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Evaluation of the Appropriate Diagnostic Tools for Intra-Mammary Infection in Lactating Dairy Goats

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ABSTRACT

Sixty milk samples of healthy, lactating dairy goats were collected by an aseptic, hand milking technique. All samples were analyzed using milk quality tests: bacterial isolation (Bac), total plate count (TPC), dye reduction test (DRT) and clot-on-boiling test (COB), as well as screening tests: the Californian mastitis test (CMT) and electrical resistance (ER), and somatic cell count (SCC) and percentage of milk composition including fat, protein, lactose and solid-not-fat (SNF). According to 34 samples for intra-mammary infection (IMI) and 26 samples for non-IMI, the results showed that Bac and TPC were the most reliable techniques for the determination of IMI with strong correlations. For an instantaneous milk quality test, CMT reaction was probably more reliable as there was a statistical significance between bacteriologically positive and negative samples ($P = 0.06$). Moreover, CMT showed a correlation with TPC, Bac, DRT, ER, ER ref, SCC, milk protein, and milk lactose ($P \leq 0.05$). Based on the range in ER, TPC showed a statistically significant difference between the ER level being less than 350 units and higher than 400 units ($P \leq 0.05$). Except for CMT reactions, SCC did not correlate to other measurements and did not indicate the IMI status of goats. These findings indicated that there was a need for future improvement in the diagnostic tools for IMI in goats.

Key words: intra-mammary infection, Californian mastitis test, electrical resistance, somatic cell count, goats

INTRODUCTION

Mastitis is the inflammation of the mammary gland. It has been classified into clinical mastitis and sub-clinical mastitis. Clinical mastitis presents with signs of localized inflammation and abnormal milk. With sub-clinical mastitis, in which no change in the milk is apparent, only a slight degree of inflammation can be found. The annual incidence of clinical mastitis in small ruminants is generally lower than 5% but the prevalence of sub-clinical mastitis has been estimated at 5–30% or even higher (Contreras *et al*., 2003; Moroni *et al*., 2005b). There are some serious risks with regard to both the production of good, hygienic-quality milk and financial loss by farmers (Leitner *et al*., 2008). Moreover, sub-clinical mastitis mostly affects milk yield and milk composition (Moroni *et al*., 2005a; Raynal-Ljutovac *et al*., 2007). Because of its importance, there has been some research using existing techniques to evaluate sub-clinical mastitis in goats.

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In dairy cows, somatic cell count (SCC) in milk is commonly used as an effective index of udder health and milk quality but it remains of arguable value in goats (Hinckley, 1990; Haenlein, 2002), although somatic cell counts in the milk of some healthy goats has been higher than in unhealthy animals. Various factors that have influenced this are the physiological differences in the process of milk secretion and the defense mechanism in the goat udder (Moroni *et al.*, 2005a). Milk secretion in goats is apocrine, producing a fluid secretion by pinching off one end of the secreting cells, compared to merocrine in cows that discharge without major damage to cells. Consequently, cytoplasmic particles are shed into the milk and these cell-like fragments can lead to erroneous SCC results. Interestingly, normal neutrophils in SCC from free intra-mammary infection goat's milk make up to 50 to 70%, whereas neutrophils only make up 5 to 20% of the total cell count in cow's milk (Paape and Capuco, 1997). Moreover, SCC is influenced by non-infectious and environmental factors, the stage of lactation, parity and stress (Dulin *et al.*, 1983; Haenlein, 2002; McDougall and Voermans, 2002). With such variances, applying the standards for cow's milk without justification seems to be inappropriate for goat's milk.

