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ORIGINAL ARTICLE

Prevalence, somatic cell count and etiology of bovine mastitis in Cuban herds from Mayabeque province using hand and machine milking

Amado Kent Ruiz, Joan Peña, Dayaimi González, Pastor Ponce

Center of Assays for Foods Quality Control (CENLAC), National Center for Animal and Plant Health (CENSA), P.O. Box 10, San José de las Lajas, CP 32 700, Mayabeque, Cuba. E-mail: kent@censa.edu.cu.

ABSTRACT: Few studies have been developed to evaluate the differences of bovine mastitis situation between hand and machine milking under the American tropic conditions. Twenty dairy herds were studied, 11 using hand milking (HM) and 9 machine milking (MM), from «San José de las Lajas» municipality, Mayabeque province. Between May 2009 and March 2012, samples from 182 cows (113 MM and 69 HM) were obtained meaning a 35.1% of total milking cows, resulting in 708 quarters sampled (435 MM and 273 HM). Samples were subjected to bacteriological diagnosis and somatic cell count (SCC). There were significant differences for the prevalence of blind quarters, subclinical mastitis and intramammary infections between hand milking, 1.1; 29.4 and 59.4% and machine milking, 3.8; 59.0 and 79.9%, respectively. A significant difference was found for somatic cell count averages, with 361 000 and 984 000 cells/ml for hand and machine milking, respectively. Only *Streptococcus agalactiae* had a difference of statistical relevance for distribution by herds, 18.2% in hand milking and 88.9% in machine milking. In samples from herds with hand milking, the pathogens of higher frequency were: *Corynebacterium bovis* 24.4 %, Coagulase Negative *Staphylococcus* 13.3% and *Staphylococcus aureus* 6.6%; those in machine milking herds were: Coagulase Negative *Staphylococcus* 33.0%, *Corynebacterium bovis* 15.8 % and *Streptococcus agalactiae* 7.4%. The somatic cell count averages for the bacteriological diagnoses showed a significant difference between milking types, for negative quarters, Coagulase Negative *Staphylococcus* and *Corynebacterium bovis*. Bovine mastitis presented a worse situation in the herds using machine milking.

Key words: bovine mastitis, coagulase negative *Staphylococcus*, *Corynebacterium bovis*, *Staphylococcus aureus*, *Streptococcus agalactiae.*

Prevalencia, conteo de células somáticas y etiología de la mastitis bovina en rebaños cubanos de la provincia Mayabeque con ordeño manual y mecánico

RESUMEN: En el trópico americano se han realizado pocos trabajos para evaluar las diferencias de la mastitis bovina entre ordeño manual y mecánico. Fueron estudiadas 20 propiedades productoras de leche bovina, 11 con ordeño manual y 9 con ordeño mecánico, del municipio San José de las Lajas, provincia Mayabeque. Entre mayo de 2009 y marzo de 2012 se tomaron muestras de 182 vacas (113 ordeño mecánico y 69 manual); del 35.1% de las vacas en ordeño, se obtuvieron 708 muestras de cuartos (435 y 273). Las muestras fueron sometidas a diagnóstico bacteriológico y Conteo de Células Somáticas (CCS). Se encontraron diferencias significativas para la prevalencia de cuartos atrofiados, mastitis subclínica e infecciones intramamarias entre ordeño manual, 1,1; 29,4 y 59,4% y ordeño mecánico, 3,8; 59,0 y 79,9% respectivamente. Existió diferencia significativa en la media del CCS, con 361 000 y 984 000 células/ml para ordeño manual y mecánico respectivamente. Solamente *Streptococcus agalactiae* tuvo una diferencia de relevancia estadística en la distribución por rebaños, con 18,2 % en ordeño manual y 88,9% en mecánico. En ordeño manual los patógenos de mayor frecuencia fueron: *Corynebacterium bovis* 24,4%, *Staphylococcus* Coagulasa Negativo (SCN) 13,3% y *Staphylococcus aureus* 6,6%; en ordeño mecánico: SCN 33,0%, *Corynebacterium bovis* 15,8% y *Streptococcus agalactiae* 7,4%. Los CCS medios según los diagnósticos bacteriológicos solamente evidenciaron una diferencia significativa entre tipos de ordeño, para cuartos negativos, SCN y *Corynebacterium bovis*. En los rebaños estudiados, la mastitis bovina presenta peor situación en el ordeño mecánico.

