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Risk factors associated with mastitis and raw milk quality in small farms of Texcoco, México

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ABSTRACT

Objective: to determine the nutritional and physicochemical quality, and the presence of aflatoxins in raw milk, as well as risk factors for developing mastitis in 20 family farms in the region of Texcoco, Mexico.

Methods: MilkoSCan FT1 was used for nutritional and physicochemical analysis of milk. Somatic cells were quantified and the cow's health status was tested using Somaticell; furthermore, the presence of Aflatoxin M1 was determined using lateral flow immunochromatography.

Results: the milk evaluated in this study reported normal nutritional values according to NMX-F-700-COFOCALEC-2012, which guarantees its quality for human consumption. The pH ranged from 5.0 to 8.4, which indicates deficient temperature control in some farms, leading to problems with acidity. The logistical analysis showed that adequate udder cleaning during milking is important to avoid it being a risk factor for an increase in somatic cells and degree of mastitis, although not the milking technique or teat sealing. The presence of aflatoxin AFM1 was not reported in raw milk.

Study implications: the Somaticell® technique renders a qualitative and efficient diagnosis of clinical mastitis.

Conclusions: raw milk quality from this region guarantees consumers with a safe and apt product for human consumption or transformation into dairy byproducts.

Key words: raw milk, Somaticell® test, risk factors.

INTRODUCTION

Small-scale dairy farms often have technological limitations to obtain raw milk or other dairy products, such as cheese, cream, or yoghurt, which meet hygiene and safety standards in order to avoid public health problems (Zumbado and Romero 2015; Villagómez and Pérez, 2017). These products can be affected by the cows' overall health, contamination with toxins or pathogenic organisms, which

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are consequence of deficient management practices and inadequate milking techniques, manipulation of milk in tanks and storage containers which can damage the milk's color, taste, physicochemical and sanitary composition (Moreno *et al*., 2007). Therefore, a periodic diagnosis of mastitis, sterilization of milking equipment, physicochemical and nutritional milk analysis, as well as the detection of toxins in cow feed and raw milk, should be standardized protocol in these farms. Among the methods used to evaluate udder health and quantify the degree of mastitis, the Somaticell test is very sensitive and allows for the classification of milk obtained from affected udders (Ruíz and Sandoval, 2018; Remón *et al*., 2019), in addition to quantifying somatic cells in storage tanks (Pereira *et al*., 2014). The presence of aflatoxins in cow feed and in milk has been reported by some studies conducted in farms in the Estado de México, reporting the presence of aflatoxin AFM1 in raw milk above permissible limits (Péres *et al*., 2008), putting at risk the health of consumers (Urban *et al*., 2009). This has been attributed to the cows consuming feed contaminated with AFB1, which is why continuous monitoring is recommended to avoid a public health problem. The nutritional and physicochemical quality of raw milk was evaluated, in order to determine the presence of aflatoxin M1, determining the risk factors during milking for developing mastitis in family owned bovine production units in the micro-region of Texcoco, Estado de México.

MATERIALS AND METHODS

A cross sectional sampling of 20 family owned small-scale production units was carried out, with a total of 565 Holstein cows (4 to 35 cows herd^{-1}) located in the municipalities of Texcoco and San Andrés Chiautla, Estado de México (19° 24' and 19° 33' N; 98° 38' and 99° 02' W) (INEGI, 2009), during the months of March to July, 2019, based on a direct poll and sampling of raw milk in storage containers and tanks.

The interview form consisted of 71 questions related to general aspects of the farm, milk production, hygiene practices, and cattle management. Milk quality was based on the Mexican norm NOM-155-SCFI-2012, which classifies degree of udder infection according to somatic cell count (Figure 1) into four degrees (healthy: 0-200,000; subclinical: 200,001-400,000; clinical: 400,001-1,000,000; acute: >1,000,000). Physicochemical and nutritional analyses were carried out using infrared spectrometry in MilkoSCan FT1, measuring: proteins (gL^{-1}), casein (%), fat (gL^{-1}) and lactose (gL^{-1}). In order to count somatic cells and overall cow health, the Somaticell test was used taking 2260 samples from udder quarters of 565 cows. In order to determine the association between somatic cell count and related risk factors (milking technique, udder cleanliness, and teat sealing), logistic regression was used analyzing the significance of regression coefficients with the Wald test (Chi squared), using PRO LOGISTIC from SAS (SAS 1992, ver. 2). In addition, the presence of aflatoxin M1 was determined in 50 milk samples, utilizing lateral flow immunochromatography, with a sensitivity of 350 ppt.

