International Food Research Journal 19 (3): 1241-1245 (2012)

Microbiological quality evaluation of goat milk collected from small-scale dairy farms in Penang Island, Malaysia

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Abstract: Microbiological qualities of fresh goat milk collected from two selected, popular dairy farms in Penang Island, Malaysia were evaluated, as a measure of food safety. Milk samples were screened for total plate counts, yeast and mould counts, psychrotrophic counts, *Staphylococcus aureus*, presumptive *Escherichia coli,* Coliforms and *Klebsiella pneumoniae,* which were in the range of (mean values) 4.2- 4.5, 4.2- 4.6, 3.1- 4.3, 2.7- 3.2, < 2- 4.6, 2.2- 4.0 and 4.1- 4.8 log CFU/ml, respectively in the two farms. Milk samples were also screened for the presence of selected foodborne pathogens such as *Listeria monocytogenes* and *Salmonella* sp. Results showed the presence of only *Salmonella* sp. (at 2.9 log CFU/ml) with the absence of *Listeria monocytogenes*. The outcome of this study assumes importance as the presence of microbial contaminants amounts indicates poor milk quality, which requires immediate consideration as it can pose serious health risk to consumers.

Keywords: Prevalence, raw goat milk, pathogens, safety, quality

Introduction

Goat milk and their products are nutritionally versatile and of late, have gained wide demand due to their potential nutraceutical properties. Goat milk can get contaminated by various pathogenic or spoilage microorganisms (mainly bacteria) during various stages of processing and storage from farm upto table. Presence of high microbial load in milk can pose major economical loss for local farmers and small hold dairies, as milk price is calculated based on the bacteria count, especially the pathogenic ones (Bonfoh *et al.*, 2003; Metz *et al.*, 2009; Suguna *et al*., 2011). Previously, some of the pathogenic and spoilage bacteria such has *Listeria monocytogenes, Salmonella* sp, *Campylobacter*, *Staphylococcus aureus, Bacillus cereus, Escherichia coli*, and species of *Streptococcus, Staphylococcus* and *Micrococcus* have been isolated from fresh raw goat milk in various parts of the world (Adesiyun *et al.*, 2007; Kagkli *et al.*, 2007). Generally, in fresh goat milk, microbial load is less. However, the count might increase upto 100 fold or more once stored at ambient temperature for an extended period of time (Chye *et al.*, 2004; Suguna *et al*., 2011). Routinely, world over, for food safety reasons, microbiological analysis is carried-out to monitor and evaluate the level of prevalent pathogenic and spoilage microorganisms.

To our knowledge, no detailed reports are available on the microbiological quality of goat milk. Hence, the main objective to undertake the present study was to screen for the microbiological quality of fresh goat milk collected from two popular, small-scale dairy farms in Penang Island, which is envisaged to provide baseline information on the level of contamination and the prevalence of pathogenic bacteria. Results generated in this study is expected to be useful for health conscious consumers as well as the local governing agency to implement appropriate food safety measures to minimize the risk factors associated.

Material and Methods

Samples collection

Six goat milk samples were collected from two local dairy farms in Penang Island, Malaysia. Traditionally, the farmer performs milking process in this region early in the morning (between 6-7 am) before distributing to customer or to the local market. In this study, milk samples were collected fresh immediately after milking with appropriate care being taken for any possible cross-contamination. The individually milked samples from different goats (~80 to 100) are pooled together in large containers. The pooled samples (as 3 individual replicates) were

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collected in sterile plastic bags and were transported back to the Food Microbiology Laboratory (Universiti Sains Malaysia, Penang) directly under aseptic conditions (in an icebox at temperature of 0°C). Milk samples were refrigerated stored (at 4°C) until all the microbial analysis was performed, which was within 2 h. Aseptic techniques were applied, wherein all the equipments were pre-sterilized prior to analysis.

Initially, 25ml of individual milk sample was dispensed into sterile bag containing 225 ml sterile peptone water and homogenized with stomacher (Bagmixer 400, Interscience). Subsequently, serial decimal dilutions of milk were prepared in peptone water for the following analysis to be performed.

