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Microbiological Quality of Raw Goat Milk in Bogor, Indonesia

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ABSTRAK

Investigasi terhadap kualitas mikrobiologis susu kambing mentah dengan menggunakan angka lempeng total bakteri (ALTB), koliform, *Staphylococcus* koagulase positif (CPS) dan *Staphylococcus* koagulase negatif (CNS) sebagai bakteri indikator, telah dilaksanakan. Sepuluh faktor resiko yang berpotensi terkait dengan adanya bakteri indikator tersebut juga telah dievaluasi. Hasil penelitian menunjukkan bahwa nilai median dari jumlah bakteri indikator dalam sampel susu asal ambing dan susu kandang untuk masing-masing bakteri indikator, yaitu ALTB, koliform, CPS, dan CNS adalah 3,74; 0,70; 1,70; dan 2,52 log cfu/ml untuk susu ambing dan 5,69; 2,98; 3,66; dan 3,32 log cfu/ml untuk susu kandang. Nilai median semua bakteri indikator dari sampel susu ambing tidak ada yang melebihi batas maksimum standar yang berlaku. Namun demikian, untuk sampel susu kandang, hanya jumlah ALTB saja yang memenuhi standar yang ada. Prevalensi total bakteri indikator, yaitu koliform, CPS, dan CNS dalam sampel susu ambing masing-masing adalah 46,3%; 37,7%; dan 66,0%, sedangkan dari sampel susu kandang adalah 86,7%; 76,7%; dan 86,7%. Kambing dari bangsa peranakan Saanen, paritas ke-5 dan ambing yang radang telah terbukti sebagai faktor resiko. Data di atas menunjukkan bahwa praktik *higiene* yang baik di peternakan masih belum optimal dilaksanakan. Kontaminasi bakteri pada susu dapat dikontrol dengan mengendalikan faktor-faktor resiko yang telah teridentifikasi.

Kata kunci: susu kambing, ALTB, koliform, CPS, CNS, faktor resiko

ABSTRACT

Milk samples were investigated for counts and prevalence of indicator bacteria, which were TPC, coliforms, coagulase positive Staphylococci (CPS), and coagulase negative Staphylococci (CNS). Ten potential risk factors were also evaluated in relation to the prevalence of indicator bacteria. The results showed that the median values of indicator bacterial counts from overall udder-half milk samples were 3.74, 0.70, 1.70, and 2.52 log cfu/ml and from bulk milk samples were 5.69, 2.98, 3.66 and 3.32 log cfu/ml for TPC, coliforms, CPS, and CNS, respectively. None of the median values of overall udder-half milk samples exceeded the maximum limit of the standards for all indicator bacteria. However, in the bulk milk samples only the median value of TPC below the maximum limit of the standards. Overall prevalence of coliforms, CPS and CNS from udder-half milk samples were 46.3%, 37.7%, and 66.0%, respectively, and from bulk milk samples were 86.7%, 76.7%, and 86.7%, respectively. Saanen crossbreed, fifth parity and udders with inflammation were found to be risk factors. This study results indicated that the hygienic practices in the dairy goat farms are still need

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to be increased. To increase the hygienic level of the milk, the identified significant risk factors must be controlled.

Key words: goat milk, TPC, coliforms, CPS, CNS, risk factor

INTRODUCTION

According to FAO (2010), Indonesia was ranked as the 7th in the list of global goat milk production and ranked as the 1st in the Southeast Asia region in 2009. The goat milk production in Indonesia in that year was 238,000 ton. Bangladesh was in the 1st position for producing goat milk globally as it reached 2,168,000 ton in the same year.

Galal (2005) reported that developing countries harbour 96% of the world goat population, but only 60% of the breeds. According to compositional differences between the milk from cows, goats and sheep, the quality standards for the milk from small ruminant animals should be adjusted and evaluated based on the individual milk source (Morgan *et al.,* 2003; Zweifel *et al.,* 2005).

Most of the reports concerning the microbiological characteristics of goat milk were dealt only with investigation on the prevalence of target pathogenic organisms, SCC and microbial quality of the milk (McDougall *et al*., 2002; Foschino *et al.,* 2002; Raynal-Ljutovac *et al.,* 2005; Wakwoya *et al.,* 2006; Contreras *et al.,* 2007; Leitner *et al.,* 2007; Hall & Rycroft, 2007, Koop *et al.,* 2010; McDougall *et al.,* 2010).

