Media Peternakan, Desember 2008, hlm. 155-165 Vol. 31 No. 3
ISSN 0126-0472
Terakreditasi B SK Dikti No: 43/DIKTI/Kep/2008

Contamination Level of *Staphylococcus* spp. in Raw Goat Milk and Associated Risk Factors

E. Taufik [a] *, G. Hildebrandt [b], J. N. Kleer [b], T. I. Wirjantoro [c], K. Kreausukon [d] & F. H. Pasaribu [e]

[a] Division of Animal Product Technology, Department of Animal Production Science and Technology, Faculty of Animal Science, Bogor Agricultural University
Jl. Agatis Kampus IPB Darmaga, Bogor 16680
[b] Institute of Food Hygiene, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany
Königsweg 69 14163 Berlin, Germany
[c] Department of Food Science and Technology, Faculty of Agro-Industrial Technology, Chiang Mai University, Thailand
155 Moo 2 Tambon Mae Hia, Muang, Chiangmai 50100 Thailand
[d] Veterinary Public Health Center for Asia Pacific, Chiang Mai University, Thailand
Mae Hia, Muang, Chiang Mai 50100 Thailand
[e] Department of Animal Disease and Veterinary Public Health, Faculty of Veterinary Medicine, Bogor Agricultural University
Jl. Agatis Kampus IPB Darmaga, Bogor 16680
(Diterima 14-05-2008; disetujui 23-09-2008)

ABSTRACT

This study was aimed to investigate the presence of pathogenic bacteria in raw goat milk by using *Staphylococcus* spp. as indicator bacteria, and also to evaluate the potential risk factors associated with them. Information regarding potential risk factors was collected by questionnaire. The conventional bacteriological method for bacterial isolation and the indirect test (California Mastitis Test (CMT)) for determining udder inflammation status were employed. A sample size of 300 udder halves milk samples from three commercial dairy goat farms in the Bogor District, West Java Province, Indonesia were investigated for counts and prevalence of indicator bacteria. Ten potential risk factors were also evaluated in relation to counts and prevalence of indicator bacteria. The results showed that the median value of indicator bacterial count from overall udder-half milk samples was 3.00 log cfu/ml. The indicator bacterial count from udder-half milk samples was significantly different ($P<0.05$) among farms. Overall prevalence of *Staphylococcus* spp. was 78.7%. As one of potential risk factors, udder inflammation status was found to be risk factor for *Staphylococcus* spp. contamination in milk. Udders with inflammation had significant association and a higher chance of having contaminated samples by *Staphylococcus* spp. as compared to udders without inflammation. Additionally, according to these study results, CMT can be used as an effective, reliable, cheap and "farm and farmer friendly test" for screening test of intramammary infection (IMI) or sub clinical mastitis in dairy goats.

Key words: goat milk, Staphylococcus spp.*, prevalence, risk factor, California Mastitis Test*

* Korespondensi:
Jl. Agatis Kampus IPB Darmaga, Bogor 16680,
email:etaufik@yahoo.com

INTRODUCTION

Milk is a nutritious food for human beings, but it also serves as a good medium for the growth of many microorganisms, especially bacterial pathogens. *Lactococcus, Lactobacillus, Streptococcus, Staphylococcus* and *Micrococcus* spp. are among the common bacterial flora of fresh milk (Chye *et al.*, 2004). The importance of various etiological agents in milkborne disease has changed dramatically over time. However, more than 90% of all reported cases of dairy-related illness continue to be of bacterial origin, with at least 21 milkborne or potentially milkborne diseases currently being recognized (Bean *et al.*, 1996).

Zweifel *et al.* (2005) stated that goats and sheep rank third and fourth in terms of global milk production from different species. According to FAO (2006), Indonesia was ranked as the 14th in producing goat milk globally and ranked as the 1st in the Southeast Asia region in 2005. It was estimated to produce around 220,000 metric tons (MT) of goat milk. In 2005, the goat population in Indonesia was 13,182,000 heads in total, out of that West Java province was ranked the 3rd in the country with 1,235,973 heads of goat (DGLS, 2005). On the other hand, unlike cow's milk, hygiene and quality regulations for production and distribution of small ruminant's milk, such as goat milk are not strict in Indonesia and are not subject to specific microbiological standards in a legal sense. So far this product has less attention in terms of quality and safety control from farmer organizations and/or government institutions than those for cow's milk and milk products. On the other hand, most of the consumers prefer to drink raw goat milk due to their belief in the benefit of raw goat milk as a health promoting agent, or even disease-relief agent.

