Original Article

Risk Factors of Subclinical Mastitis in Small Holder Dairy Cows in Khon Kaen Province

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Abstract

This cross-sectional study was introduced to determine the risk factors of subclinical mastitis in dairy farms. Sixteen small holder farms and 285 milking cows in Khon Kaen province were selected for the study. The mastitis investigation, feeding information and clinical inspection were applied in the farm visits. Milk samples from each quarter with a CMT score of 3 and bulk milk were collected for bacterial identification and somatic cell counts. The prevalence of subclinical mastitis and associated factors were analyzed. Results found that the prevalence of subclinical mastitis was 36.14%. Major microorganisms isolated from infected quarters were *Streptococcus* spp. and coagulase negative staphylococci. The odds ratio of vacuum pressure and teat liner were strongly related with subclinical mastitis (2.18 and 2.00, respectively). In addition, the milking management had a higher impact on subclinical mastitis with odds ratios of 1.83, 3.37 and 2.18 in no strip cup, no single towel and no clean and dry before milking, respectively (p<0.05) at 95% confidential intervals. High odds ratios in subclinical mastitis were also found in poor body condition score and abnormal hoof score accounted for 2.66 and 1.78, respectively (p<0.05) at 95% CIs. Dairy cows from the 1st to 3rd lactation had a significantly lower prevalence of subclinical mastitis than those in higher parities. In conclusion, the study demonstrated that milking management, body condition score, and hoof score were significantly related to subclinical mastitis in small holder dairy cows.

Keywords: dairy cows, risk factor, subclinical mastitis

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บทคัดย่อ

ปัจจัยเสี่ยงของโรคเต้านมอักเสบแบบไม่แสดงอาการในฟาร์มโคนมรายย่อยในจังหวัดขอนแก่น

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การศึกษาแบบ cross-sectional เพื่อวัดปัจจัยเสี่ยงของโรคเต้านมอักเสบแบบไม่แสดงอาการในฟาร์มโคนม รายย่อยจำนวน 16 ฟาร์ม จากแม่โคทั้งหมด 285 ตัวในเขตสหกรณ์โคนมขอนแก่น จำกัด โดยใช้การประเมินจากแบบสอบถาม ข้อมูลอาหารและการตรวจประเมินในทุกครั้งที่เข้าเยี่ยมฟาร์ม ทำการเก็บตัวอย่างน้ำนมจากเต้านมที่ตรวจพบว่ามีผลบวกต่อระดับ CMT ที่ระดับ 3 ขึ้นไปเพื่อทำการตรวจนับจำนวนเซลล์โซมาติกและเพาะแยกชนิดเชื้อแบคทีเรีย ความชุกของการพบโรคเต้านมอักเสบแบบไม่แสดงอาการและปัจจัยเสี่ยงถูกนำมาวิเคราะห์หาความสัมพันธ์ พบว่าความชุกของการเกิดเต้านมอักเสบแบบไม่แสดงอาการอยู่ที่ร้อยละ 36.14 ชนิดเชื้อแบคทีเรียที่แยกได้จากเต้านมที่ติดเชื้อ คือ กลุ่มสเตรปโตคอคคัสและกลุ่มสเตรปโคคอคคัสที่ให้ผลลบต่อ coagulase ค่า odds ratio ของแรงดันสูญญากาศและยางในรีดนมที่ไม่เรียบมีความเสี่ยงในระดับ 2.18 และ 2.00 ตามลำดับ (p<0.05, 95% CIs) นอกจากนี้การไม่รีดนมต้นทิ้ง การไม่ใช้ผ้าเช็ดเต้าหนึ่งผืนต่อหนึ่งตัว หัวนมไม่แห้งและสะอาดก่อนการสวมหัวรีดนมก็เป็นปัจจัยเสี่ยงต่อการเกิดที่ 1.83, 3.37 และ 2.18 (p<0.05, 95% CIs) ค่าคะแนนร่างกายที่ต่ำกว่าเกณฑ์และความผิดปกติของกีบเป็นปัจจัยเสี่ยงที่ 2.66 and 1.78, (p<0.05,CIs= 95) แม่โคนมที่มีระยะรีดนมไม่เกินระยะที่ 3 จะมีความเสี่ยงของการเกิดโรคน้อยกว่าแม่โคนมที่มีระยะให้นมที่มากกว่า จากการศึกษานี้แสดงว่าขั้นตอนการรีดนม ค่าคะแนนร่างกาย และความผิดปกติของกีบแม่โคมีความสัมพันธ์ต่อการเกิดโรคเต้านมอักเสบแบบไม่แสดงอาการในโคนมในฟาร์มรายย่อย