Microbiological analysis

Goat milk samples were analyzed for the prevalence prevalence of selected bacterial pathogens. Enumeration of total plate count (TPC), Psychrotrophic count, Coliforms, Presumptive *Escherichia coli*, *Staphylococcus aureus* and yeast and mould counts were carried out by employing standard methods (FDA, 2001; BAM, 2003; Yousef and Carlstrom, 2003).

Enumeration of Coliforms, *E. coli* and *K. pneumoniae* in goat milk was performed by employing three-tube most probable number (MPN) technique. Positive tubes from MPN were streaked onto eosin methylene blue (EMB) agar (Merck) and incubated at 37ºC for 24h. The typical colony found was confirmed based on their IMViC pattern based on BAM method (FDA, 2001). While, Baird-Parker agar (BPA, Merck) was used to enumerate *Staphylococcus aureus* in the samples. Characteristic black colonies surrounded by a clear zone were selected and subjected to coagulase and thermonuclease tests for confirmation of *S. aureus* (BAM, 2004).

For determination of *Salmonella* in samples, International Standard Organization protocol (ISO, 1990) was employed. Presence of *Salmonella* colonies were confirmed using API 20E test kit (Biomerieux, France). For identification of *Listeria* spp., modified method described by the Food and Drug Administration (FDA) was employed (Westoo and Peterz, 1992; FDA, 2001). Selected colonies from each plate of *Listeria* selective agar (Merck) and Palcam Listeria selective agar (Merck) were streaked onto Trypticase soy agar (TSA, Merck) and incubated 37ºC for 24 h. Presumptive *Listeria* species isolates were confirmed based on Gram reactions and catalase tests. Isolates, which were Gram-positive and catalase-positives were sub-cultured and identified with API *Listeria* test kit (BioMerieux, France).

Statistical analysis

The bacterial counts of milk samples were converted into logarithm of number of colony forming units per ml (log CFU/ml) for statistical analysis. Means were compared by employing analysis of variance (ANOVA, SPSS version15.0) followed by t-test to determine difference among means at 95% confidence level (significance level at $P \leq 0.05$).

Results and Discussions

Goat milk can easily get contaminated and spoiled due to poor hygienic conditions maintained at 'on farm' levels or due to improper handling, inadequate storage and transport conditions encountered. The reported outbreak of foodborne illness on consumption of raw goat milk has been attributed to presence of favorable nutrients, which in turn encourages the growth and proliferation of microorganisms (Seifu, 2004; Suguna *et al*., 2011). Providing adequate details on the prevalence of pathogenic or spoilage microorganisms in goat milk might be useful to identify and implement appropriate HACCP (Hazard Analysis Critical Control Point) along with good GAP and GMP (good agricultural and manufacturing practices) at the farm level, to benefit both consumers and the dependent dairy industry.

Results obtained for prevalence of microbial population in goat milk samples collected from the two farms is shown in Table 1. The total plate counts (TPC) in samples collected from farm 1 (4.5 log CFU/ml) had highest bacterial count compared to all other samples under study. In farm 1, the psychrotropic counts, yeast and mould counts, *Staphylococcus aureus* and Coliform counts were found to be 4.3, 4.2, 3.2 and 2.2 log CFU/ml, respectively. However, presumptive *E. coli, Salmonella* spp. and *L. monocytogenes* were not present in goat milk samples collected from this farm. Whereas, in the second farm, *K. pneumoniae* (4.8 log CFU/ml) counts were recorded to be high compared to all other bacteria under study. Followed by this were the yeast and moulds, presumptive *E. coli,* TPC, Coliforms, Psychrotrophic counts, *Salmonella* spp. and *S. aureus*, which were 4.6, 4.6, 4.2, 4.0, 3.1, 2.9 and 2.7 log CFU/ml, respectively.