Investigation on the presence of pathogenic bacteria of goat milk together with some risk factors affecting these microorganisms in Indonesia was very rare. In view of food hygiene and consumer health as well as animal health protection, however evaluation of the microbiological status and presence of pathogenic bacteria in goat milk, which can cause adverse health effects on the animals as well as pose a high risk of causing foodborne disease in humans, is of central importance.

Staphylococcus spp. are the main aetiological agents of small ruminant's intramammary infections (IMI), the more frequent isolates being *Staphylococcus aureus* (coagulase-positive staphylococci [CPS]) in clinical cases and coagulase-negative staphylococci (CNS) in subclinical IMI (Bergonier *et al.*, 2003). *Staphylococcus* spp. can be found widely distributed in animals, and it is a contagious pathogen that can be transmitted from doe to doe during unhygienic milking procedures. High prevalence of CPS such as *S. aureus*, or CNS can be of veterinary public health concern. Both groups of bacteria are important zoonotic bacterial pathogens, which can also be transmitted to humans through goats' raw milk and cause food poisoning associated with enterotoxin production (Wakwoya *et al.*, 2006).

The objectives of this study were to investigate the presence of *Staphylococcus* spp. in raw goat milk and to evaluate the potential risk factors associated with them in dairy goat farms in Bogor District, West Java Province, Indonesia.

MATERIALS AND METHODS

Study Design

This study was a cross sectional survey to investigate the presence of *Staphylococcus* spp. in raw goat milk and the association of possible risk factors with this group of bacteria.

Study Location

Three dairy goat farms with herd sizes of 600, 400 and 200, respectively, in the Bogor District, West Java Province, Indonesia were conveniently selected as sampling sites.

Questionnaire

Questionnaires were used for collecting information regarding possible risk factors, which reflected udder, teat and the goat's general condition. The questionnaires were completed during the farm visits.

Type of Sample

The milk sample that was obtained in this study was individual udder half (left and right udder) milk of lactating goats, which was collected at the time of sampling visits.

Laboratory Investigation Standard Procedures

General rules for the preparation of the initial suspension and dilutions were based on ISO 6887-1 (1999). To investigate the presence and enumeration of *Staphylococcus* spp. in the sample, the ISO 6888-1 (1999) standard technique using Baird Parker agar medium was used. The California Mastitis Test (CMT) was conducted according to Shearer & Harris (2003) to determine the inflammation status of the udder.

Sample Size Determination

The Win Episcope® Version 2.0 1998 program was used to determine sample size based on estimate prevalence. As the most prevalent intramammary infection (IMI) causing agent according to many scientific reports, the prevalence of CNS in goat milk was used as the expected prevalence. Since no report was found from Indonesia regarding CNS prevalence in goat milk, the prevalence of 25% of CNS reported by Bergonier *et al.* (2003) was used. At a 95% level of confidence and 5% accepted error, the sample size of 288 was obtained and then it was rounded up to 300 samples. If the prevalence turns out to be the expected value, the true prevalence will be between 20.78 and 29.22%.

Sampling Strategy

Three dairy goat farms in the Bogor District, West Java Province, Indonesia with herd sizes of 600, 400 and 200 were included in the study. The sampling period was done in the rainy season, starting from early December 2006 until the end of March 2007. The sample size was distributed to all farms equally, therefore from each farm 100 udder-half milk samples (from 50 different individual lactating goats) were collected. The equal distribution of sample size to each sampling farm was due to the fact that the object of this study was only the lactating goats. The number of lactating goat varied among farms during sampling time, it depend on the stocking and reproduction management and also herd size composition (number of bucks, does and kids) of each farm. The apparently healthy lactating goats were selected conveniently in a studied farm during a visiting time. Each lactating goat was marked after milk sampling to avoid redundancy in the sample collection.