คำสำคัญ: โคนม ปัจจัยเสี่ยง โรคเต้านมอักเสบแบบไม่แสดงอาการ

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Introduction

Mastitis is one of the most important diseases that causes economic loss in dairy industry worldwide, and also in Thailand. The mean annual incidence is 41.6 cases per 100 cows and affected cows suffered a mean of 1.5 cases and 16.4% of quarters suffered at least one repeat case (Bradley and Green, 2001). Bennett et al. (1999) estimated the total economic impact of clinical mastitis to be £119 per cow-case. Generally, pathogen-infected quarters of dairy cows may be clinical or subclinical depending on the degree of inflammation. In addition, the subclinical form is 15 to 40 times more prevalent, thus constituting a reservoir of microorganisms and leading to the infection of other animals within the herd (Philpot and Nickerson, 1999). The prevalence of subclinical mastitis on the dairy farms can range from 19 to 78% (Tuteja et al., 1993). The economic loss from subclinical mastitis alone mainly from reduction in milk production in affected lactating cows has accounted for 15 to 45% (Dohoo and Meek, 1982). Furthermore, milk production has decreased by 1.2%, 6.3%, and 33% in quarters with California mastitis test (CMT) scores of 1, 2, and 3, respectively (Mungube et al., 2005).

Philpot and Nickerson (1999) previously described many risk factors for bovine mastitis including environment, human, management, microorganisms, and dairy cows. Factors associated with dairy cows are age, parity, breed, lactation stage, body condition score and diet deficiency (Brand et al., 1997). Some vitamins and minerals have been reported to be associated with the prevalence and epidemiology of subclinical mastitis (Bartlett et al., 1992; Chassagne et al., 1998). Vitamin E and selenium are essential to the immune function of the cells, whereas vitamin A serves as a cell component preventing in the initiation of fatty acid peroxidation chain reactions (Weiss, 2002). However, these factors can be divided into two main levels, farm and milking management. Farm management consists of bedding, milking parlors, and feeding. Factors from milking management are considered to play an important role in subclinical mastitis. The milking machine directly affects new infection in dairy farms (IDF 215, 1987; Baxter et al., 1992; Rasmussen et al., 1994). Cleaning udders and teats before milking prevents intramammary infection (IMI) in the lactating cows (Köster et al., 2006). Effective teat dipping at post-milking and dry cow therapy decreases the incidence of subclinical mastitis (Hogan et al., 1994; Lam et al., 1995).

In Thailand, most dairy cows are kept in small holder dairy farms which are typically found in tropical areas. Each farm possesses approximately 10-20 milking cows which are commonly milked by the bucket-type milking machine. Apparently, subclinical mastitis has been considered to have a great impact on udder health and milk production for Thai dairy farmers. Aiumlamai et al. (2000) reported that the prevalence of subclinical mastitis in small holder dairy farms in Khon Kaen, located in the northeast of Thailand, was up to 62.8%, of which 16.8% of the subclinical mastitis cases were caused by *Staphylococcus aureus*. A longitudinal study of subclinical mastitis in some epidemiology studies in the North has also been documented (Boonyayatra and Chaisri, 2004). However, evidence supporting subclinical mastitis and various factors affecting subclinical mastitis in dairy cows in Thailand are very limited and should be further investigated. Therefore, a cross-sectional study was conducted to assess the occurrence of subclinical mastitis in lactating dairy cows and to investigate the relationship between the prevalence and risk factors for subclinical mastitis in lactating cows in small holder farms in Khon Kaen province.

Materials and Methods

Study Area: The study was carried out in dairy farms in Khon Kaen Province in the north-east of Thailand. The climate of this area is mostly hot and humid with an average temperature of 27°C, 70% humidity, and an average annual rainfall of 1,039 mm (TMD, 2003). Generally, there are three seasons including rainy (June-September), winter (November-January), and summer (February-May), respectively.

Experimental design: The cross-sectional study was carried out in 16 out of 150 dairy farms. A total of 285 lactating cross-bred Holstein-Friesian milking cows in each farm were used in this study. Each farm was visited at least twice during the study period and a comprehensive questionnaire was administrated at every visit.