RESULTS AND DISCUSSION

General description of the farms. The dairy herds of the family milk farms (FMF) in the region of study included a total of 565 cows, 58% of which are in production and 19% of which are dry. This indicates fertility planning throughout the year, in order to have a continuous offer for the market and a useful life of 5 to 6 births. Artificial insemination and replacement production are common practice. The milk is sold without any processing on the farm to consumers, intermediaries, or artisanal collection centers where it is transformed into fresh cheese, yoghurt and creams, with results similar to those reported by de Espinosa *et al*. (2010). Average production is 6 kg animal-1d^{-1} (Sánchez *et al*., 2015; Álvarez *et al*., 2012).

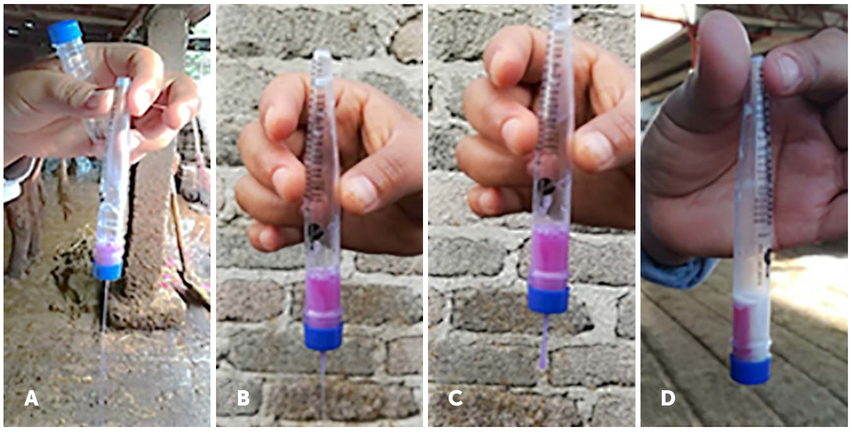

Figure 1. Aspect of the milk samples tested with Somaticell in order to determine presence of mastitis. A: Healthy, B: Subclinical, C: Clinical, D: Acute.

Milk production. On average each FMF produced 16±1.9 L animal^{-1} day^{-1}, which is sold without any processing in the region directly on the farm to consumers, collection centers, and artisanal companies who make fresh cheese, yoghurt, and creams. It is common for small-scale producers to sell raw milk door to door and get a higher price per liter compared to warehouse prices. Since these small-scale farms are family run, the production cost is lower, and they are able to establish a labor chain that includes local buyers, intermediaries, processing plants (cheese factories) and final distributor, as reported by Ruiz *et al*. (2017). This payment system based on volume offered is not adequate, since there is no additional compensation for milk quality, such as fat percentage and amount of total solids.

Mastitis diagnosis. In this region hand milking is more common than mechanical milking, and it is associated with a higher percentage of mastitis when compared to the mechanical process ($p<0.05$), due to presenting higher degree of clinical mastitis (29.2% and 26.6%, respectively), compared to other degrees of mastitis (Table 1). These results demonstrate that the absence of good milking practices, and deficient sanitary conditions at the time of cleaning and udder sterilization, can be underlying causes of a higher incidence of mastitis in these herds. This is inconsistent with findings by Ruiz *et al*. (2011), who reported a higher incidence of clinical mastitis associated with mechanical milking. There is also a higher frequency with afternoon milking (55%), with clinical type showing the highest degree of mastitis.

Mastitis diagnosis according to udder quarter. There was no difference reported when evaluating each quarter individually ($P>0.05$). The average prevalence of each quarter was 14% (Table 2). It was found that the highest presence of mastitis in all of the quarters was clinical mastitis (55.8%), and 23.7% reported acute mastitis. These percentages represent the most severe degrees of mastitis. This demonstrates that the lack of a cleaning routine and adequate udder hygiene are associated with an increase in mastitis.