Observation of the general condition of the selected dairy herd, including examination of variation in teat and udder conformation, udder cleanliness and any abnormalities of the individual goat was recorded. Approximately 10 ml of pre-milking milk samples were collected into sterile bottles from each udder half (left and right). Milk samples were directly kept at $\leq 4^{o}$C and transported in an icebox to the laboratory for microbiological analysis within 3 hours. The milking process was done by the farmer/milker, teat disinfection was carried out prior to the milk sampling using alcohol and fore-stripping was done before the main sample collection.

CMT was done by mixing 3 ml of milk sample with 3 ml of CMT reagent (provided by Faculty of Veterinary Medicine, CMU) in the CMT paddle. By gently rocking the CMT plate, the sample and reagent were carefully mixed and the result was observed within around 20 seconds. The CMT scores were 0, trace, +1, +2 and +3 (Shearer & Harris, 2003).

Table 1. List of potential risk factors related to goats, udder and teat condition

Factors	Description
Breed	Breed of animal
Parity	Parity number
Lactation stage	Stage of lactation
Udder symmetry	Symmetry of udder
Udder hygiene	Score of udder hygiene
Teat end condition	Score of teat end condition
Teat skin condition	Score of teat skin condition
Teat shape	Teat shape condition
Udder inflammation status	Inflammatory status of the udder (based on CMT test result)
Milk appearance	Normality of milk appearance

For determining the status of udder inflammation, the score of CMT was further classified into two categories: negative and positive. The negative score was represented by CMT scores of 0 and "trace" and the positive score (indicator of subclinical mastitis/ intramammary infection) by CMT scores of +1, +2 and +3 (Wakwoya *et al*., 2006).

Information Regarding Potential Risk Factors from Questionnaire Survey

Information regarding potential risk factors related to goats and udder, as well as teat condition, was collected by using the questionnaire. The investigators collected all information during farm visits. There were 10 potential risk factors obtained in this study and listed in Table 1. Some of the risk factors were subjectively scored by the investigator based on an adoption of the available scoring standard for dairy cows.

Data Management and Statistical Analysis

Laboratory and questionnaire data were managed by using Microsoft Office Excel version 2003. Databases were prepared for each type of data and later merged into one. Descriptive statistics were used to describe enumeration and prevalence data. The prevalence estimates were determined by using the standard formula (i.e. the number of positive samples divided by the number of total samples examined). Chi-square univariate analysis was performed to evaluate the impact

Table 2. Selected statistical values of *Staphylococcus* spp. count from udder half milk samples (n= 100/farm)

Indicator bacteria	Selected statistical value	Overall [n=300]	Farm 1	Farm 2	Farm 3	P-value (among farms)
		log cfu/ml				
Staphylococcus spp.	Median	3.00	2.59	3.34	2.83	0.029
	Maximum	6.51	6.10	6.51	5.70	
	Minimum	1.70	1.70	1.70	1.70	
	IQR*	2.10	2.48	1.70	2.00	

* IQR = Inter Quartile Range.

of each potential risk factor (derived from the questionnaire responses) to the pathogenic outcomes (present or not present) in samples. McNemar Chi-square test was used to compare the true proportion of positive results among two testing methods, whilst Cohen's kappa coefficient was used to evaluate the agreement between two test results. The multiple logistic regression model was carried out to evaluate the impacts of particular risk factors without interaction of each factors. Mann-Whitney U test or Kruskall-Wallis one way ANOVA (depending on the number of data groups) were used to evaluate statistical significance in bacterial population among farms and within the evaluated potential risk factors (Petrie & Watson, 1999; Dawson & Trapp, 2004).

RESULTS AND DISCUSSION

Results of Bacterial Isolation and Enumeration

Table 2 shows the compilation of some selected statistical values of *Staphylococcus* spp. count data from udder-half milk samples. The median values of overall samples (n=300) was 3.00 log cfu/ml. Within the farm level (n=100/farm), the highest median value of *Staphylococcus* spp. was 3.34 log cfu/ml (Farm 2) and the lowest was 2.59 log cfu/ml (Farm 1). Statistically significant difference ($P<0.05$) of this indicator bacterial count was observed among farms ($P= 0.029$).

