Scientific Paper

Prevalence of subclinical mastitis and associated microorganisms

Flavia García-Sánchez, Tania Sánchez-Santana, Onel López-Vigoa and Miguel Ángel Benítez- Álvarez

Estación Experimental de Pastos y Forrajes Indio Hatuey, Universidad de Matanzas, Ministerio de Educación Superior Central España Republicana, CP 44280, Matanzas, Cuba
flavia.garcia@ihatuey.cu

Abstract

The study was conducted in a dairy farm of the Genetic Animal Husbandry Enterprise Los Naranjos, in the Caimito municipality –Artemisa province, Cuba–, in order to evaluate the prevalence of subclinical mastitis and the associated microorganisms. Milk samples were taken from each animal (10 mL), in sterile flasks, for the determination of the fat percentage and the contents of protein, lactose and total solids (TS). Likewise, a diagnosis was made of the disease through the California test, with count of somatic cells, and the microbiological agents that cause this pathology were determined. A high prevalence was found in the herd (60 %) during April and May, as well as a significantly higher index ($p < 0{,}001$) in May with regards to March. The main etiological causative agents of the high prevalence of subclinical mastitis in the herd in April and May were *Staphylococcus aureus* and *Enterobacter* spp. From the animals, 50 % showed counts of somatic cells higher than 200 000 cells/mL. No significant differences were found between the count of microorganisms and total coliforms during the evaluated period. It is concluded that the pathology had high prevalence in the herd and that the contagious etiological agents prevailed, with a 50 % frequency. The milk had good nutritional quality; however, it showed high counts of somatic cells, which bring about bad hygienic-sanitary quality.

Keywords: Bovinae, cells, milk production

Introduction

Subclinical mastitis is a disease with high prevalence in dairy cattle, and it is one of the most important pathologies that affect the dairy industry and cause economic losses to milk producers in the world. For such reason, it has been acknowledged for some time as the most costly illness in dairy herds (Calderón and Rodríguez, 2008; Ruiz-Gil *et al*., 2016).

According to Ponce *et al*. (2010), subclinical mastitis does not entail visible changes in milk or the udder, and it is characterized by the reduction in production, alteration in milk composition and presence of inflammatory components in it.

The etiology of mastitis can be infectious, traumatic or toxic. The causative bacteria can be larger or smaller pathogens of the mammary gland. The larger pathogens include *Staphylococcus aureus*, *Streptococcus agalactiae* and *Actinomyces pyogenes*, and other bacteria such as *Escherichia coli*, *Klebsiella* spp. and *Enterobacter* spp. The smaller pathogens include *Mycoplasma* ssp., *Pasteurella* ssp., *Nocardia* ssp., *Listeria* ssp. and some fungi and yeasts, among others (Ericsson *et al*., 2009; Trujillo *et al*., 2011).

According to Ruiz-Gil (2016), reducing the prevalence of mastitis is one of the important tasks in Cuban animal husbandry, and to study the economic losses associated to the disease is recommended. Hence the objective of the study was to evaluate the presence of subclinical mastitis in cows and determine the microorganisms associated to it in a cattle herd.

Materials and Methods

Location of the farm. The study was conducted in a farm of the Genetic Animal Husbandry Enterprise Los Naranjos, in the Caimito municipality –Artemisa province, Cuba–, during the period March-May, 2016.

Soil. The soil is classified as Ferralitic Red (Hernández-Jiménez *et al*., 2015).

Animals. The farm had 57 clinically healthy Holstein animals, from which 66,7 % were cows to guarantee the productive purpose; 16,0 % were milking and 38,3 % were dry, and they were distributed in two groups (a milking one and the other with dry animals). The system used for both groups was continuous grazing.

Herd management and feeding. The dairy farm had a total area of 32 ha, from which 26 ha were dedicated to pastures, 3 ha to *Cenchrus purpureus* and 1 ha to *Saccharum officinarum*.

The cows grazed from 6:00 a.m. to 10:30 a.m.; then they were taken to the sheds, where they received forage and stayed until the milking time (3:00

p.m.). At the end of milking they were transferred to an area close to the farm, where they remained until 6:00 p.m.; later, they were taken to the shed until the next milking time (from 4:00 to 5:00 a.m.).