As sub-clinical mastitis cannot be visually detected and is sometimes hard to evaluate, for economic and practical reasons, easy-to-apply, indirect methods and equipment have been developed to assess intra-mammary infection (IMI) for commercial cow milk production. The tests include: the Californian mastitis test (CMT), SCC, electrical resistance (ER), milk composition analysis and other milk quality tests. To date, there is no proper procedure available for testing goat's milk. Although bacterial culture is recognized as the gold standard to detect IMI, it is a costly, complicated, time-consuming procedure and impractical for fieldwork. As significant differences exist among dairy ruminants, any approach to mastitis control in goats should be carefully thought out and have a specific aim. The objectives of the present investigation were to compare milk from IMI and healthy udders as determined by different diagnostic tools and to determine the possible effect of the mammary bacteriological status on CMT, ER, SCC, milk quality and milk composition as well as any correlations.

MATERIALS AND METHODS

Animals and milk sampling

The animals studied were mixed breed dairy goats, managed under a semi-intensive system. A total of 60 milk samples from three farms in three different districts in Kanchanaburi province, Thailand were collected from goats without clinical mastitis. For the sampling procedure, teat ends were cleaned with 70% alcohol before sampling. The first streams of foremilk were discarded prior to performing ER and CMT respectively. The next 30 ml of milk were collected into a sterile container using an aseptic technique for hand milking (one udder per vial) and were kept at 4°C until bacteriological procedures, SCC, milk quality and milk composition analysis were carried out.

Electrical resistance (ER) and Californian Mastitis Test (CMT)

A mastitis detector (Dramiński®) was utilized to detect the ER of milk from each udder half. CMT was conducted according to Gomes *et al.* (2006). Each 2 ml of foremilk was drawn into a testing paddle, mixed with 2 ml of CMT reagent by a gentle circular motion of the paddle in the horizontal plane and the observed reaction scores were recorded within 10 sec. The scores of reactions were graded as negative, trace, 1+, 2+ and 3+, where negative and trace meant clean and slightly accepted, and 3+ represented the most affected status.

SCC, milk composition and milk quality

Milk samples were homogenized at 40°C, then the composition of fat, protein, lactose and solid-not-fat (SNF) was measured using automated infrared analysis (MilkoScan™, IDF and AOAC approved), while SCC was analyzed using a Coulter® Counter. Milk quality was assessed by analyzing the samples using a dye reduction test (methylene blue reduction test) and clot-on-boiling was conducted using the Thai FDA standard.

Bacteriological procedures

A tenfold serial dilution of each milk sample was prepared in sterile NSS + 0.1% peptone up to 10^{-3} dilution. Subsequently, 1 ml of each dilution was poured onto blood–agar plates (5% defibrinated horse blood) and MacConkey plates. The plates were incubated aerobically at 37°C and examined at 24 h post-seeding. The colonies were provisionally identified based on morphology, haemolysis pattern and Gram stain. The numbers of each distinct colony type were recorded. Representative colonies were subcultured on blood–agar plates and MacConkey plates and incubated aerobically at 37°C to obtain pure colonies. Gram-positive cocci were tested for catalase and coagulase production. An IMI was diagnosed when ≥100 cfu/ml (2 colonies/plate) of the same organism was found. Cultures with two or more identical colonies were classified as positive (Contreras *et al*., 1999).

Statistical analysis

As the counts were on non-parametric data, the Mann–Whitney test was used, and values were expressed as a median. Spearman's analysis was used to analyze correlations among various measurements. All statistical data were processed and analyzed with the SPSS program. Tests were accepted as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

In the present study, sixty samples were analyzed using various techniques including: total plate counts (TPC), bacterial isolation (Bac), the dye reduction test (DRT), the clot-on-boiling test (COB), the Californian mastitis test (CMT), electrical resistance (ER), electrical resistance reference (ER ref), somatic cell count (SCC) and other milk composition analysis (percentage of fat, protein, lactose and SNF). The percentages of these reactions classified by the number of 100 cfu per ml TPC (Contreras *et al*., 1999) are presented in Table 1, with 34 samples for IMI positive (TPC ≥ 100 cfu/ml) and 26 samples for IMI negative (TPC < 100 cfu/ml).