Figure 1 shows the descriptive statistics of *Staphylococcus* spp. counts in each sampling farm. *S. aureus* is a member of *Staphylococcus* spp. group of bacteria, therefore *Staphylococcus* spp. was compared with the *S. aureus* maximum limit standard from available food standards which have specific microbiological quality for raw goat milk, i.e. European Council (EC) Directive 92/46/EEC (1992) and German "Milchverordnung" (BGBl Teil I Nr. 58 S 2794, 2004). There is also an Indonesian national standard, i.e. Standar Nasional Indonesia (SNI) for microbiological quality of fresh milk but it was designed for fresh cow milk (SNI 01-3141) (BSN, 1998). There is no specific Indonesian national standard for microbiological quality of goat or sheep milk exists up to date. The median values of *Staphylococcus* spp. counts from farm 1 and 3 (Table 2) did not exceed the maximum limit of *S. aureus* in EC Directive 92/46/EEC (1992) and German "Milchverordnung" (BGBl Teil I Nr. 58 S 2794, 2004) which is 3.30 log cfu/ml. The median value of *Staphylococcus* spp.

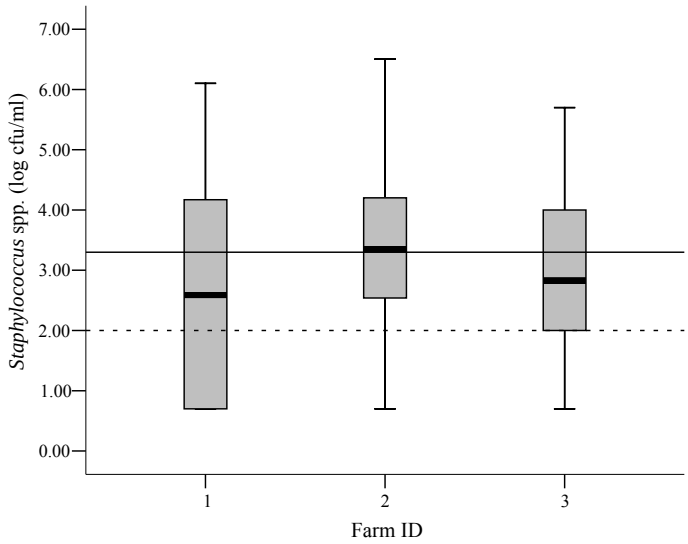

Figure 1. Box and Whisker plots of *Staphylococcus* spp. counts in three farms compared to the maximum limit of available standards (—— = SNI, ····· = EC Directive and "Milchverordnung").

Table 3. *Staphylococcus* spp. count from udder-half milk samples among level of each factor (n = 300)

Factors/Level	n	Median (log cfu/ml)	P-value
Breed			
Ettawa Crossbreed (EC)	176	3.30	0.014
Saanen Crossbreed	102	2.68	
Jawarandu (Local crossbreed)	22	2.77	
Parity			
First	66	2.48	0.000
Second	114	2.82	
Third	80	3.70	
Fourth	32	3.15	
Fifth	8	4.01	
Lactation stage			
First	183	3.10	0.004
Second	66	2.48	
Third	51	3.30	
Udder symmetry			
Yes	176	3.09	0.774
No	124	2.90	
Udder hygiene			
Free of dirt	296	3.02	0.257
Slightly dirty	4	2.09	
Teat end condition			
No ring	225	2.80	0.003
Smooth rough ring	75	3.75	
Teat skin condition			
Free from scales/smooth	295	3.00	0.406
Shows some scaling	5	3.92	
Teat shape			
Normal	166	2.79	0.052
Dilated	117	3.34	
General dilated	17	3.42	
Udder inflammation status			
Yes	186	3.56	0.000
No	114	2.48	
Milk appearance			
Normal	293	3.00	0.738
Abnormal	7	3.53	

count at farm 2 (3.34 log cfu/ml) was slightly exceeding the above mentioned standards.

The median values of *Staphylococcus* spp. counts from all farms exceeded the maximum limit of *S. aureus* in SNI 01-3141 (BSN, 1998) which is 2.00 log cfu/ml. However, all farms had maximum values of *Staphylococcus* spp. counts exceeding the maximum limits of those standards.

Data regarding *Staphylococcus* spp. count for each level in every factor are shown in Table 3. *Staphylococcus* spp. count was significantly different among breeds of goats. A statistically significant difference ($P<0.05$) was observed among count of *Staphylococcus* spp. bacteria within level of parity, lactation stage, teat end condition and udder inflammation status. There was no significant difference observed within udder symmetry, udder hygiene status, teat skin condition, teat shape and milk appearance. Based on milk appearance, the indicator bacterial counts were not significantly different between the normal and abnormal milk appearance, but numerically, the abnormal had higher count of bacteria than the normal one.

