reported the possibility of finding mastitis pathogen in individual milk sample but did not found in BTM sample, was 58.1%. The finding probably resulted from the high dilution of pathogen in normal milk of the herds which reduced chance of discovery. Moreover, the negative

Table 1 The association of isolation of pathogen with average bulk milk somatic cell count (BMSCC) in bulk tank milk samples from 55 dairy cattle herds in Khon Kaen province, Thailand.

	Mean SCC (x10 ⁶ cells/ml)		Difference (x10 ⁶ cells/ml)	P-value
	Isolated (#herds)	None isolated (#herds)		
Any contagious pathogen	1.619 (14)	0.899 (41)	0.720	0.03
<i>Mycoplasma bovis</i>	4.481 (1)	1.080 (54)	3.461	na
<i>Streptococcus agalactiae</i>	1.771 (12)	0.890 (43)	0.881	0.01
<i>Staphylococcus aureus</i>	1.063 (4)	1.083 (51)	0.000	0.49

results on identification of mycoplasma are not definitively indicated that herd is being free from mycoplasma infection (Farnsworth, 1993; Gonzalez and Wilson, 2003; Kunkel, 1985; Olde Riekerink et al., 2006). Some infected cows are intermittently shedders of mycoplasma in the milk, with as many as 40% of cows shedding < 10 CFU/ml of this organism from infected glands (Biddle et al., 2003). Thus herds that had high BMSCC could not discard the udder infection with mycoplasma. Furthermore, the high mean BMSCC of the studied population indicated the uncovered udder trouble.

In conclusion, this first report of *M. bovis* in dairy milk sample suggested another vigilant mastitis pathogen among Thai dairy cattle. Further study should be done to investigate the source of the organism in dairy herds and also investigated the infection route; i.e. milking machine and procedure, to provide the complete picture of bovine mastitis in dairy herds in Thailand which helps to prevent and correct the udder problem in the future.

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