The quantitative diagnostic method Somaticell allowed for a more accurate somatic cell count (SCC). Figure 2 shows the distribution of the variation of mastitis in all of the udder quarters evaluated, where 57% of the variation corresponds to values between 400 and 1000 SCC (clinical mastitis), while 24% ranged between 1001 and 1970 SCC (acute mastitis). The sum of these two figures represented 81% of the cases reported, these being the most severe degrees of mastitis (Gómez *et al*., 2015).

Calculating possible risk factors according to possible causes of mastitis

In this region hand milking is more common than mechanical milking, with 56% of done manually and the rest mechanically. Hand milking has been associated with higher percentage of mastitis when compared to mechanical milking.

The whole set of data about milking variables, udder cleanliness, and teat sealing were not statistically significant (X^2, $p>0.0.5$), therefore they could not be taken into account for a model of risk factors for degrees of mastitis in small-scale dairy herds in this region. On its own, the variable of udder cleanliness is very important (Pr>ChiSq 0.05); that is, the risk of presenting mastitis is higher if appropriate udder cleanliness is not carried out, which indicates that when the hygiene is more complete in the milking routine, the risk of presenting mastitis and udder contamination is 3.66 times lower (Table 3), with a 1 to 15 confidence interval. These findings are consistent with Ramírez (2015) who also reports that udder cleanliness is the most important factor associated with mastitis. The milking technique and teat sealing did not increase or decrease mastitis development.

Table 1. Frequency of mastitis (%) in Holstein cows depending on time of milking.

	Mastitis Classification				
	Healthy	Subclinical	Acute	Clinic	Total
Milking type					
By hand	3.0	8.5	29.2	13.4	54.2^a
Mechanics	1.8	7.2	26.6	10.4	45.8^b
Milking time					
Morning	3.0	7.2	22.8	12.2	45.1^b
Afternoon	1.8	8.5	33.0	11.6	55.0^a

($P<0.001$, Chisq test).

Table 2. Frequency of the degree of mastitis (%) in each of the four udder quarters of dairy cows in family owned production units.

Degree of mastitis	Udder quarter				
	CDD	CDI	CTD	CTI	Total
Healthy	1.0	0.9	1.4	1.5	4.8^d
Subclinical	3.7	4.4	4.0	3.7	15.7^c
Clinic	14.2	13.9	14.0	13.8	55.8^a
Acute	6.5	5.7	5.6	5.9	23.7^b

Columns with different letters are different ($P<0.01$, Chisq test); CDD: Right front quarter; CDI: Left front quarter; CTD: Right rear quarter; CTI: Left rear quarter.

Figure 2. Manual and mechanical milking system.

Nutritional quality of raw milk. It is fundamental for milk to conserve its nutritional and hygienic quality, even if this is related to a higher cost to the consumers. The estimated values for fat content varied between 3.6 and 4.42, due to the amount of fiber included in the cow's diet; with higher fiber content values, there is higher fat percentage in the milk. In these small herds, the fodder/concentrate ratio is high, with the proportion most likely increasing due to elevated concentrate costs and the fact that many produce their own fodder. The average lactose content was 4.21 and protein was 3.01, while the highest values were 4.63 and 3.87, respectively. These values are very similar to those reported in other studies, and they comply with milk quality norms and are not modified by the presence of mastitis.