Sampling: Post-milking quarter milk samples were collected for the California Mastitis Test (CMT) following Philpot and Nickerson (1999). Approximately 2-5 ml of quarter milk samples with a CMT score of 3 were aseptically recollected for bacteriological procedures. In addition, approximately 15-ml of bulk milk sample were also collected from each farm. All samples were placed in ice-cooled containers and transported to the laboratory within 2 hours after the collection for further analysis.

Sample analysis: All selected quarter milk samples were determined for somatic cell count (SCC) using the direct microscopic count (DMC) modified from IDF (1995) and reported as cells/ml. Bacterial isolation and identification were also carried out according to Quinn et al. (1994) and NMC (1987) procedures. Infected quarters were reported if at least one of mastitis-causing pathogens such as *Streptococcus agalactiae*, *Streptococcus* spp., *Staphylococcus aureus*, *Escherichia coli*, coagulase-negative Staphylococci (CNS), and yeast was isolated from the selected milk sample. Whereas uninfected quarters were classified if no microorganisms were isolated. Bulk milk samples were also analyzed for SCC, bacterial identification and total bacterial count (TBC) (Quinn et al., 1994).

Questionnaire administration: The mastitis was investigated by KKU-mastitis and a herd health management questionnaire modified from Ryan and Barton (1994). The questionnaire under the primary objective was administered to each visited farm to determine risk factors for subclinical mastitis, including all possible information which were herd, reproduction, nutrition and mastitis (Table 1). Observation and inspection was done along with the questionnaire. The feeding information in concentrate and roughage was also estimated by interviewing, inspecting and weighing. The feeding composition and nutritional values were analyzed by computer software (Wachirapakorn, 2003).

Hoof scoring and body condition scoring: Hoof scoring was done during inspection and evaluation. The hoof score was classified in a 5 present scale: 1, 2, 3, 4, and 5, as normal, unilateral overgrowth, bilateral overgrowth, long hoof, and showing signs of lameness, respectively, following the KKU hoof score investigation, which had been modified from Andrews (2000). During milking, all milking cows were assessed for body condition scores (1-5, Brand et al., 1997).

Statistical analysis: The prevalence of subclinical mastitis based on the laboratory results of individual quarter sample from milking cows with various risk factors was compared using chi-square analysis for independence. Explanatory variables that were graded at more than two levels in the questionnaire were dichotomized and coded as 0 or 1 (Table 2). The body condition score was classified according to Brand et al. (1999). The targeted body condition score was different depending on the lactation stage and categorized by good, fair and poor as ±0.5, ±1.0 and ±1.5 of the targeted scores, respectively. Hoof scoring of 1 and 2 were considered as a normal score while scores of 3, 4, and 5 were classified as abnormal hoof scores. Subclinical mastitis in milking cows was defined as positive bacterial identification in selected milk samples (De Graaf and Dwinger, 1996; Philpot and Nickerson, 1999). Quarters with a positive CMT score more than 2 were considered as suspected subclinical mastitis in that animal. However, latent infection was classified in quarters with a CMT score of 3 and a negative bacterial culture. In addition, a multivariate statistical model was employed to analyze the data. A logistic regression model was also calculated to illustrate the magnitude of association between selected factors and subclinical mastitis. This was done by deriving odds ratios, *p*-value and 95%

confidence intervals (CIs) of those selected risk factors when entered as explanatory variables of subclinical mastitis. Odds ratios that were greater than unity supported by P-values less than 0.05 were considered as significant at 95% confidence intervals.

Table 1 Summary of the variables in KKU-Mastitis questionnaire

Herd, reproduction and nutrition
Part 1 Herd and reproductive information
• Herd information: lactating cow, dry cows, heifer and young stock rearing, average milk production
Part 2 Nutrition information
• Concentrate information : ingredients and proportion in feed formula
• Roughage information : kinds of grass, by products and other materials for roughage
• Feeding information: amount of concentrate, roughage and supplements.
Mastitis investigation and inspection
Part 1 Data analysis: history of mastitis, mastitis treatment and management, knowledge of mastitis control and milk quality, and history of culling caused by mastitis
Part 2 Mastitis control: CMT test, drying off procedure, mastitis case control.
Part 3 Milking machine assessment
• Milking machine compartment, vacuum test, regulator, milking unit, liner and claw, pulsation. The Alpha Tonic® (DeLaval, Sweden) was used in milking machine inspection.
• Cleaning milking machine: timing, type of detergent, alkaline and acid application.
Part 4 Milking management: investigation and intensive inspection during milking time including start and finish milking time, feeding at milking parlor, washing udder and teat before milking, chlorine preparation, ordering, strip cup, towel, clean and dry udder, manual stimulation, weights used, claw attachment and detachment, and teat dipping.

