Microbiological Quality of Raw Goat's Milk

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Abstract

A study was conducted to determine the microbiological status of raw goat's milk from a few sources in Selangor and to detect milk-borne pathogens; especially *Staphylococcus aureus*, *Salmonella* spp., *Campylobacter* spp., and *Brucella melitensis*. Forty samples from nine different sources in Selangor were collected. The study found that the samples had a mean Total Plate Count (TPC) of $5.2 \pm 1.36 \times 10^5$ cfu/mL. The levels of coliform of all the samples were high with the mean of $1.5 \pm 4.17 \times 10^6$ cfu/mL. *Staphylococcus* aureus were detected in 14 of 40 samples of raw goat's milk (35%). *Salmonella* spp., *Campylobacter* spp. and *Brucella melitensis* were not isolated from any of the samples.

Keywords: Microbiological quality, TPC, CPC, raw goat's milk, pathogens

Introduction

Milk is an essential food for newborn and is rich in proteins, carbohydrates, fats, minerals and vitamins. Milk spoils easily and the reasons for milk spoilage are numerous which includes infected milking animals, unhygienic milking processes and improper milk storage methods. Microorganism not only can cause spoilage of milk but may also cause milk-borne infections to humans. Milk is a good medium for the growth of many microorganisms, including pathogens (Bishop and White, 1986; Sorhaug and Stepaniak, 1997).

More than 90% of all reported cases of dairy-related illness are of bacterial origin. At least 21 milk-borne or potentially milk borne diseases has been identified (Bean *et al*., 1996). In the past 20 years, illness from dairy consumption have been predominantly associated with *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes* and *Escherichia coli* O157:H7, which can be present in milk obtained from apparently healthy looking animals, typically as a consequence of contamination that occurs during and after milking. The present study examines the microbiological quality of raw goat's milk and attempts to detect a few important milk-borne pathogens.

Materials and Methods

Sample and data collection

Raw goat's milk was purchased from nine different sources in Selangor (either farms or retail shops). Forty samples of raw goat's milk were obtained between 29th November and 22th December 2010 for the purpose of this study. From the goat farm, the samples were collected from individual goats and stored in 30 mL bottles. The sample collection for 3 farms was performed by the author of this study and for the other 3 farms was performed by the workers in the farm. All samples were placed in a styrofoam box filled with ice and transported to the Veterinary Public Health Laboratory in UPM, Serdang Selangor.

Bacteriological Analysis

Total Plate Count

Plate Count Agar (PCA), (OXOID, Basingstoke, U.K.) was used to determine the Total plate count of the raw goat's milk. One mL of milk was pipetted aseptically and transferred into universal bottles containing 9 mL 0.1% Buffered Peptone Water (BPW). Serial dilutions were carried out and 0.1 mL was spread onto the PCA. The plates were incubated at 30ºC for 48 h. At the end of incubation period, plates containing colonies between 30 and 300 were selected for colony count.

Coliform Plate Count

Violet Red Bile Agar (VRBA), (OXOID, Basingstoke, U.K.) was used to detect the presence of coliform in the raw goat's milk. Serial dilution was carried and 1 mL was pipetted into the plate and mixed well with the VRBA using pour plate method. Then a thin layer of VRBA was poured onto the solidified agar. The plates were incubated at 37ºC for 48 h. At the end of the incubation periods, coliform appear as typical red colonies. At the end of incubation period, plates containing colonies between 30 and 300 were selected for colony count (Harrigan, 1998).

Staphylococcus aureus

The presence of *S. aureus* was determined by surface plating the samples on Mannitol Salt Agar (MSA) (OXOID, Basingstoke, U.K). One loopful of milk samples were cultured onto the MSA. Then the plates were incubated at 37ºC for 24 h. The presence of positive isolates is indicated by the presence of yellow colonies and the agar turning colour to yellow.

Salmonella spp.

One mL of milk samples was pipetted into 10 mL of BPW, and then incubated at 37ºC for 24 h. Then, one mL of sample was transferred into 10 mL of Rapapport – Vassiliadis (RV) (OXOID) and was incubated at 42ºC for 24 h. After the incubation periods, one loopful of enrichment was streaked onto the Chromogenic Agar (CA)

(OXOID) and XLT4 Agar (OXOID). The plates were incubated at 37ºC for 24 h. On the CA plates, purplish colonies indicates *Salmonella* spp. while on XLT4 agar plates, black colonies is indicative of *Salmonella* spp. Biochemical test were perform to confirm the presence of *Salmonella* using Triple Sugar Iron (TSI), Lysine Iron Agar (LIA), and urease. To confirm for *Salmonella*, agglutination test using polyvalent O and H antiserum was carried out.

Campylobacter spp.