In this study 64.7% (97/150) of goats had infections in both of their udders (positive bacteriological isolation of *Staphylococcus* spp.). Table 4 shows overall and farm level prevalence of *Staphylococcus* spp. from udder-half milk samples. Overall prevalence of *Staphylococcus* spp. was 78.7%, whereas in the farm level the highest prevalence was 86% (farm 2). There was no statistically significant difference observed for the prevalence of *Staphylococcus* spp. among farms.

Prevalence of *Staphylococcus* spp. from udder-half samples from this study (78.7%) was higher to the prevalence of this group of bacteria reported previously from other countries by Kalogridou-Vassiliadou (1991); Contreras *et al.* (1995); White & Hinckley (1999); Sanchez *et al.* (1999); Ndegwa *et al.* (2001); Leitner *et al.* (2004); Moroni *et al.* (2005) and Leitner *et al.* (2007), which were 3.1% (Greece), 4.1% (Italy), 38.2% (USA), 70.0% (Spain), 60.3% (Kenya), 32.9% (Israel), 1.6% (Italy) and 28.8% (Israel), respectively.

Table 4. Proportion of positive samples for *Staphylococcus* spp. from udder-half milk samples

Indicator bacteria	Factor/level	n	No. of positive/ contaminated samples	Prevalence (%)	P-value
Staphylococcus spp.	Overall	300	236	78.7	0.053
	By Farm				
	Farm 1	100	72	72	
	Farm 2	100	86	86	
	Farm 3	100	78	78	

Summary results of the assessment of associations between prevalence of *Staphylococcus* spp. with each level of potential risk factors in univariate analysis are shown in Table 5. The results indicated that only "lactation stage" and "udder inflammation status" were significantly associated (P<0.05) with the contamination of *Staphylococcus* spp. in the samples. Those two factors were then subjected to multiple logistic regressions.

The results of logistic regression of two risk factors which were significantly associated with sample prevalence of *Staphylococcus* spp in univariate analysis are shown in Table 6. The second stage of lactation had significantly lower *Staphylococcus* spp. contamination in the samples than the first lactation stage (OR=0.392, P=0.005). The odd ratio (OR) of udder inflammation status factor was greater than one, meaning that factor was positively associated with the presence of *Staphylococcus* spp. in the samples. Therefore the udder with inflammation had significantly higher results of *Staphylococcus* spp. positive samples than udder without inflammation (OR= 2.490, P=0.002). Hence the risk of having *Staphylococcus* spp. positive milk samples was 2.49 times more likely in goats with udder inflammation than in goats without it.

The status of udder inflammation was based on a CMT score by following the method suggested by Wakwoya *et al.* (2006). Udder inflammation status was found to have a significant association with the presence of *Staphylococcus* spp. Moreover results of logistic regression confirmed that the udder inflammation status was a risk factor for the presence of these bacteria in the milk samples. The udders with inflammation had a strong and significant association with and higher prevalence of *Staphylococcus* spp. [OR= 2.490, P= 0.002, 95%CI= 1.403, 4.418] as compared to the udders without inflammation.

The Comparison between California Mastitis Test (CMT) Results and Conventional Bacteriological Isolation

CMT was conducted on 300 udder-half milk samples for determining the udder inflammation status as well as for an indicator of the presence of subclinical mastitis or IMI. Regarding the CMT score, 62.7% (188/300) of the samples were CMT positive and 37.3% (112/300) of the samples were CMT negative. This result was different from the study result reported by Wakwoya *et al.* (2006) in Ethiopia, that showed from 680 udder-half goat milk samples, 278 (40.9%) milk samples were CMT positive, while 402 (59.1%) samples were CMT negative. On the other hand, 28 (10.1%) of the 278 CMT positive milk samples yielded no bacterial growth while the remaining 250 (89.9%) samples were also culture positive in which diverse bacterial pathogens were identified. They did not present a further proportion of the bacteria growth in CMT positive-negative samples for each identified bacteria.