As expected, no bacteria could be isolated from the IMI negative samples. Although the same situation also occurred in some IMI positive samples (35.29%), most samples could be isolated (64.71%). Most of the pathogens isolated from the infected milk samples were coagulase-negative staphylococci (CNS) species (38.24%), similar to figures reported previously (Deinhofer and Pernthaner, 1995; Poutrel *et al*., 1997; McDougall *et al*., 2002; Ajuwape *et al*., 2005; Moroni *et al*., 2005a).

The evaluation of microbial activity using DRT, indicated grading excellence in most populations of non-infected milk samples (86.96%), whereas grading was fair and poor in infected milk samples, with 11.76% for each, respectively. DRT evaluations between non-infected and infected milk samples were significantly different ($P = 0.009$). Another milk quality test, COB, had a lower reaction indicating the IMI in this study.

CMT reactions with values of 2+ and 3+ were rarely observed in uninfected milk samples (4.35%), whereas they were high in IMI milk (32.36%). In addition, CMT scores of negative and trace were higher in the bacteriologically negative samples. These results were in agreement with

Table 1 The percentage of evaluated results from various measurements according to the total plate count (TPC) in goat's milk.

	TPC (cfu/ml)	
	< 100	≥ 100
Identify		
N/A	100.00	35.29
Staphylococcus aureus	0.00	8.82
CNS	0.00	38.24
Gram negative	0.00	17.65
Dye reduction test		
Excellent	86.96	52.94
Good	8.70	23.53
Fair	0.00	11.76
Poor	4.35	11.76
Clot on boiling		
Negative	100.00	97.06
positive	0.00	2.94
Californian Mastitis test		
negative	78.26	58.82
trace	13.04	2.94
1+	4.35	5.88
2+	0.00	14.71
3+	4.35	17.65
Electrical resistance (unit)		
<250	0.00	0.00
250-300	0.00	0.00
300-350	13.04	50.00
350-400	47.83	14.71
>400	39.13	35.29
Electrical resistance reference		
<50	100.00	5.88
≥50	0.00	94.12
Somatic cell count		
<800,000	82.61	58.82
800,000-1,500,000	4.35	17.65
1,500,000-3,500,000	8.70	14.71
>3,500,000	4.35	8.82

previous reports that lower percentages were recorded in samples of normal goat's milk with higher positive CMT scores (Haenlein, 2002) and the positive bacteriology in the milk samples was associated with high CMT score (Boscos *et al.*, 1996). Even though there was no statistical significance, the difference in CMT scores between infected and non-infected milk samples from lactating goats tended to indicate sub-clinical mastitis ($P = 0.06$) in this experiment.

By using the mastitis detector (equipment that was specifically invented for cows), the ER value exceeded 300 units in all samples. This result indicated that the standard range used with cows was not appropriate for the determination of sub-clinical mastitis in goats. (Electrical resistance interpretation for cows: < 250 units indicates acute subclinical mastitis or high risk of subclinical mastitis; 250-300 units indicates the risk of sub-clinical mastitis; and > 300 units indicates good quality milk or low risk of subclinical mastitis.) Even though new additional ranges for goats were assigned with values of 300-350 units, 350-400 units and more than 400 units, no significant difference was found between bacteriologically positive and bacteriologically negative samples. However, by using the ER reference[b] interpretation, all of the non-bacteriologically active samples were lower than 50 units, whereas 94% of bacteriologically active samples were higher than 50 units. (Electrical resistance reference (ER ref) is the difference in ER units between the highest ER unit and the lowest ER unit that indicated the risk of sub-clinical mastitis in cows: < 50 units for good quality milk and ≥ 50 units indicates a risk of sub-clinical mastitis.) These results indicated that the ER detector might be useful in a screening test for sub-clinical mastitis in goats.