Palabras clave: Mastitis bovina, *Staphylococcus* Coagulasa Negativo, *Corynebacterium bovis*, *Staphylococcus aureus*, *Streptococcus agalactiae.*

INTRODUCTION

Bovine mastitis is an inflammatory response of the mammary gland. It has a major impact on animal production, animal welfare and milk quality. Mastitis is one of the biggest problems for dairy because of the highest morbility and significant economic losses (1,2).

The results of microbiological tests can be used for the adoption of specific control measures, identification of emerging pathogens, culling animals with chronic infection, evaluation of proficiency tests for treatments, and to establish antimicrobial susceptibility profiles (3). Especially important is to identify the circulating microorganisms and their characteristics before considering a mastitis control program (4). The term «intramammary infection» (IMI) is not strictly synonym of mastitis; IMI is commonly used in the etiology context defined, using complex diagnostic procedures (5).

There are few studies in order to evaluate differences between machine and hand milking under American tropic conditions, however mastitis is a serious problem for both milking systems, and prevalence is normally high (6); there are significant differences concerning IMI, clinic, subclinical mastitis prevalence and incidence, total counts of aerobic mesophilic and coliform bacteria and the isolated IMI pathogens; with a higher subclinical mastitis in animals using hand milking, in Venezuela and Mexico (7, 8). However, some preliminary results indicate the opposite situation in Cuba (9).

The present study was conducted to microbiologically and epidemiologically differentiate mastitis in a Cuban municipality for each type of milking systems.

MATERIALS AND METHODS

The study included 20 dairy herds, 11 under hand milking system (HM) and 9 using machine milking (MM). All herds were located in «San José de las Lajas» municipality, Mayabeque province. Each herd had heterogeneity in breed, animal age and stage of lactation. Herds were visited between May 2009 and March 2012. Samples from 182 cows (113 MM and 69 HM) were obtained, meaning a 35.1 % of total milking cows, resulting in 708 quarters sampled (435 MM and 273 HM). During sampling, the milking routine was observed in each herd. If a quarter did not produce milk, it would be considered blind or atrophied. The clinical mastitis diagnostic was based on the examination of cow´s, udder and forestrip test result. The sampling methodology was according to NMC guidelines (10). Samples from very far herds were frozen until arriving to the laboratory.

In each sample, 0.1ml was streaked into blood agar (Columbia agar base supplemented with 5% defibrinated sheep blood), incubated from 48 to 72 hours at 37°C. A valid isolation was considered when more than three identical colonies per sample were found; samples with more than three types of colonies were considered a contaminated one. Pure cultures were tested by: catalase, oxidase and Gram stain for a presumptive diagnosis until genera level: *Staphylococcus*, *Streptococcus*, *Corynebacterium*, *Bacillus*, *Candida* and *Prototheca.* For *Corynebacterium bovis* identification, the differential growth was used on unsupplemented Tryptic Soy Agar (TSA) and TSA supplemented with 1% of Tween 80. *Staphylococcus* species differentiation was supported by coagulase and Voges-Proskauer test; divided in to *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 *Streptococcus agalactiae* identification, Edward medium was used following the manufacturer indications.

Somatic cells in milk samples were counted by the Fossomatic Minor equipment (Foss, Hillerød, Denmark). The resulting values were transformed into Somatic Cell Score (SCS) (11).

For data analysis, there was a comparison of binomial proportions using hypothesis testing and t-test for comparing two sample means using the program Statgraphics Plus 5.1 for Windows.

RESULTS AND DISCUSSION

Days under lactation and parity averages of all herds were 157.5 and 3.7 (115.2; 3.5 for MM and 176.8; 3.9 for HM), several studies (12, 13) mention these variables as risk factors.

There is a significant difference between hand and machine milking systems regarding the prevalence of atrophied quarters, subclinical mastitis (if SCS>4) and IMI; being better in general for hand milking (Table 1). There is no significant difference for clinical mastitis prevalence between the milking systems.