Table 2 Recoding of risk factor for logistic regression models

Risk factors	No. of Levels	Code
Mastitis control		
CMT screening test	2	yes= 0, no= 1
Dry cow therapy	2	yes= 0, no= 1
Milking machine		
Vacuum pressure	2	45-50 kPa= 0, more or lower = 1
Pulsation	2	45-60 times/minute= 0, more or lower= 1
Liner	2	change in time, no crack= 0, crack & over due = 1
Milking managements		
Washing	2	yes= 0, no= 1 (washing udder and teat)
Using chlorine	2	yes= 0, no= 1 (using chlorine at milking time)
Strip cubs	2	yes= 0, no= 1
Towel	2	yes= 0, no= 1 (using towel individual cow)
Clean and dry	2	yes= 0,no= 1 (clean and dry udder before milking)
Slip claw	2	no= 0, yes= 1
Weights used	2	no= 0, yes= 1 (weights used during milking)
Claw detachment	2	yes= 0, no= 1
Teat dipping	2	yes= 0, no= 1
BCS	3	good= 0, fair= 1, poor= 2
DIM	3	0-60= 0, 61-120= 1, >120= 2
Hoof score	2	normal= 0, abnormal= 1
Parity	3	1-3= 0, 4-6= 1, >7= 2
Feeding	2	concentrate in ration > 60% = 1 concentrate in ration < 60% = 0

Results

From 16 small holder dairy farms, the average number of dairy cows and milking cows were 48 (28-75) and 19 (11-31), respectively. The average of milk production was 12.91 kg/cow/day. The cows were fed with a concentrate from agricultural products (rice bran, cassava chip, and corn meal or soya-bean meal). Fresh grass was cut and brought to feed as roughage source in the rainy season and was commonly replaced by rice straw during dry season. Crude protein and metabolizable energy were different among dairy farms depending on lactation number and days in milk. Two hundred and eighty five milking cows were tested by CMT reagent and yielded only 103 milking cows with a CMT score of 3. Average SCC was 5,337,202 cells/ml. Approximately 83.8% of milk samples (119/142) were positive bacterial culture. Milk samples from quarters with a CMT score of 3 and identified bacteria were more likely to indicate the bacterial infection (odds ratio = 5.18, *p*=0.00, CI= 3.26-8.23). Bulk milk analysis revealed the contamination of bacteria from the environment and possibly infected quarters. The milk sample analyses were summarized in Table 4.

The liner slip also significantly increased the prevalence of subclinical mastitis. Moreover, abnormal vacuum pressure was strongly associated in subclinical mastitis problems in the dairy cows. Milking management was affected directly to the prevalence of subclinical mastitis. Using a single towel, cleaning and drying udders and checking the milk by strip cubs before milking were significantly associated with the prevalence of subclinical mastitis. The odds ratios, *p*-values and 95% confidential intervals for subclinical mastitis associated with each risk factor were summarized in Table 5. The cows with a poor body condition scores and abnormal hoof score had significantly higher subclinical mastitis than the others. However, lower subclinical mastitis was found in low parities as compared with high parities. The odds ratios for subclinical mastitis associated with health factors were recorded (Table 6).