Whereas reports on the evaluation of different factors concerning farm management and milking practices as well as other predisposing factors from goat condition in association with microbial quality and the prevalence of pathogenic bacteria in goat milk were very limited (Ndegwa *et al.,* 2001; Zweifel *et al*., 2005; Kyozaire *et al*., 2005; Moroni *et al.* 2005; Lilenbaum *et al*., 2008; Megersa *et al.* 2010; Oliviera *et al*., in press).

In Indonesia, so far raw goat milk has less attention in terms of quality and safety control from farmer organizations and/or government institutions than those for cow milk and milk products. Whereas the most of the consumers are prefer to drink raw goat milk due to their belief in its benefit as a health promoting or even disease-relief agent. This condition is in line with the report from Oliver *et al.* (2005), they stated that although numerous studies have documented that foodborne pathogens of public health significance have been isolated from bulk tank milk and are capable of causing disease in humans, people continue to consume raw milk.

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. *Staphylococcus* spp. are the main etiological agents of small ruminant's intramammary infections (IMI) (Stuhr & Aulrich, 2010), 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).

High prevalence of CPS such as *S. aureus*, or CNS can be of veterinary public health concern (Silanikove *et al*., 2010). 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). Gonzalo *et al.* (2002) and Koop *et al.* (2010) also reported that subclinical mastitis in goats decrease the milk yield and especially CNS can changed the milk composition especially its curd yield Leitner *et al.* (2004).

Investigation on the microbiological quality such as Total Plate Count (TPC), coliforms as quality and safety indicators, and the presence of pathogenic bacteria of goat milk together with some risk factors affecting these microorganisms in Indonesia was very rare. Therefore the objective of this study were to investigate the microbiological quality of raw goat milk by using several indicator bacteria and to evaluate the potential risk factors associated with them in dairy goat farms in the Bogor District, West Java Province, Indonesia.

MATERIALS AND METHODS

Study Design and Location

This study was a cross sectional survey to investigate the microbiological quality and the association of possible risk factors with the microbiological status of raw goat milk. 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 by the investigator during the farm visits.

Type of Sample

The main milk sample that was used in this study was individual udder half (left and right udder) milk of lactating goats, which was collected at the time of sampling visits. Two parallel bulk milk samples were taken from each farm at every visiting time as an additional sample. Ten milliliters of milk was collected for each sample, either from udder-half or bulk milk.

Laboratory Investigation Standard Procedures

General rules for the preparation of the initial suspension and decimal dilutions were based on ISO 6887-1 (1999). US FDA-BAM (United States Food and Drug Administration–Bacteriological Analytical Manual) online for analysis of Aerobic Plate Count (FDA, 2001) was followed for conventional culture of TPC, whereas for the total coliforms count, the 3M™ Petrifilm™ interpretation guide for the total coliforms count (Petrifilm™, 2008) was followed. To investigate the presence and enumeration of coagulase positive and negative staphylococci in the sample, the ISO 6888-1 (1999) standard technique by using Baird Parker agar medium was used. The California Mastitis Test (CMT) was done according to Shearer & Harris (2003).

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 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.

Sampling Strategy and Evaluation of Potential Risk Factors

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 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 repetition 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 ≤4°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 forestripping 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). 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).

There were 10 potential risk factors examined in this study, some of them were subjectively scored by the investigator based on an adoption from the available scoring standard for dairy cow. The potential risk factors were breed of animal (three breeds were found in the field during investigation i.e. Ettawa crossbred, Saanen crossbred and Jawarandu), parity number (first to fifth), stage of lactation (first to third), udder symmetry (yes or no), score of udder hygiene (Ruegg, 2002), score of teat end condition (Mein *et al*., 2001), score of teat skin condition (Goldberg *et al.,* 1994), teat shape condition (normal to general dilated), udder inflammation status (yes or no), and milk appearance (normal or abnormal).

Statistical Analysis

Descriptive statistics were used to describe enumeration and prevalence data. Chi-square univariate analysis was performed to evaluate the impact 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 were used to evaluate statistical significance in bacterial population among evaluated variables (Dawson & Trapp, 2004).