With regard to the prevalence of *Staphylococcus* spp.in the samples, 84.0% (158/188) of CMT positive samples yielded bacterial growth and the rest [16.0% (30/188)

Table 5. Summary results of the assessment of associations between sample prevalence of *Staphylococcus* spp. with potential risk factors (Univariate Analysis)

Factors/level	n	n (+)	n (-)	% (+)	P-value
Breed					
Ettawa Crossbreed (EC)	176	144	32	81.8	
Saanen Crossbreed	102	78	24	76.5	0.117
Jawarandu (Local crossbreed)	22	14	8	63.6	
Parity					
First	66	47	19	71.2	
Second	114	86	28	75.4	
Third	80	67	13	83.8	0.100
Fourth	32	28	4	87.5	
Fifth	8	8	0	100	
Lactation stage					
First	183	152	31	83.1	
Second	66	44	22	66.7	0.021
Third	51	40	11	78.4	
Udder symmetry					
Yes	176	138	38	78.4	1.000
No	124	98	26	79.0	
Udder hygiene					
Free of dirt	296	234	62	79.1	0.427
Slightly dirty	4	2	2	50.0	
Teat end condition					
No ring	225	171	54	76.0	0.073
Smooth rough ring	75	65	10	86.7	
Teat skin condition					
Free from scales/smooth	295	231	64	78.3	0.533
Shows some scaling	5	5	0	100.0	
Teat shape					
Normal	166	124	42	74.7	
Dilated	117	100	17	85.5	0.066
General dilated	17	12	5	70.6	
Udder inflammation status					
Yes	186	157	29	84.4	0.003
No	114	79	35	69.3	
Milk appearance					
Normal	293	230	63	78.5	1.000
Abnormal	7	6	1	85.7	

of CMT positive samples] yielded no bacterial growth. Whereas 69.6% (78/112) of CMT negative samples yielded bacterial growth and the rest [30.4% (34/112)] yielded no growth of *Staphylococcus* spp.

Table 6. Logistic regression of the risk factor associated with sample prevalence of *Staphylococcus* spp.

Factors/level	OR*	P-value	95% Confidence interval
Lactation stage			
First	1	-	0
Second	0.392	0.005	[0.203, 0.755]
Third	0.662	0.305	[0.301, 1.456]
Udder inflammation status			
Yes	2.49	0.002	[1.403. 4.418]
No	1	-	0

Note:
*OR = Odd ratio
OR = 1: no association exits between presence of indicator bacteria and factor
OR > 1: the factor is positively associated with the presence of indicator bacteria (risk factor)
OR< 1: the factor is negatively associated with the presence of indicator bacteria (protective factor).

Table 7 shows the comparison between CMT results (positive and negative) and conventional bacteriological isolation results by using McNemar's test. McNemar's Chi-square test was used to test the null hypothesis that the true proportions of successes (positive results) using the two methods in the same sample were equal (Petrie & Watson, 1999). The isolation of indicator bacteria by using conventional bacteriological method was considered a gold standard. Further epidemiological and statistical analyses were done by calculating sensitivity, specificity for CMT, and Cohen's kappa coefficient (κ) for measuring agreement between results of the two diagnostic tests/methods.

Statistically significant differences (P<0.05) between the two test results were observed for *Staphylococcus* spp. isolation results. It meant that the ability of two tests/methods to detect true proportions of indicator bacteria in the samples was significantly different.

The study result showed that the proportion of CMT positive samples which yielded bacterial growth was higher compared to CMT positive samples with no bacterial growth. Wakwoya *et al.* (2006) explained that

Table 7. The comparison between CMT and conventional bacteriological isolation results of *Staphylococcus* spp. from udder-half milk samples

Indicator bacteria	CMT results	n	n (+)#	Percent of positive samples	Se *	Sp*	κ**	P-value***
Staphylococcus spp.	(-)	112	78	69.6	0.67	0.53	0.158	0.000
	(+)	188	158	84.0				

Remarks: #Positive sample based on conventional bacteriological isolation
* Sensitivity (Se) and Specificity (Sp)
** Cohen's kappa coefficient
*** From McNemar's Chi-square test

Cohen's kappa coefficient interpretation of agreement between two tests (Petrie & Watson, 1999):
- "Poor" if $\kappa \leq 0.20$;
- "Fair" if $0.21 \leq \kappa \leq 0.40$;
- "Moderate" if $0.41 \leq \kappa \leq 0.60$;
- "Substantial" if $0.61 \leq \kappa \leq 0.80$;
- "Good" if κ exceeds 0.80.

the CMT positive and culture negative samples (those which yielded no bacterial growth) could be partly explained in that the udder could be injured and was recovering from infection or the infection could be not due to a bacterial pathogen. It could also be due to an organism such as mycoplasma, which requires special media and cannot be detected using routine bacterial isolation techniques.