The analysis of SCC results indicated there were 4 categories for goats, namely: cells lower than 0.8×10^6 SCC/ml (high-quality milk); 0.8×10^6 to 1.5×10^6 SCC/ml (medium-quality milk); 1.5×10^6 to 3.5×10^6 SCC/ml (low-quality milk); and higher than 3.5×10^6 SCC/ml (unacceptable milk) (Leitner *et al.*, 2008). The SCC data showed that the greater number of cells did not indicate the IMI status in goats. In addition, no significant difference was found between bacteriologically positive and bacteriologically negative samples. These phenomena can be explained by the apocrine milk secretion procedure of mammary cells in goats, which produces cytoplasmic particles and goat's milk contains a large number of the epithelial cells (Zeng and Escobar, 1995). It has been reported that the use of a Coulter counter might be considered inappropriate for goat's milk (Boscos *et al.*, 1996; Haenlein, 2002). Moreover, a Coulter counter has been suggested to be appropriate only for the prediction of a major pathogen such as *S. aureus* in goat's milk (Boscos *et al.*, 1996). Although, SCC determined using Fossomatic has been reported to be associated with bacterial infection of the mammary gland (McDougall *et al.*, 2002), unfortunately, this method was not included in this study.

The correlation coefficients of tested variables are summarized in Table 2. The analyses indicated that, except for COB, most of the milk quality tests (Bac, TPC and DRT) had a relationship. Bac had a positive relationship with TPC ($P < 0.001$) and DRT had a negative relationship with Bac and TPC ($P = 0.006$ and $P = 0.001$, respectively). This observation indicated that these were appropriate, reliable methods for goat's milk evaluation. However, they have been considered as inappropriate methods for instantaneous milk quality testing as they require expertise or access to laboratories or involve time-consuming procedures. CMT showed a relationship with nearly all measurements (Table 2). This report implied that CMT might be facilitated for sub-clinical mastitis as an appropriate screening test for goats. As the CMT showed it could be classified according to non-

infected and infected udders in prior discussion ($P = 0.06$), this measurement seemed to be more reliable in a leukocyte count and for detecting infection. This phenomenon has been explained by CMT accounting specifically for deoxyribonucleic acid recognition (Galina *et al.*, 1996).

For further consideration, various measurements according to CMT reactions are listed in Table 3. Milk composition analysis showed that protein had a positive relationship ($P = 0.02$) but lactose had a negative relationship ($P < 0.001$) with CMT reactions (Table 2). However, classification by CMT reactions only, showed a statistically significant difference between CMT negative or trace to 3+. These activities were similar to the previous report that lactose concentration was significantly lower while the whey protein concentration was significantly higher in the CNS-infected mammary gland of goats. This can be explained by an increase in the plasmogen activator and plasmin activities as a result of the accelerated conversion of plasminogen to plasmin in the infected glands (Leitner *et al.*, 2004). Interestingly, though statistical differences were reported, a higher score of CMT reaction did not indicate a greater number of somatic cells at each level (Table 3). The results

Table 2 Correlation coefficients among total plate count (TPC), bacterial isolation (Bac), dye reduction test (DRT), clot-on-boiling test (COB), Californian mastitis test (CMT), electrical resistance (ER), electrical resistance reference (ER ref), somatic cell counts (SCC), percent fat, percent protein, percent lactose and percent solid-not-fat in the milk of healthy goats.

	Bac	DRT	COB	CMT	ER	ER ref	SCC	Fat	Protein	Lactose	SNF
TPC	0.724**	-0.422**	0.066	0.319*	-0.219	-0.023	0.174	-0.121	0.091	-0.236	-0.031
Bac		-0.357**	0.150	0.360**	-0.144	0.070	0.262	-0.115	0.037	-0.195	-0.041
DRT			0.092	-0.336*	-0.007	-0.126	-0.138	-0.033	-0.200	0.144	-0.226
COB				0.184	-0.171	0.094	0.154	0.162	-0.008	-0.130	-0.130
CMT					-0.276*	0.349**	0.281*	0.006	0.306*	-0.418**	0.116
ER						-0.196	-0.175	0.271*	0.073	0.627**	0.324*
ER ref							0.165	-0.046	0.149	-0.275*	0.067
SCC								0.247	0.130	-0.344**	0.023
Fat									0.493**	-0.127	0.385**
Protein										-0.268*	0.863**
Lactose											0.057

*$P \leq 0.05$; **$P \leq 0.01$

Table 3 Various measurements parameters according to CMT reactions in the milk of healthy goats.