The observed difference in the microbial load in both farms might have been influenced by extrinsic factors such as level of hygienic condition during handling of milk, season and geographical location of the dairy farm as opined earlier by Millogo *et al*. (2010). Contamination by *Salmonella* spp. and *E. coli* can be attributed directly to the surrounding environmental conditions (unhygienic environment

Table 1. Prevalence of microorganisms in goat milk samples collected from two popular dairy farms in Penang Island, Malaysia (log CFU/ml) (n=3 ± S.D.)

Parameter	Farm 1 (log CFU/ml)	Farm 2 (log CFU/ml)
Total plate count (TPC)	4.5±0.0	4.2±0.0
Yeast and Mould counts	4.2±0.0	4.6±0.0
Psychrotropic counts	4.3±0.0	3.1±0.0
Staphylococcus aureus	3.2±0.2	2.7±0.1
Coliforms	2.2±1.9	4.0±0.0
Presumptive *E. coli*	NP	4.6±0.0
Klebsiella pneumoniae	4.1±0.0	4.8±0.0
Salmonella spp.	NP	2.9±0.0
Listeria monocytogenes	NP	NP

NP, not present

such as presence of feces or organic matter) (Kousta *et al*., 2010). Additionally, the lack of awareness among the farmers with regard to the possible source of entry of these pathogens might contribute for contamination upto certain extent.

According to Cempirkova (2002), in Europe, milk qualities are monitored based on the presence of total bacterial count, which should not exceed 4.5 log CFU/ml. While in United States, bacterial count in goat milk is allowed upto 5.0 log CFU/ml with somatic cell count of 6.0 log CFU/ml. The limitation in goat milk is same with that of cow milk, except for somatic cell (Zweifel *et al*., 2005). In the present study, the total plate counts was 4.5 log CFU/ml, which is on par to the Malaysians Food Act 1983 and Food Regulations 1985, which states that the load of total bacteria should not exceed 5.0 log CFU/ml in every milliliter (1ml) of the milk sample (Food Act 1983 and Food Regulations 1985- Act 281, 2005). Based on this, our results indicate that bacteria load recorded in goat milk samples collected from two farms are still considered to be at a safe range. However, still there are high chances that the bacterial load might be enhanced if storage and handling conditions are not appropriate and of not international standards. Factors such as infected udder of goat, unhygienic milking procedure, poor water quality used for cleaning, use of unsterilized equipments and milk storage conditions are considered some of the common factors responsible for cross-contaminations (Chye *et al.*, 2004; Suguna *et al*., 2011).

Psychrotrophic microorganisms mainly belong to the genus Pseudomonas, which are capable of producing heat resistance enzymes (proteolytic and lipolytic) at low temperatures, which can hydrolyze milk fat and protein structures leading to development of off-flavors (Ercolini *et al*., 2009). Occurrence of Psychrotrophic bacteria in both the farm highlights the tendency of this bacterium to grow and multiply once stored at low temperature. If the farmers fail to store the milk under cooling conditions after milking for long duration of time, rapid contamination might occur by this bacterium (Champagne *et al*., 1994). Hence, adequate care should be taken to minimize the risks associated with this bacterium.

The presence of yeasts and moulds in goat milk samples collected from the 2 farms in this study highlights improper sanitary conditions encountered in the milking area as well as the storing equipments (containers) used in farm. Previously, in one of the studies reported by Torkar and Vengust (2008) on raw milk and cheese, 95% of raw milk samples showed the presence of yeasts (mean concentration of 1.7 log CFU/ml) and moulds (63.3% with a mean concentration 0.6 log CFU/ml). The authors have attributed the presence of these microbes to improper hygienic practice at manufacturing environment such as walls and shelves of container, air, equipments, water and milk brine.