Table 3 Estimated feeding composition in the feedings

Farm	Nutritional values				Rations
	Crude protein (%)		Energy (Mcal/kg)		Concentrate : Roughage
	Feeding	Requirements	Feeding	Requirements	
1	10.85	11.18	2.40	2.27	65.6 : 34.4
2	10.50	10.76	2.64	2.23	67.6 : 32.4
3	11.03	12.29	2.52	2.29	59.7 : 40.3
4	8.79	11.45	2.63	2.28	77.5 : 22.5
5	10.26	12.19	2.84	2.34	84.7 : 15.3
6	9.06	11.84	2.72	2.32	62.2 : 37.8
7	10.31	10.81	2.57	2.24	67.4 : 32.6
8	13.58*	11.01*	2.55	2.25	67.6 : 32.4
9	11.48	11.84	2.89	2.32	74.5 : 25.5
10	8.48	9.42	2.21	2.12	37.1 : 62.9
11	10.45	11.92	2.38	2.25	42.4 : 57.6
12	8.93	11.15	2.79	2.26	65.4 : 34.6
13	***10.48****	***9.63****	2.59	2.14	58.4 : 41.6
14	9.63	12.06	***2.27*****	***2.34*****	49.0 : 51.0
15	9.63	11.86	2.46	2.46	61.9 : 38.1
16	10.16	11.74	2.46	2.24	61.8 : 38.2

* Higher percentage of crude protein and **lower energy than NRC recommendations

Table 4 Milk sample analysis

Variables		Results	
Quarter milk analysis			
Prevalence of subclinical mastitis			
CMT negative		7.35%	
+1 and higher		92.64%	
+2 and higher		67.64%	
+3		36.14%	
Bacterial identification			
Contagious pathogens		9.85%	
CNS		24.64%	
Streptococcus spp.		26.76%	
Others		14.78%	
SCC in CMT 3	mean±SD	5,337,202±3,346,600	cells/ml
	maximum	18,058,000	cells/ml
	minimum	990,000	cells/ml
	uncountable	17.5%	(24/137)
Bulk milk analysis			
Mean bulk milk SCC (±SD)		708,000±440,000	cells/ml
Total bacterial count		435,743 ± 713,862	cfu/ml
Bacterial identification			
Contagious pathogens		31.25%	
CNS		68.75%	
Streptococcus spp.		37.50%	
Enterobacter spp.		50.00%	
Others		37.50%	

CMT: California mastitis test, CNS: coagulase negative staphylococci, cfu: colony-forming unit, SCC: somatic cell count

Table 5 Summary of statistical analysis of potential risk factors from mastitis control program, milking machine and milking managements on subclinical mastitis.

Variables	Odds ratio	*P* values	95 % CIs
Mastitis control			
CMT screening test	0.92	0.82	0.48-1.77
Dry cow therapy	0.66	0.41	0.24-1.79
Milking machine			
Vacuum pressure	2.18**	0.01**	1.16-4.10**
Pulsation	1.12	0.63	0.69-1.80
Liner	2.00**	0.00**	1.22-3.26**
Milking management			
Prepare udder			
Washing	1.23	0.41	0.74-2.04
Chlorine	1.75	0.09	0.90-3.37
Strip cubs	1.83 *	0.01**	1.12-2.98**
Towel	3.37**	0.00**	2.05-5.55**
Clean and dry	2.18**	0.01**	1.16-4.10**
Milking time			
Slip claw	2.83**	0.00**	1.64-4.90**
Weight used	1.32	0.35	0.73-2.40
Claw detachment	0.91	0.78	0.48-1.71
Teat dipping	1.19	0.51	0.70-2.03

** Significant

Table 6 Relationship between occurrences of subclinical mastitis and risk factors

Health indices	Odds ratio	*P* values	95 % CIs
BCS			
Good	0.89	0.65	0.51-1.58
Fair	0.64	0.09	0.38-1.07
Poor	2.66 **	0.01**	1.22-5.76**
DIM (days)			
0-60	0.61	0.16	0.30-1.22
60-120	1.25	0.52	0.63-2.47
> 120	1.15	0.59	0.67-1.97
Hoof Score			
Normal	0.56	0.02	0.33-0.99
Abnormal	1.78**	0.02**	1.06-2.98**
Parity			
1-3	0.54**	0.04**	0.30-0.90**
4-6	1.41	0.28	0.74-2.68
>6	2.37	0.09	0.86-6.49
Feeding			
Concentrate : Roughage	1.15	0.59	0.68-1.94