In the farm they were supplied commercial concentrate feed and forage (*C. purpureus*); the quantity of feedstuff supplied depended on the category and productive status of the animal. In the case of the milking group concentrate feed was supplied at a rate of 0,450 kg^{-1} kg of milk, in addition to 33 kg of chopped forage per animal.

In the floristic composition of the pastureland the following prevailed: *Dichanthium annulatum* (24,4 %), *Paspalum notatum* (44,5 %), *Megathyrsus maximus* (14,7 %) and 16,4 % of weeds.

Sampling and inspection of the animals. The udder and the milking routine were inspected. From each animal, milk samples (10 mL) were taken, in sterile flasks; they were transferred, in a thermos with ice, to the laboratory of the National Center of Agricultural Health –Mayabeque, Cuba– for the determination of milk quality, the diagnosis of subclinical mastitis and the microbiological diagnosis.

Experimental measurements

Milk quality. The samples were obtained from all the milking cows in the first 150 days of lactation. The fat percentage, protein content, lactose and total solids (TS) were determined by the infrared method (FIL-141: B, 1997), through the MilkoScan 104 A/S Foss Electric, according to Kent-Ruiz *et al*. (2014).

Diagnosis of subclinical mastitis. The California Mastitis Test (CMT) was carried out, which was used as qualitative method; and the content of somatic cells present in the milk was determined, according to the methodology described by Gómez-Quispe *et al.* (2014), as quantitative method.

Based on the results of the CMT a subclinical mastitis index was determined, where: negative: 1; traces: 2, +: 3, ++: 4 y +++: 5 (Gómez-Quispe, 2014), which was used for the data analysis.

The prevalence of subclinical mastitis was calculated by the following formula:

$$\text{Prevalence} = \frac{\text{Number of positive animals}}{\text{Number of sampled animals}} \times 100$$

Microbiological diagnosis. The procedure described by Kent-Ruiz (2014) was followed, which is described below:

From each sample 0,1 mL was taken, planted in Blood Agar (Columbia agar base supplemented with 5 % defibrinated sheep blood), and incubated at 37 ºC for a period of 48-72 h. The isolation was valid when more than three identical colonies were found per sample, and the samples were considered contaminated when more than three colony types appeared.

The pure cultures were tested by: catalase, oxidase and Gram staining for a presumptive diagnosis to the genus level: *Staphylococcus*, *Streptococcus*, *Corynebacterium*, among others. For the identification of *Corynebacterium* the differential growth in Tryptic Soy Agar (TSA) and TSA supplemented with 1 % of the Tween 80 species was used.

For *Staphylococcus* the species differentiation was supported by coagulase and the Voges-Proskauer test, and it was divided in *S. aureus*, coagulase-positive *Staphylococcus* (CPS) and coagulase-negative *Staphylococcus* (CNS) if the results were positive-positive, positive-negative and negative-negative/positive for both tests, respectively. For the identification of *Streptococcus* to species level the Edward medium was used following the manufacturer's indications.

The somatic cells were counted in the Minor Fossomatic (Foss, Hillerød, Dinamarca) equipment.

Statistical processing. The variables milk quality, total microorganisms and total coliforms were processed through a simple classification variance analysis (ANOVA), to determine the differences between the sampling months; the means were compared through Duncan's test (1955) for 5 % significance, after verifying they fulfilled the normality (Kolmogorov-Smirnov goodness-of-fit test) and variance homogeneity (Levene's test), using the statistical package SAS®. A frequency analysis was performed on the somatic cells and etiological agents.

Results and Discussion

The milk quality during the studied period is shown in table 1. No significant differences were found for the percentages of fat, protein, lactose, total solids and non-fatty solids.

The fat values were similar to the ones obtained by Hernández and Ponce (2000) for this breed (3,78 %), but it is valid to state that these authors calculated the physical-chemical indicators of the milk of Holstein-Friesian cows in silvopastoral systems; while the protein values were similar to the ones reported by Peraza-González *et al.* (2015).

Table 1. Nutritional quality of milk during the evaluated period.