The proportion of CMT negative samples yielded bacterial growth from this study was also higher compared to the study results in Kenya by Ndegwa *et al.* (2000), who reported that 22.5% of 568 CMT negative samples yielded bacterial growth. They suggested that bacterial organisms isolated from the CMT negative samples were either a latent cause of infections or did not stimulate any significant increase in somatic cell counts. Winter & Baumgartner (1999) reported from their study results in Austria regarding the evaluation of CMT reaction in goat milk that CMT was not specific for infected udder halves, but can be used as an additional diagnostic tool concerning goat mastitis without overestimation, due to the influence of different factors in cell counts.

Schaeren & Maurer (2006) evaluated the relationship of subclinical udder infection and individual SCC as well as CMT in three dairy goat herds in Bern, Switzerland. They concluded that the relationship between CMT reactions and udder infections was not very close. More than 20% of mammary halves infected with CNS showed negative CMT reactions. Their result was comparable with ours. On the other hand, 25% of the samples from mammary halves without a proven infection reacted positively.

However, this study result showed that despite of statistical significance, numerically the proportion of CMT positive samples which yielded bacterial growth was higher as compared to CMT negative samples which yielded bacterial growth. Based on this study result and the above mentioned previous reports from other countries, it could be stated that CMT can be used as an effective, reliable, cheap and "farm and farmer friendly test" for screen testing of IMI or subclinical mastitis in dairy goats.

CONCLUSION

Results of this study showed that based on median values of *Staphylococcus* spp. counts from all studied farms, the microbiological quality of raw goat milk from udder-half milk samples complied with the available specific standards for goat milk EC Directive 92/46/EEC (1992) and German "Milchverordnung" (BGBl Teil I Nr. 58 S 2794, 2004), but exceeded the maximum limit of *S. aureus* in SNI 01-3141 (BSN, 1998) which is actually designated for fresh cow milk. However, all farms had maximum values of *Staphylococcus* spp. counts exceeding the maximum limits of those standards. Whereas the prevalence of *Staphylococcus* spp. in raw goat milk, was relatively higher compared to the majority of other study results from other countries. Therefore from the food safety and public health protection point of view, more efforts should be taken by the farmers to increase sanitary and hygienic level of all components and processes within the farm to produce safer product for the consumer. With regard to the prevention of intramammary infection (IMI) which can cause bacterial contamination of the milk, udder with inflammation must be considered as risk factor. Additionally, CMT can be used as an effective, reliable, cheap and "farm and farmer friendly test" for screening tests of intramammary infection (IMI) or subclinical mastitis in dairy goats

REFERENCES

Bean, N. H., J. S. Goulding, C. Lao, & F. J. Angulo. 1996. Surveillance of foodborne disease outbreaks-United States, 1988-1992. Morbidity and Mortality Weekly Rep. 45 (SS-5): 1.

Bergonier, D., R. De Cremoux, R. Rupp, G. Lagriffoul & X. Berthelot. 2003. Mastitis of dairy small ruminants. Vet. Res. 34: 689-716.

BGBl (Bundesgesetzblatt). 2004. Milchver-ordnung-Verordnung über Hygiene-und Qualitätsanforderungen an Milch und Erzeugnisse

auf Milchbasis. Neugefasst durch Bek. v. 20. 7.2000 I 1178; zuletzt geändert durch Art. 5 V v. 9.11.2004 I 2791. Bundes-ministerium der Justiz, Bundesrepublik Deutschland.

BSN (Badan Standardisasi Nasional). 1998. Susu Segar. SNI 01-3141-1998, Jakarta.

Chye, F. K., A. Abdullah & M. K. Ayob. 2004. Bacteriological quality and safety of raw milk in Malaysia. Food Microbiol. 21 : 535-541.

Contreras, A., J. C. Corrales, D. Sierra & J. Marco. 1995. Prevalence and aetiology of nonclinical intramammary infection in Murciano–Granadina goats. Small Rumin. Res. 17: 71–78.

