MONITORING OF *BRUCELLA SP., COXIELLA BURNETII* AND AFLATOXIN M1 IN GOAT MILK FROM JOHOR

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ABSTRACT. Forty-nine fresh goat's milk samples produced by local farmers and sold in market for public consumption as well as raw goat milk in Johor, Malaysia were analysed for total plate count (TPC), *E. coli, coliform, Brucella melitensis*, *Brucella abortus, Coxiella burnetii* as well as aflatoxin M1 (AFM1) content, as measures for food safety. The mean counts per ml for TPC were 4.90 x 10^5, 6.50 x 10^5, 1.60 x 10^5 and 1.48 x 10^6 for pasteurised, unpasteurised and unknown (status of pasteurisation) milk sold in the market as well as the raw milk from milk collection center (MCC), respectively. Among pasteurised samples, only one had TPC count higher than the permitted level whereas the rest were all within the permitted level. The mean counts per ml for *E. coli* were <1.00 x 10^2 for pasteurised and unknown milk whereas 1.67 x 10^1 for unpasteurised and 1.18 x 10^2 for raw milk. The mean counts per ml for *coliform* were 9.53 x 10^3, 9.76 x 10^3, 1.20 x 10^2 and 1.16 x 10^4 for pasteurised, unpasteurised, unknown milk and raw milk, respectively. Overall, no significant differences on the bacterial counts in both pasteurised and unpasteurised milk. All milk samples were negative of *B. melitensis* and *B. abortus*, but one unknown sample from the market and two raw samples from MCC were positive of *C. burnetii* through the ELISA test. The unknown sample from the market showed the presence of *C. burnetii* when further analysed microscopically. Meanwhile, no sample exceeded the permitted level of AFM1 in milk.

Keywords: goat's milk, brucellosis, *Brucella melitensis*, *Brucella abortus, Coxiella burnetii*, Q-fever, aflatoxin M1, unpasteurised milk

INTRODUCTION

Milk is a universal food used by humans of all ages and nationalities. Malaysia produces fresh milk for local consumption both from smallholder farmers as well as commercial dairy farms. Thus it is important that the milk quality is excellent for human consumption as fresh milk as well as to produce other by products.

With respect to food safety and public health concerns, fresh milk for human consumption must be pasteurised as the raw milk may contain pathogenic or spoilage microorganisms that may harm the consumers. Pathogenic bacteria that can be found in milk are *Brucella sp.* and *Coxiella burnetii*, where both bacteria can cause zoonotic disease and are harmful to human.

Animal brucellosis (commonly caused by *Brucella abortus* or *Brucella melitensis*) can be transmitted to humans through ingestion of unpasteurised milk of infected animals and dairy products prepared from such milk. It can also be transmitted through direct contact with fluids and carcasses of infected animals, consumption of its undercooked meat, and through inhalation of airborne infectious particles. From 2011 to 2012, an outbreak of human brucellosis was reported in Penang, Malaysia involving 79 patients who had consumed unpasteurised goat's milk from the same farm (Leong *et al.*, 2015). As for *Coxiella burnetii,* the bacterium is the causative agent for Q-fever disease and is endemic in cattle, sheep and goats. Data has shown that there are epidemiological evidences relating the Q-fever cases in human with consumption of unpasteurised milk (Gale *et al.*, 2015).

The microbiological load of milk will determine its quality although the bacteria may not be pathogenic. This is because the shelf life of milk will become shorter and thus could cause economical loss especially for local farmers and smallholder dairies. Microbiological analysis such as Total Plate Count (TPC), *Escherichia coli* and *coliform* count are commonly conducted to monitor the milk quality and the effectiveness of pasteurisation.

Another food safety concern is regarding the aflatoxin M1 (AFM1) contamination in milk. AFM1 is a mycotoxin secreted in the milk of mammals that have consumed aflatoxin B1-contaminated foods (Suthep *et. al*, 2013). Aflatoxins are produced by certain species of fungi from the genus *Aspergillus* and AFM1 has been classified in type 2B (probable) human carcinogens by International Agency for Research on Cancer (IARC, 1993).

As local data is limited on the safety and quality of fresh goat's milk in the local market, a small study with the objectives to monitor the *Brucella* sp., *C. burnetii* as well as other microbiological quality and AFM1 content in fresh goat's milk produced and sold by local farmers in Johor was carried out. Results from the study could be useful to evaluate the safety, quality and pasteurisation status of the fresh goat's milk sold for public consumption as well as for the local governing agency to ponder whether further appropriate food safety measures needed to be implemented.