RESULTS AND DISCUSSION

The median values of indicator bacterial counts from overall udder-half milk samples were 3.74, 0.70, 1.70, and 2.52 log cfu/ml and from bulk milk samples were 5.69, 2.98, 3.66, and 3.32 log cfu/ml for TPC, coliforms, CPS and CNS, respectively (Table 1). The indicator bacterial counts from udder-half milk samples were significantly different ($P<0.05$) among farms, except for the CPS count, whereas from bulk milk samples, it was only the coliforms count that was observed to have statistically significant difference among farms. None of the median values of overall udder-half milk samples exceeded the maximum limit of available microbiological standards for TPC, coliforms and *Staphylococcus aureus* (for the Indonesian standard, since no special standard for goat milk available yet, standard for cow fresh milk was used as a comparison), however the samples had maximum values which exceeded the maximum limits within those standards. In bulk milk samples, it was only the median value of TPC that was below the maximum limit of standards. All indicator bacteria had maximum values beyond the maximum limits (Table 1).

Overall prevalence of coliforms, CPS and CNS from udder-half milk samples were 46.3%, 37.7%, and 66.0%, respectively (Table 2), and from bulk milk samples were

86.7%, 76.7%, and 86.7%, respectively. Results of this study showed that based on median values of indicator bacteria (Table 1), the microbiological quality of raw goat milk from udder-half milk samples complied with the available standards, whereas for the samples from bulk milk do not complied with the standards except for TPC. Prevalence of all indicator bacteria was relatively higher compared to the majority of other study results from other countries (Table 2).

The results of the evaluation of several potential risk factors in its association with the presence of indicator bacteria in the samples show that some of those potential risk factors could be considered to be risk factors, which significantly increased the risk of presence of each indicator bacteria i.e. Saanen crossbreed for coliforms, parity for CPS and udder inflammation for CPS (Table 3).

The data in Table 3 shows that the breed of goats was only significantly associated with the presence of coliforms in the samples. Saanen crossbreed had a significantly higher chance of coliforms contamination (OR= 3.942, P= 0.000, 95%CI= 2.348, 6.618) than other breeds. It was likely to be associated with low resistance and adaptability of Saanen crossbreed to the environment condition as compared to two others breeds which are local and tropical breed. A significant association of the parity factor with the presence of indicator bacteria was observed only in CPS. Further analysis by logistic regression test showed that only goats in fifth parity had a significantly positive association and higher risk of having CPS in the samples (OR= 6.033, P= 0.050, 95%CI= 0.999, 36.455) (Table 3).

This result was also comparable to some previous reports, i.e. Boscos *et al.* (1996), who also found that no parity differences in the prevalence of type of bacteria isolated, but the proportion of positive samples was significantly higher in multiparous than in primiparous Saanen goats; Sanchez *et al.* (1999) who reported that prevalence of IMI (which was mostly caused by *Staphylococcus* spp. [P= 70%]) increased with the age of the goats, and they also found positive statistical association between subclinical intramammary infection and goats in greater than fifth parity (PR= 1.80; 95% CI= 1.21, 2.68); McDougall *et al.* (2001) reported that significant association in infection prevalence was found between goats older than 4 years and less than 4 years old and Moroni *et al.* (2005) who reported that goats in third and fourth parities had significantly more infection than

Table 1. Overall bacterial count from udder-half milk (n= 300) and bulk milk (n= 30) samples compared to the maximum limits of available standards

Selected statistical value	Bacterial count (log cfu/ml)			
	Total plate count	CPS	Coliforms	CNS
Median	3.74 (5.69)*	1.70 (3.66)	0.70 (2.98)	2.52 (3.32)
Indonesian National Standard for Fresh Milk (SNI 01-3141-1998) (for comparison)	6.00	2.00 (for *S. aureus*)**	1.30	-
EU Council Directive 92/46/EEC (raw goat and sheep milk) (EC, 1992)	5.70	3.30 (for *S. aureus*)**	2.00	-
German "Milchverordnung" of "raw goat/sheep milk" for making raw based milk products (BGBl, 2004)	5.70	3.30 (for *S. aureus*)**	2.00 ("Vorzugs-milch"/special raw milk)	-
Minimum	0.70 (3.74)	1.70 (1.70)	0.70 (0.70)	1.70 (1.70)
Maximum	6.96 (7.50)	6.18 (5.65)	5.99 (4.45)	6.41 (5.54)

Note: * Numbers in parentheses are for bulk milk samples; ** *S. aureus* is the most dominant species in CPS group of bacteria, in this case the CPS count was compared to *S. aureus* standard; CPS= coagulase-positive Staphylococci; CNS= coagulase-negative Staphylococci.