Dawson, B. & R. G. Trapp. 2004. Basic and Clinical Biostatistics. 4th ed. Mc Graw Hill. International Edition, New York.

DGLS (Directorate General of Livestock Services). 2005. Livestock statistics of Indonesia. DGLS-Ministry of Agriculture, Republic of Indonesia.

EC (European Council). 1992. EC Directive 92/46/EEC of 16 June 1992 laying down the health rules for the production and placing on the market of raw milk, heat-treated milk and milk-based products, Luxembourg.

FAO (Food and Agricultural Organization). 2006. Major food and agricultural commodities and producers. Country by commodity. http://www.fao.org/es/ess/top/commodity.html?lang=en& item=1020&year=2005. [25 Agustus 2006].

ISO (International Organization for Standardization) 6887-1 1999. International Standard: Microbiology of food and animal feeding stuffs – Preparation of test samples, initial suspension and decimal dilutions for microbiological examination - Part 1: General rules for the preparation of the initial suspension and decimal dilutions. Geneve.

ISO (International Organization for Standardization) 6888-1. 1999. International Standard: Microbiology of food and animal feeding stuffs – horizontal method for the enumeration of coagulase-positive staphylococci (Staphylococcus aureus and other species). Part 2: Technique using Baird Parker agar medium, Geneve.

Kalogridou-Vassiliadou, D. 1991. Mastitis-related pathogens in goat milk. Small Rumin. Res. 4: 203–212.

Leitner, G., U. Merin, & N. Silanikove. 2004. Changes in milk composition as affected by subclinical mastitis in goats. J. Dairy Sci. 87: 1719–1726.

Leitner, G., U. Merin, Y. Lavi, A. Egber & N. Silanikove. 2007. Aetiology of intramammary infection and its effect on milk composition in goat flocks. J. of Dairy Research 74: 186–193.

Moroni, P., G. Pisoni, M. Antonini, G. Ruffo, S. Carli, G. Varisco, P. J. Boettcher. 2005. Subclinical mastitis and antimicrobial susceptibility of *Staphylococcus caprae* and *Staphylococcus epidermidis* isolated from two Italian goat herds. J. Dairy Sci. 88: 1694-1704.

Ndegwa, E. N., C. M. Mulei & S. J. Mynyua. 2000. The prevalence of subclinical mastitis in dairy goats in Kenya. J.S. Afr. Vet. Assoc. 71: 25-27.

Ndegwa, E. N., C. M. Mulei & S. J. Mynyua. 2001. Prevalence of microorganism associated with udder infections in dairy goats on small-scale farms in Kenya. J.S. Afr. Vet. Assoc. 72: 97-98.

Petrie, A. & P. Watson. 1999. Statistics for Veterinary and Animal Science. Blackwell Science Ltd., London.

Sanchez, A., A. Contreras & J. C. Corrales. 1999. Parity as a risk factor for caprine subclinical intramammary infection. Small Rumin. Res. 31: 197-201.

Schaeren W. & J. Maurer. 2006. Prevalence of subclinical udder infections and individual somatic cell counts in three dairy goat herds during a full lactation. [Article in German]. Schweiz Arch Tierheilkd. 148: 641-648.

Shearer, J. K. & B. Harris Jr. 2003. Mastitis in dairy goats. IFAS Extension. University of Florida, USA.

Wakwoya, A., B. Molla, K. Belihu, J. Kleer & G. Hildebrandt. 2006. A cross sectional study on the prevalence, antimicrobial susceptibility patterns and associated bacterial pathogens of goat mastitis. Intern. J. Appl. Res. Vet. Med. 4: 169-176.

White, E. C. & L. S. Hinckley. 1999. Prevalence of mastitis pathogens in goat milk. Small Rumin. Res. 33: 117-121.

Winter, P. & W. Baumgartner. 1999. Evaluation of the California mastitis test reaction in goat milk and its interpretation. [Article in German]. Dtsch. Tierarztl. Wochenschr. 106: 30-4.

Zweifel, C., J. E. Muehlherr, M. Ring & R. Stephan. 2005. Influence of different factors in milk production on standard plate count of raw small ruminant's bulk-tank milk in Switzerland. Small Rumin. Res. 58: 63-70.