**Significant

Discussion

In 14 farms (87.5%), the crude protein in feed was lower than the nutritional requirement for dairy cows (NRC, 2001). However, 15 farms (93.8%) had higher metabolizable energy than the NRC recommendation (NRC, 2001). The ratio of concentrate to roughage was higher than 65% in 8/16 farms in the study. The estimated nutritional values were calculated by computer software containing information of all local feed ingredients (Wachirapakorn, 2003), which was very helpful for both farmers and practitioners. The prevalence of subclinical mastitis was 36.14% (103/285), which was 26.06% less than the previous report (62.2%) in the same area in 1999 (Aiumlamai, 2000). The prevalence of subclinical mastitis in CMT positive and more than score 1 was 92.64%, 67.64% respectively. However, the previous study was done by collecting milk samples from every quarter; thus, possibly resulting in a high prevalence of subclinical mastitis. The risk factor of the mastitis control program for both the screening for CMT and the administration of dry cow therapy on subclinical mastitis was insignificant. Only 5.76% (17/295) of the milking cows were not managed by dry cow therapy; therefore, this risk factor on subclinical mastitis was not found in this study, which can be explained by too small sample size affecting the a statistical analysis model (STATA, 2001). However, most studies showed that dry cow therapy potentially prevent new IMI in postpartum and treated cows with subclinical mastitis at drying off

(Sol et al., 1994; Shpigel et al., 2006).

The vacuum pressure in the pipe-line of the milking system had a great impact on subclinical mastitis, which corresponded to most other studies that showed high risk between vacuum pressure and mastitis (Dahl et al., 1993; Rasmussen and Madsen, 2000). Higher or lower vacuum pressure directly involve teat tissue and the teat canal, thus, leading to a decreasing natural protection of the udder (Zecconi et al., 2000) and predisposing factors for teat duct colonization by environmental pathogens (Zecconi et al., 1992). Previous studies reported an increasing new IMI in high liner slips (Rasmussen and Madsen, 2000; Baxter et al., 1992). This study did not show the effect of pulsation on subclinical mastitis. However, Capuco et al. (1994) reported the changes of mean somatic cell count in the presence or absence of pulsation. Milking management was an important part of the mastitis control and was directly related to subclinical mastitis. Checking udders before milking by strips cup, and using single towels could prevent new IMI. Using single towels made udders clean and dry before milking. Cows in low parities had a lower risk of subclinical mastitis than those in high parities. Janosi and Baltay (2004) reported that milking cows with high lactation number had high milk SCC. The condition of liner was significantly associated with the prevalence of subclinical mastitis. A cracked liner and low elasticity caused teat lesions and new IMI (Bakke and Binde, 1984; Miltenburg et al., 1997).

This study demonstrated that poorly conditioned dairy cows had a high average SCC, which was possibly related to low immune function (Heuer et al., 1999; Berry et al., 2007). The dairy cows' health factors significantly affected subclinical mastitis especially in cows with a poor body conditions and abnormal hoof scores. Although the concentration in a ratio of more than 60% was not associated with subclinical mastitis, abnormal hoof score and poor body condition score were high odds ratio in prevalence of subclinical mastitis. The feeding management directly affected the body condition score and hoof score. Apparently, feeding with high concentration was associated with acidosis condition and laminitis in the dairy cows. Inflammation of hoof tissue and laminitis stimulated the hoof tissue overgrowth in the dairy cows (Nocek, 1997). The high incidence of laminitis was associated with decreased milky yield in the dairy cows (Nocek, 1997; Hernandez et al., 2005). The feeding management before calving influenced the body condition scores at postpartum. Overfeeding and high body condition score at prepartum created high problems of negative energy balance in postpartum cows (Rukkwamsuk et al., 1999). Relationship between negative energy balance and mastitis incidence in the dairy cows were reported (Janosi et al., 2003).

Conclusion

Subclinical mastitis is still one of the most important health problems in small holder dairy farms in Thailand and can be found at any stage of lactation. *CNS* and *Streptococcus* spp. are major isolated microorganisms. CMT can be commonly used as a routine screening test for subclinical mastitis in dairy cattle because milk samples with high SCC (CMT score of 3) have more opportunity to yield positive culture of mastitis-causing bacteria. Furthermore, many factors caused risks on subclinical mastitis in this study. No strip cups and single towels had a high correlation to subclinical mastitis demonstrated by a high odds ratio. As a key to mastitis control, the udder should be clean and dry before milking. The vacuum pressure and liner highly affects the prevalence of subclinical mastitis. The body condition score and hoof sore might be useful in the prediction of the prevalence of subclinical mastitis in dairy farms as well.

Acknowledgements

This study was supported by the Faculty of Veterinary Medicine, Khon Kaen University, Thailand. Asst Prof Varaporn Sakulpong and staff from the Department of Pathobiology are also acknowledged for their contributions in the microbiological processes.

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