MATERIALS AND METHODS

Samples collection

Thirty-two fresh goat's milk samples (without flavour) were purchased from 16 different locations such as groceries, *Pasar Tani*, night market, booths, from the farmers as well as individual agents around Johor from October to November 2016 (duplicate samples). Apart from that, analyses were also carried out on 17 raw goat's milk from Milk Collection Centre (MCC) that was sent for milk quality checks to Makmal Veterinar Johor Bahru (Regional Veterinary Laboratory Johor Baru) from October to December 2016.

Laboratory Analysis

Goat milk samples were analysed for total plate count (TPC), *coliform* and *E. coli*

following the standard methods (AOAC International 2012). Direct isolation of *B. melitensis* and *B. abortus* on selective media were following the method by OIE Terrestrial Manual 2016, whereas the analysis of *C. burnetii* in milk was carried out by ELISA method (LSIVet™ Ruminant Q Fever-Serum/Milk, Life Technologies) and staining procedure for positive samples (modified Ziehl-Neelsen method) were following the OIE Terrestrial Manual 2015. Quantitative analysis of AFM1 content in milk was performed by ELISA method (MaxSignal® Aflatoxin M1, Bioo Scientific Corp.).

RESULTS

Milk samples were collected from 16 locations, 10 had labels and brand names on the milk bottle, whereas the remaining six were not labelled. Five brands had labels stating that the fresh goat's milk was pasteurised. Six claimed that it was unpasteurised: (i) stated on the label, (ii) labeled with direction to cook the milk prior for consumption, and (iii) unlabelled but admission by the farmer that it had not been pasteurised. Another five were classified as unknown: (i) no pasteurisation status stated on the label, (ii) no label and the vendor did not know whether the milk had been pasteurised, and (iii) no label on the bottle but the seller claimed it has been pasteurised.

Table 1 shows the total plate count (TPC), *E. coli* and coliform count as well as positive cases of *Brucella* sp. and *C. burnetii* in goat's milk samples from Johor (n = 49).

Table 1. Total plate count (TPC), *E. coli* and Coliform count as well as positive cases on *Brucella* sp. and *C. burnetii* on goat milk samples from Johor (n = 49)

Sample	Colony count (CFU/ml)			positive of *B. abortus* or *B. melitensis*	positive of *C. burnetii*	
	TPC	*E. coli*	Coliform		ELISA test	Staining
Pasteurised (n = 10)						
mean	4.90×10^5	$<1.00 \times 10^2$	9.53×10^3	0	0	0
max	4.50×10^6	$<1.00 \times 10^2$	4.10×10^4			
Unpasteurised (n = 12)						
mean	6.50×10^5	1.67×10^1	9.76×10^3	0	0	0
max	4.50×10^6	$<1.00 \times 10^2$	9.20×10^4			
Unknown (n = 10)						
mean	1.60×10^5	$<1.00 \times 10^2$	1.20×10^2	0	1	1
max	9.00×10^5	$<1.00 \times 10^2$	5.00×10^2			
MCC (Raw milk, n = 17)						
mean	1.48×10^6	1.18×10^2	1.16×10^4	0	2	0
max	1.00×10^7	2.00×10^3	1.20×10^5			

Key: Fresh goat milk sold for consumption were categorised as pasteurised, unpasteurised and unknown (status of pasteurisation). MCC: milk collection centre

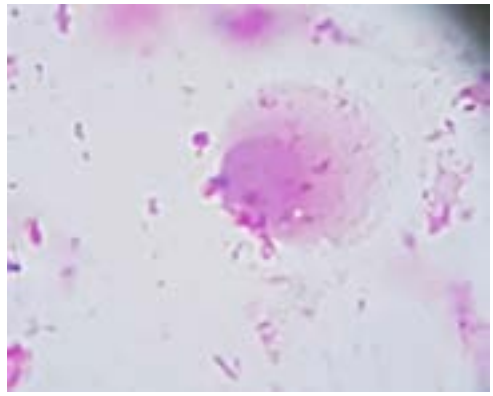

Figure 1. *C. burnetii* in goat's milk sample (modified Ziehl-Neelsen staining)

Results were categorised according to the pasteurisation status after evaluation of the samples purchased. The mean counts (cfu/ml) for TPC were 4.90×10^5, 6.50×10^5, 1.60×10^5 and 1.48×10^6 for pasteurised, unpasteurised and unknown (status of pasteurisation) milk sold in the market as well as the raw milk from MCC, respectively. Nine out of 10 pasteurised samples showed TPC count below 1.00×10^5 cfu/ml, and one sample had 4.50×10^6 cfu/ml. The mean counts (cfu/ml) for *E. coli* were $<1.00 \times 10^2$ for pasteurised and unknown milk whereas 1.67×10^1 for unpasteurised and 1.18×10^2 for MCC milk. The mean counts (cfu/ml) for *coliform* were 9.53×10^3, 9.76×10^3, 1.20×10^2 and 1.16×10^4 for pasteurised, unpasteurised, unknown milk and MCC milk, respectively. Overall, no significant differences on the microbiological counts for these bacteria between the pasteurised and unpasteurised milk.