Indicator (%)	March		April		May		Sig.
	Mean (± SE)	VC (%)	Mean (± SE)	VC (%)	Mean (± SE)	CV (%)	
Fat	3,5 (± 0,193)	20,5	3,5 (± 0,260)	19,5	3,6 (± 0,246)	21,6	
Protein	3,0 (± 0,061)	7,9	3,0 (± 0,097)	8,6	3,2 (± 0,134)	13,4	
Lactose	4,3 (± 0,089)	7,8	3,7 (± 0,341)	24,4	4,1 (± 0,268)	20,8	NS
TS	11,5 (± 0,270)	8,8	11,1 (± 0,512)	12,3	11,7 (± 0,230)	6,2	
NFS	8,0 (± 0,127)	5,9	7,4 (± 0,385)	13,7	8,1 (± 0,290)	11,4	

TS: total solids, NFS: non-fatty solids.

Table 2 shows the prevalence and index of subclinical mastitis in the March-May period. The highest prevalence occurred in May (60 % of the animals). There was significant difference for the subclinical mastitis index; the highest values were found in May (2,6) and the lowest ones in March (1,2).

The prevalence of subclinical mastitis in April and May was higher than the one found by Ruiz *et al*. (2011) in Brazil; between 39,3 and 54,8 % for CMT in a mechanized milking system.

In a study conducted in Cienfuegos, Cuba, it was reported that among the main factors which caused the appearance of this disease were the slipping of the liners and the incorrect udder squeezing, as well as others related to management practices, mainly with the milking routine, and those depending on the animal, especially lactation days and number (Novoa *et al*., 2005).

In this sense, in the evaluation of the farm it could be observed that the milking routine was not fulfilled, which is one of the main causes in the prevalence of this disease. In addition, the final antisepsis of the nipple was not performed and it constitutes the entrance door for microorganisms.

In turn, the values found in this research are lower than the ones reported by Bonifaz and Colango (2016) in the community of Paquiestancia Cayambe canton, Ecuador, from an epidemiological study of bovine mastitis prevalence through the field California Mastitis Test. It was found that from the sampled quarters, 64 % was affected by some degree of mastitis.

Santivañez-Ballón *et al.* (2013) stated that Holstein cows, three to four years old and without hygiene in milking, have 74 % of probability of suffering subclinical mastitis.

A similar conclusion was arrived at by Vidales-Curequia *et al*. (2017), who determined the influence of breed and coincided in the fact that Holstein was the breed with higher prevalence of subclinical mastitis, when comparing it with different crossings

Another way of determining subclinical mastitis is through the count of somatic cells. The main factor that determines the increase of somatic cells in the milk is mastitis (McDougall *et al*., 2009; Green *et al*., 2014).

Figure 1 shows the number of somatic cells. The counts higher than 200 000 per milliliter (for 50 % of the sampled animals) mean a high percentage of subclinical mastitis in the herd (approximately 50 % of the total animals), which brings about considerable losses in milk production (Fonseca-Sánchez, 2015).

Table 2. Prevalence of subclinical mastitis during the study.

California test, CMT	Prevalence (%)		
	March	April	May
Negative	80	50	40
Traces	20	10	20
+	-	-	30
+	-	20	-
+++	-	30	10
CMT index	1,20 (0,1333)^{b}	1,90 (0,4333)^{ab}	2,60 (0,4761)^{a}

a, b: Different letters in the same row differ at $p<0,05$

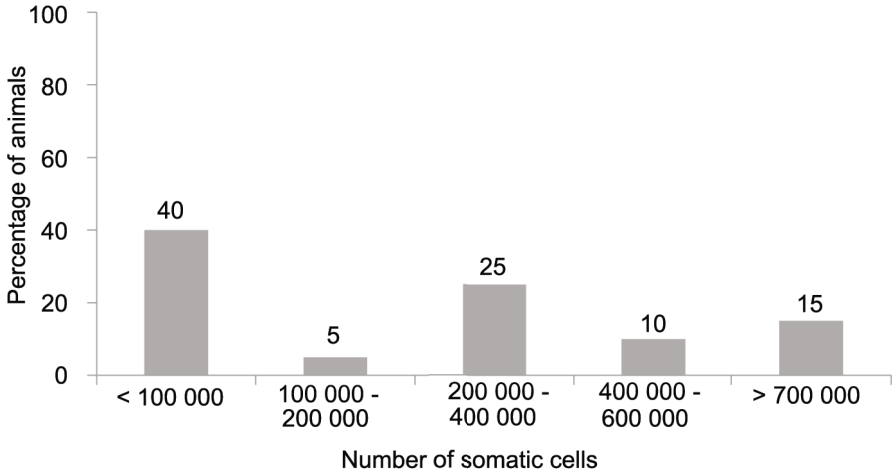

Figure 1. Count of somatic cells

Table 3 shows the count of total microorganisms and coliforms. There were no significant differences between April and May for any of the evaluated indicators.