CMT	Median [a]							
	TPC (cfu/ml)	ER (unit)	ER ref [a] (cell/ml)	SCC (cell/ml)	milk composition			
					Protein (%)	Lactose (%)	Fat (%)	SNF (%)
Neg.	409 [a]	400 [a]	0 [a]	462,400 [a]	3.51 [a]	4.56 [a]	4.64 [a]	9.01 [ac]
Trace	0 [a]	445 [ab]	20 [ac]	289,050 [ac]	4.34 [bc]	4.53 [ac]	6.08 [a]	9.72 [bd]
1+	181 [ad]	380 [ab]	30 [bc]	1,650,800 [ac]	4.76 [ac]	4.39 [ac]	5.07 [a]	9.90 [ab]
2+	22,364 [bd]	330 [ab]	10 [ac]	1,114,700 [bc]	3.52 [a]	4.21 [ac]	4.18 [a]	8.60 [a]
3+	51,273 [cd]	350 [b]	10 [ac]	448,500 [ac]	4.03 [ac]	4.17 [bc]	4.45 [a]	9.06 [bc]

[a,b,c,d] Different letters show significant statistical differences ($P \leq 0.05$).

implied that even though SCC correlated with CMT, it did not show any evidence related to mastitis, which was in agreement with the previous report that total SCC did not correlate with mastitis, but correlated with CMT (Galina *et al.*, 1996).

In addition to the CMT reactions, ER was considered to be another option for instantaneous milk testing. According to the CMT reactions, the greater the CMT reactions, the lower the ER levels. However, a statistical significance was only observed with the negative and 3+ CMT scores (Table 3). When the range of ER levels and ER ref were used in the analysis of TPC and SCC, the statistical difference in TCP have promoted the ER equipment more confidential with the statistical significantly difference between the lesser than 350 unit and the higher than 400 unit (Table 4). Moreover, the ER measurement correlated to the changes in the milk content of lactose and SNF ($P < 0.001$ and $P = 0.01$ respectively) without any significant correlations between the level of SCC and ER in this experiment. This was similar to a previous report (Haenlein, 2002). As SCC did not show any statistically significant difference and did not relate to any measurements, this phenomenon indicated that SCC was an insufficient measurement to classify the bacteriological status of goat's udders. However, further investigations are required.

CONCLUSION

The most reliable methods to classify the bacteriological status in a goat's udder were milk quality tests including bacterial culture, TPC and DRT. As these require skilled technicians to carry out the complicated laboratory procedures, which are also time-consuming, these are considered inappropriate procedures for instantaneous milk quality testing. For this purpose, the CMT reaction was a more simple, reliable and practical procedure for fieldwork. In addition to CMT, ER was also considered as another screening test of choice, though adjustment procedures and some calibrations specifically for goat's milk are required. SCC did not perform well in this study, suggesting that DNA-specific methods would have to be employed to obtain accurate results. These outcomes suggested that there was a need for improvement in existing methods or the adoption of other methods for future IMI diagnosis in lactating goats.

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Table 4 Total plate count (TPC) and somatic cell count (SCC) according to the ranges of electrical resistance (ER) in the milk of healthy goats.

Unit	Median [a]	
	TPC(cfu/ml)	SCC (cell/ml)
ER		
<350	18,136 [a]	605,700 [a]
350-400	91 [ab]	475,300 [a]
>400	182 [b]	440,100 [a]
ER ref		
<50	1000 [a]	476,400 [a]
≥50	30,909 [a]	301,300 [a]

[a,b] Different letters show significant statistical differences ($P \geq 0.05$)

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