Table 2. Overall prevalence of indicator bacterial from udder-half milk samples and its comparison with prevalence from other study results

Indicator bacteria	Overall prevalence	Prevalence from other studies
Coliforms	46.30%	· 12.0%, UK (Little & de Luvois, (1999)
CPS	37.70%	· 18.5%, Greece (Boscos *et al*., 1996)
		· 45.34%, Pakistan (Ali *et al*., 2010)
CNS	66.00%	· 66.7%, USA (McDougall *et al*., 2002)
		· 9.6%, Ethiopia (Wakwoya *et al*., 2006)
		· 17.9%, Israel (Leitner *et al*., 2007)
		· 47%, UK (Hall & Rycroft, 2007)

Note: CPS= coagulase-positive Staphylococci; CNS= coagulase-negative Staphylococci.

goats in first or second parities. McDougall *et al.* (2002) stated that increasing prevalence with age may be due to the increased length of exposure to pathogens in older compared to younger animals. The recent report from Megersa *et al.* (2010) stated that according to univariable analysis of potential risk factors, the goat with parity more than five had a significant association with having subclinical mastitis (OR= 2.3, P= 0.028, 95%CI= 1.1, 4.9).

Udder inflammation status was found to have a significant association with the presence of CPS. Moreover results of logistic regression in the Table 3 confirmed that the udder with inflammation had a strong and significant association with and higher prevalence of CPS [OR= 2.622, P= 0.001, 95%CI= 1.487, 4.623] compared to the normal udders. In this case, the report by Megersa *et al.* (2010) also in agreement with this study results, they showed that goat's udders with length more than 2 cm had significantly 2.2 times higher chance to get mastitis as compared to those with lenght less than 2 cm (OR= 2.2, P= 0.019, 95%CI= 1.1, 4.2).

The data of CMT results as compared to the positively contaminated samples by indicator bacteria based on plate count method are shown in the Table 4. Despite of statistical significance, numerically the proportion of CMT positive samples which yielded bacterial growth was higher compared to CMT negative samples which yielded bacterial growth in all indicator bacteria (Table 4).

Based on this study results in Table 4, it can be stated that CMT can become cheap and handy useful tool for the dairy goat farmer to do screen testing of IMI or subclinical mastitis in dairy goats. The positive CMT result can be used as an indicator of pathogenic bacterial contamination in the milk. Therefore the farmer can easily differentiate between contaminated and uncontaminated individual milk, in order to guarantee the quality and safety of their milk production. The high quality and safety of milk production can ensure their business sustainability.

CONCLUSION

The number of bacteria in individual raw goat milk samples exceeded the maximum limit of the available

Table 3. Results of the potential risk factors evaluation

Indicator bacteria	Factors/Level	OR	P-value	95% Confidence interval
Coliforms	Breed			
	· Ettawa Crossbreed (EC)	1.000	-	0
	· Saanen Crossbreed	3.942	0.000	[2.348, 6.618]
	· Jawarandu (Local crossbreed)	1.305	0.564	[0.528, 3.226]
CPS	Parity			
	· First	1.000	-	0
	· Second	0.900	0.778	[0.431, 1.876]
	· Third	1.853	0.118	[0.856, 4.013]
	· Fourth	1.764	0.253	[0.666, 4.672]
	· Fifth	6.033	0.050	[0.999, 36.455]
	Udder inflammation			
	· Yes	2.622	0.001	[1.487, 4.623]
	· No	1.000	-	0

Note: CPS= coagulase-positive Staphylococci; OR= odds ratio.

Table 4. Comparison between California Mastitis Test (CMT) and conventional bacteriological isolation results of indicator bacteria from udder-half milk samples

Indicator bacteria	CMT Results	n	n (+)*	Percent of positive samples	P-value**
Coliforms	(-)	112	50	44.6	0.000
	(+)	188	89	47.3	
CPS	(-)	112	30	26.8	0.000
	(+)	188	83	44.1	
CNS	(-)	112	67	59.8	0.419
	(+)	188	131	69.7	

Note: * Positive samples based on conventional bacteriological isolation; ** McNemar Chi-square test; CPS= coagulase-positive Staphylococci; CNS= coagulase-negative Staphylococci.

standards. Overall prevalence of the indicator bacteria from udder-half milk samples of this study were relatively higher compared to the other reports from other countries. The bacterial contamination associated mainly with the intramammary infection. More efforts and measures must be taken in order to increase sanitary and hygienic level of all components and processes within the dairy goat farms to produce safer goat milk for the consumer.

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