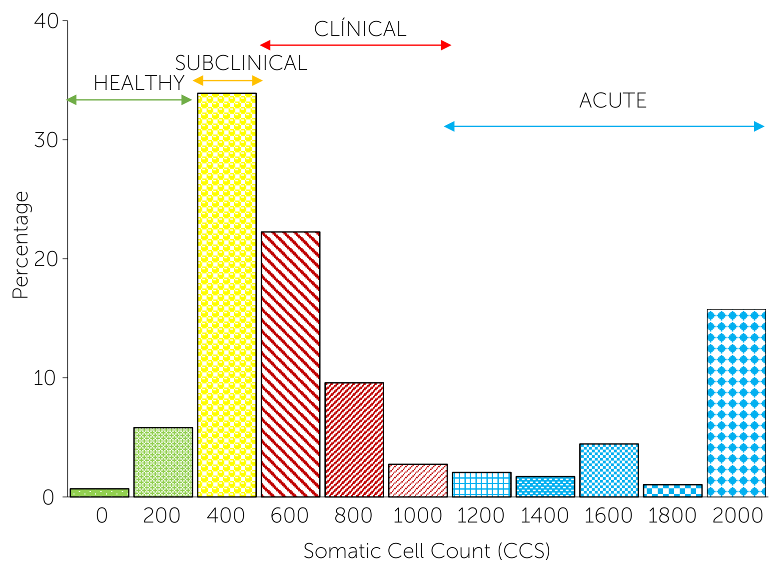

Figure 3. Distribution of mastitis variation in udder quarters of Holstein dairy cows in the Texcoco, Estado de México.

Table 3. Logistic regression analysis associating degree of mastitis with milking technique, udder cleanliness and teat sealing.

Variable	GL	Est*	E.E.	ChiSq. Wald	Pr>ChiSq	Parameter Estimater	ODD RATIO	
							Confident	Límites
							95%	
Intercept	1	1.15	0.30	15.07	0.01			
Milking Type	1	0.33	0.50	0.45	0.50	1.39	0.53	3.73
Udder cleaning	1	1.30	0.66	3.82	0.05	3.66	1.07	14.93
Sealing nipples	1	−0.25	0.52	0.23	0.63	0.78	0.277	2.18

*Est=estimator.

Other studies have reported that mastitis decreases the percentage content of fat and milk solid nonfat (MSNF), as well as reduces lactose levels (Bramley, 1996). The average MSNF value was reported at 7.96, with the highest value reported at 8.72 (Table 4).

A good indicator of milk adulterated with water is the cryoscopic point, which corresponds to milk freezing temperature, which normally ranges between −0.553 and −0.551 °C (Table 4), due to the presence of water soluble dairy components, mainly minerals and lactose.

Table 4. Nutritional and physicochemical composition of raw milk in dairy farms in Texcoco.

Variable	Minimum	Mean	Máximum	Standard error
Nutritional composition				
Fat	2.38	3.61	4.42	0.046
Lactose	3.29	4.21	4.63	0.035
Protein	2.51	3.01	3.87	0.034
NFS	6.4	7.96	8.72	0.064
TS	9.94	12.49	13.345	0.08
Density	1030.31	1037.56	1044.65	0.392
Casein	1.805	2.29	2.65	0.029
Urea	−0.1	0.01	0.02	0.003
Physicochemical composition				
Cryoscopic Point	0.39	0.5	0.55	0.004
pH	5.0	7.06	8.4	0.134

The value reported in this study is within this interval, and this adulterating practice should be avoided because it could cause a public health problem from contamination with water microorganisms.

The pH values reported ranged between 5.0 and 8.4 (Table 4), with a higher tendency towards more acidic levels; however higher, more neutral pH levels, were also found with a trend toward alkalinity values, similar to what is reported by Negri (2005), who mentioned that higher alkalinity pH could be an indicator of high incidence of mastitis. Therefore, it is important to be aware of the pH value, considering that alkaline levels are linked to high permeability of membranes in cows' mammary glands, which leads to higher Na^+ and Cl^- ion concentrations, as well as a reduction in lactose and inorganic P (Negri, 2005; Asif and Sumaira, 2010).

Aflatoxin M1 presence. Analysis of n=50 samples of raw milk to determine presence of aflatoxin M1 did not result in positive readings, which coincides with results reported by Ortiz (2009), in production units in Arequipa, Peru. However, they are not similar to reports by Pérez *et al*. (2008) in the Texcoco region, who reported levels above those established by the European Union (0.05 μg kg^{-1}), and above the daily recommended intake in Mexico for raw and pasteurized milk (0.5 μg L^{-1}). The results obtained could be attributed to the absence or very low levels of AFLAB1 present in the fodder and grains consumed by the animals.

CONCLUSIONS

In the study area, milk producers are characterized by planning the reproductive activities of herds during the entire year, which has the benefit of maintaining a constant milk offer in the market, which is sold without processing and directly to the consumer. The sanitary and physicochemical quality of milk complies with established norms. No aflatoxin AFM1 levels were found, which guarantees the consumer with a product suitable for human consumption.

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