All milk samples from the current study were negative for *B. melitensis* and *B. abortus*, but one unknown sample from the market and two raw milk from MCC were positive for *C. burnetii* through the ELISA test. These three positive samples were further analysed through staining and the unknown sample showed the presence of *C. burnetii* as shown in Figure 1.

Concentration of aflatoxin M1 in the milk samples were quantitated using matrix-matched calibration curve at five working standards of 0.005, 0.015, 0.030, 0.090 and 0.270 ppb (µg/L). The linearity of the regression (r^2) was 0.921, mean recovery for AFM1 in milk was 96%, and detection limit was 0.005 ppb. Results showed that AFM1 content in the milk samples ranges from below detection limit to 0.082 ppb.

DISCUSSION

Fresh goat's milk produced by local farmers were usually sold in small scale, where some of them bottled and sold the fresh milk individually, whereas some farmers bought the milk from other farmers in order to get a larger quantity of milk before bottling and selling it. Some of the farmers also market the milk through a cooperative (*Koperasi*) with help from Department of Veterinary Services (DVS) in terms of consultation,

awareness and methods of pasteurisation as well as in the use of a pasteurisation machine. Commonly, fresh goat's milk is sold in 250 ml bottles with prices from RM5 to RM7 in its original flavour, and higher prices for chocolate, strawberry or corn-flavoured milk at around RM6 to RM9. Milk was also bottled in 500 ml or 1L.

The fresh goat's milk is usually sold at agriculture bazaars (*pasar tani*), night market, booths, direct from the farmers as well as individual agents, advertised through the social media, and some individual agents even sell it from home. Despite that, the market for fresh goat's milk is smaller than fresh cow's milk. Sellers or farmers claimed that demand for fresh goat's milk is growing. They also claimed some of the reasons consumers bought the fresh goat's milk is due to its health benefit, as an alternative for children with allergies towards cow's milk and also the local popular belief which is for the treatment of jaundice in babies.

From the study, only five out of 16 locations (31%) of the milk sampled were pasteurised and stated on the label. Another 37.5% were not pasteurised, only a few was labeled with directions to cook before consumption. About 31% had no known pasteurisation status where some sellers claimed it had been pasteurised but most did not know. Some were even unaware of the importance of milk pasteurisation and confessed that there were parents who bought it to be given to their jaundiced babies.

The mean TPC counts for pasteurised milk in the current study was higher than the permitted level (1 x 10^5 cfu/ml) stated in the Food Act 1983 (Act 281) & Food Regulation 1985 (updated 2014). However, only one sample had high TPC result exceeding the level, whereas the rest of the pasteurised samples were all within the permitted level. Despite the fresh milk being pasteurised, storage and selling of milk at inappropriate temperatures could be the reason for the high bacteria count. Another possible reason is perhaps the pasteurisation process had not been carried out properly.

The presence of *C. burnetii* in one unknown sample might suggest that the sample was actually unpasteurised. Previous studies have found that the bacterium in bulk milk tank samples as well as unpasteurised milk samples (Loftis *et al.*, 2010; Kim *et al.*, 2005), and humans consuming milk contaminated with it could acquire Q-fever disease. Humans with Q-fever may develop pneumonia, hepatitis, risk of miscarriage during pregnancy and can be fatal (CDC, 2016). Meanwhile, no sample exceeded the permitted level of AFM1 in milk at 0.5 ppb (Food Act 1983 (Act 281) & Food Regulation 1985, (updated 2014)).

The selling of unpasteurised fresh goat's milk for consumption in the local market due to negligence or lack of knowledge could expose the consumers to harmful diseases and pose a potential public health risk. The local governing agencies ought to evaluate and take the necessary steps to protect the public health and educate the farmers and consumers on the importance of milk pasteurisation.

CONCLUSION

This study indicates that local goat's milk sold for public consumption has

microbiological load and aflatoxin M1 level within the permitted level, but some are still sold as unpasteurised. Further work is needed to analyse more samples to ensure safe milk for human consumption.

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