The presence of Coliforms in goat milk samples collected from both farms in this study is identified to be fecal Coliforms, which were confirmed by MPN method. Malaysian Food Act (1983) and Food Regulations Act (1985) have stated that Coliform count should not exceed 1.7 log CFU/ml and *E. coli* should not be present in one ml of a milk sample (Food Act 1983 and Food Regulations 1985- Act 281, 2005). Therefore, presumptive *E. coli* detected in this study is confirmed to be an origin from fecal contamination, which indicates the presence of other enteric pathogen such as *K. pneumoniae*. However, feces alone might not be a cause of contamination, wherein other contributing factors such as poor hygiene and sanitary practice at farm level can also play a significant role. As dairy farms have complex surroundings, Coliforms might be omnipresent in feces, manure and soil, which enables easy dispersal of pathogens throughout the farm (Son *et al*., 2009; Lingathurai *et al*., 2010). This holds true for present observations too.

Staphylococcus aureus in goat milk showed counts of < 30 colonies in milk samples collected from both farms. The possible contamination by *S. aureus* in raw milk might occur from infected mammary glands (Kousta *et al.*, 2010). In the present study, the milking process was performed by hand (by the farmer), and hence the transmission of this pathogen through contaminated hands to mammary glands of the goat might have occurred. According to FDA (2001), presence of this pathogenic bacterium

in milk will start to produce toxins at 6.0 log CFU/ml and hence anything above this limit is unacceptable.

Outbreak of 'Salmonellosis' is considered to be uncommon in dairy farms. However, dairy products are recognized as one of the main source of *Salmonella* contamination, which can occur from either feces or unhygienic environment encountered in the farms vicinity (Lanzas *et al*., 2010). As *Salmonella* spp. are considered a potential foodborne pathogen, their presence even in threshold levels is not acceptable, considering the effects rendered on consumers. By identifying the actual source of contaminant (mainly cross-contaminants) along with adopting appropriate post-processing treatments, this pathogen could be prevented in goat milk.

The annual outbreak of human listeriosis (caused by *L. monocytogenes*) on consumption of contaminated milk and cheese is estimated to be between < 2 to 12 per 100 people in North America and Europe (Broseta *et al*., 2003). However, in Japan cases pertaining to listeriosis outbreak in milk is reported to be lower in comparison to other regions of the world. This has been attributed to the increased awareness among the consumers regarding the possible outcome of human listeriosis (Okutani *et al*., 2004). In our present study, *L. monocytogenes* was not present in the goat milk samples collected from both farms. As the surrounding environment in the dairy farm plays a significant role for contamination by *Listeria*, there might not be favorable conditions for the growth of this pathogen in both the farms evaluated in this study. Contamination by *Listeria* sp. in a particular food commodity generally occurs via farm soil, manure or from wastewater from industries (Broseta *et al*., 2003).

Since there is an ever-increasing demand being witnessed for goat milk, enhancing their shelf-life is the need of the day. As raw milk consumption with a possible contamination by food borne pathogens can pose high risks for human health, this can be avoided through good farming practices such as breeding healthy animals, waste management at farm level, appropriate milk handling with reducing storage times, and by employing appropriate refrigerated temperatures. Good hygienic practices aiming towards reduction of pathogens and spoilage bacteria is also considered beneficial (FAO/WHO, 1997; Kousta *et al*., 2010; Ercolini *et al*., 2009). Additionally, thorough hand washing (especially in the developing countries) in between milking of the goat during pre-milking and post-milking stages by using safe disinfectants can enhance the safety of fresh milk (Oliver, 2005). Heat treatment such as pasteurization and sterilization before consumption are also vital to manage the microbial contaminants in goat milk.

Conclusions

Microbiological quality evaluation of goat milk collected from 2 popular dairy farms in Penang Island, Malaysia, revealed fresh milk to be contaminated by foodborne pathogens. As there is every chance that this might pose serious health risks to the local population as well as in the surrounding regions (in Malaysia), adequate steps need to be undertaken by the local governing bodies to minimize the risks associated, by providing necessary training to the farmers as well as educating health conscious consumers. This will surely ensure long term benefits to the local dairy industries considering the local demand as well as export market of goat milk.

Acknowledgement

The first author thanks Universiti Sains Malaysia, for the financial support provided (as graduate assistantship).

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