The count of microorganisms was 3,24 x 10^5 CFU/mL in the two months the experiment lasted, value which is considered high; the milk is classified as of bad quality when such count exceeds 300 x 10^3 CFU/mL (Calderón *et al*., 2006). The total coliforms reached 2,30 x 10^3 and < 10^3 CFU/mL for April and May, respectively; and although no statistical differences were found, these values are considered high in good-quality milk.

The presence of coliforms is an indicator of the degree of fecal contamination which, in the case of milk, becomes an evaluator of the cleaning degree of the milker's hands, of the cleaning and disinfection of nipples and liners (Calderón, 2006). It is stated that more than 1 000 coliforms/mL can be found, and the values detected in April are over this quantity.

In order to decrease these values good animal husbandry practices should be promoted, such as the implementation of hygienic practices that guarantee clean, dry and healthy nipples, which is the first rule to obtain milk of good bacteriological quality.

The microorganisms isolated with higher frequency are shown in table 4. The most frequent pathogen was coagulase-negative *S*., with 50,0 and 53,3 % for April and May, respectively; followed by *Enterobacter* spp. with 28,6 and 26,7 % in April and May, respectively. The values of coagulase-negative *S*. exceed the ones found in Boyacá (14,5 %) by Hernández-Jiménez (2015) and Hernández *et al*. (2016).

According to Yera-Pompa and Ramírez (2016), the presence of Enterobacteriaceae in a dairy herd could be associated to the fact that they are germs that come from the intestinal tract of the animals and can be found in dung, soil and water, which indicates deficiencies in the hygiene of the environment where the milking cows are placed.

Bovine subclinical mastitis, according to the etiological agent, is classified in contagious and environmental; in the case of this study the one produced by contagious agents, whose main reservoir is the bovine (infected) mammary gland, prevailed. This mastitis can be controlled through good milking practices and drying treatments.

However, the results of this study differ from the report by Aguilar-Aldrete *et al*. (2014) when

Table 3. Count of total microorganisms and coliforms.

Month	Total microorganisms CFU/mL × 10^5 (± SE)	Total coliforms CFU/mL (± SE)
April	3,24 (± 1,399)	2,30 (± 0,7394)×10^3
May	3,24 (± 1,115)	< 10^3
Sign.	NS	NS

Table 4. Frequency of microorganisms during the study.

Microorganism	April		May	
	Absolute frequency	Relative frequency (%)	Absolute frequency	Relative frequency (%)
Streptococcus agalactiae	-	-	1	6,67
Strepococcus spp.	2	14,29	1	6,67
Coagulase-negative *Staphylococcus aureus*	7	50,00	8	53,33
Enterobacter spp.	4	28,57	4	26,67
Corynebacterirum bovis	1	5,88		
Shigella spp.	1	5,88		
Escherichia coli			1	6,67
Total	14	100	15	100

evaluating the prevalence and associated pathogens of subclinical mastitis in dairy cows in the Ciénega region of Jalisco state –Mexico–, because the microbiological analysis showed that 100 % of the samples had presence of *S. aureus* and *Salmonella* spp.

In this sense, Karimuribo *et al*. (2008) stated that the predominance of contagious pathogens could be correlated to the type of milking and/or its bad hygiene. Similar performance was obtained in Brazil where the microorganisms *Staphylococcus* spp., *Corynebacterium* spp., *Micrococcus* spp. and *Streptococcus* spp. prevailed (Ruiz *et al*., 2011).

It is concluded that subclinical mastitis showed high prevalence and that contagious etiological agents prevailed, among them coagulase-negative *S.* with 50 % frequency.

The milk had good nutritional quality; nevertheless, it showed high counts of somatic cells, which brings about bad hygienic-sanitary quality.

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