One mL of milk samples was pipetted into 9 mL of enrichment media that was prepared according to the manufacturer's instruction with *Brucella* broth (Becton Dickinson, Germany) and supplemented with 5% lysed horse blood, 1 vial of growth supplement (SR023) and 1 vial of CCDA selective supplement (SR155). The samples were then incubated at 42ºC for 48 h. Then, one loopful of the enrichment media was streaked onto Campy Cefoperaxone Deoxycholate Agar (CCDA) (OXOID, U.K) and incubated at 42ºC under microaerobic atmosphere for 48 h. Then, motility test were carried out on the small, greyish, translucent colonies and observed under microscope. *Campylobacter* appeared as motile, small, curved rod organism (seagull-shaped). Those samples with positive motility test were subcultured on Columbia Blood Agar (CBA) and incubated further. Then, another motility test was done before further confirmation test including oxidase test, catalase test, hippurate hydrolysis and indoxyl acetate hydrolysis tests were performed.

Brucella melitensis

One mL of milk samples were pipetted into a micro centrifuge tube. Then, the tubes were centrifuged at 2000 x g for 15 min. Then, the milk cream was separated from the sediment part. The milk cream and the milk sediments were transferred and streaked onto *Brucella* Agar (BA) (OXOID). The plates were incubated at 37ºC for at least 2 to 3 d to allow the organism to grow. Small, honey colour, translucents colonies were subcultured onto BA and were incubated at 37ºC for another 2 to 3 d. Then Modified Acid Fast staining was done to check for *B. melitesis* which should appear as red and small coccobacilli.

Results

Bacterial counts

Fifteen representative samples out of 40 samples of raw goat's milk were examined for TPC and coliform count. The TPC number ranges from <1 x 10 to 5.2 x 10^6 cfu/mL. Coliform was found in all samples examined. All samples show a moderate count of coliform ranging from 10^3 to 10^6 cfu/mL.

Isolation of pathogens

Of the 40 samples tested, 14 (35%) were positive for *S. aureus*. *Salmonella* spp., *Campylobacter* spp. and *Brucella* were not detected in this study.

Discussion

In this study, the wide range of bacterial counts between sources could be due to pre-and post-milking hygienic practices because based on the author's observation during sample collection, neither pre-milking nor post-milking hygiene routine were practiced among the milkers in all 3 farms where milk collection was performed by the farm workers. This may result in increased bacterial contaminations from the udder. The practice may also increase the risk of intramammary infections that directly increase the TPC in milk. In farms where the author milked the goat, a very low number of counts were found.

Inferior microbiological quality of the water used for cleaning the utensils could have contributed to the high TPC of the milk samples. However, most of the sampled farms uses tap water, therefore reduces the possibility of water-borne contaminations.

The presence of coliform is associated with faecal and environmental contamination and the counts in raw milk should be less than 50 cfu/mL. The existence of coliform bacteria in the milk may not necessarily indicate a direct faecal contamination of milk, but may indicate poor hygiene and sanitary practices during and after milking. In the present study, we suspect that the high coliform count could be due to the poor hygiene and sanitary practices during milking. This is because the goats were kept in houses with raised-slatted flooring which generally are easily cleaned and remained clean for longer periods. Besides, the faeces of the goat are in pelleted form and are drier as compared to the cow dungs. Thus, contamination due to the direct faecal contact is much reduced as compared to that that would occur in cow's milk.

Staphylococcus aureus was found in 35% of raw milk sampled. The finding is in agreement with other studies that reported *S. aureus* isolation rate of 12- 32% in raw goat's milk (Ekici *et al*., 2004) and 37 to 70% in other type raw milk (sheep, cow and camel) (El-Ziney and Al-Turki, 2006).

The author speculate that the high percentage of positive samples might be due to subclinical mastitis as *S. aureus* is the major causative pathogen that causes the disease (Chye *et al*., 2004). As observed during the milking of animals in the present study, the milkers did not perform any basic sanitary precaution before and after milking and the milker did not wash his hands between milking different animals. Therefore, this increase the chances of bacterial transmission during the milking process as contaminated hands and milking equipment come into contact with uninfected mammary glands (Moroni *et al*., 2005). Thus, Oliver and Gillespie. (1999) suggested that post-milking teat disinfection is an effective procedure to reduce the number of contagious mastitis pathogens such as *S. aureus* on the teat skin immediately after milking.

Failure to isolate other pathogens targeted in the study does not necessarily mean that goat's milk is free from the pathogens. However, it may suggest that the prevalence or concentration of the organisms in the milk is low and was not detectable with the study sample size. The low prevalence of the bacteria, the fastidious characteristic of the some of these organisms can affect the success of

its isolation from the raw goat's milk. Rollins and Colwell, (1986) reported that *Campylobacter* might be present in the raw goat's milk, but in a non-culturable state. In addition, for *Brucella*, the sensitivity of the bacteriological culture methods depends on the viability and numbers of the bacteria in the sample, and the nature of other contaminating bacteria in the same samples. Thus, culture methods may not always be successful.

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