Following similar conditions, other authors could not find significant differences regarding atrophied quarters; i.e. 3.9 % and 3.0 % for hand and machine milking, respectively (9). The explanation for our findings could be the non-optimal technical conditions of milking machines, taking into account frequently electric

TABLE 1. Comparison of prevalence of atrophied quarters, clinical and subclinical mastitis by SCS and IMI, between hand and machine milking./ *Comparación de la prevalencia de cuartos atrofiados, mastitis clínica y subclínica, según SCS e IIM; entre ordeño manual y mecánico.*

Milking system	Atrophied quarters	Clinical mastitis	SCS (Somatic Cells Score)	IMI (Intra-mammary Infections)
Machine milking	0.038[a]	0.018[a]	0.590[a]	0.799[a]
Hand milking	0.011[b]	0.007[a]	0.294[b]	0.594[b]

Proportions with different superscripts in the same column are significantly different ($p<0.05$).

failures, generating more atrophied quarters.

The values of clinical mastitis reported here were very inferior with respect to other studies in recent years (6, 14, 15), all greater than 4%.

Subclinical mastitis is 15-40 %, more prevalent than clinical mastitis (16). In these results, the difference was close to 40%, a possible explanation is the underestimated diagnostic of clinical mastitis since there was a lack of forestrip test in some herds.

The highest prevalence of subclinical mastitis was found in machine milking and can be associated to non-well-functioning machines used in these herds, more specifically: old and porous teat cup, out of frequency pulsation system, out of order vacuums and lack of cleaning supplies; besides there was a delay or suspended milking by electrical power failures.

Another study made in Colombia showed smooth differences in subclinical mastitis prevalence by comparing the milking systems, 23.6% in hand milking and 30.0% in machine milking; the mastitis status was determined by California Mastitis Test (CMT) and less herds were used (6). A research in Mexico showed a higher prevalence in hand milking (57%) than in machine milking (33%), but just one herd was included per milking system (7). The highest prevalence using machine milking (35%) over hand milking (25.9%) was previously demonstrated in Cuba (9), but with less numbers of herds and quarters than in this study.

The fact that IMI prevalence was higher in machine milking than in hand milking was found in other countries like Venezuela (17, 18). A possible explanation for the difference is that milking machines become fomites and traumatic agents if the periodical maintenance fails (17).

According to other researchers, the proceedings in machine milking pre-disposed the bacteria entrance throw the teat channel (19). Based on the mammary gland tissue reaction to the milking machine, researchers found that the machine represented a higher risk to get IMI, compared to the use of calf (20). Machine milking provided an opportunity for bacterial transmission among cows and cow's quarter, due to variation in the pressure vacuum, wear of the teat cups and over-milking (18).

There was a high difference (higher than 20 units) of prevalence between subclinical mastitis and IMI in both milking systems, but in the case of machine milking, that difference was more reduced and both values were higher, suggesting that machine milking contributed to the increase of mammary inflammation and predisposition of bacterial infection.

The inflammatory reaction was measured by electronic count and average values were significantly lower in hand milking (Table 2). This was the first time that SCC was used in Cuba in order to compare both milking systems.

TABLE 2. Comparison between hand and machine milking using average values of SCS and SCC./ *Comparación de la media de CCS y SCS entre ordeño manual y mecánico.*

Parameter	Average ± Standard Error	
	Machine milking	Hand milking
SCS	4.57[a] ± 0.14	2.40[b] ± 0.16
SCC (10^3 cells/mL)	984.4[a] ± 88.2	361.2[b] ± 49.0

Means with different superscripts in the same row are significantly different ($p<0.01$).

SCC methodology is very important and more effective to characterize mastitis disease and its pathogen under our conditions, thus the increase in SCC associated to the volume reduction in milk production; SCC has been used to measure the quality at herd or region level and mastitis prevalence (5). SCC has been an important component of milk in the

assessment of aspects such as quality, hygiene and mastitis control (12).

In Brazil, the same difference was found in SCC between both milking systems with values very similar to our results for hand milking (373 000 cells/mL) and lower for machine milking (530 000 cells/ml) (21). The authors suggested as causes, the problems in the cleaning process and the lack of equipment maintenance. These criteria reinforced our findings and points of view. It is well known that a proper maintenance and operation of any milking system are key aspects for a successful milking (22).

Table 3 shows the percent of herds where each microorganism was present. For all pathogens, there was a higher distribution in machine milking; *S. agalactiae* was the only one with significant difference. The prevalence, biological and epidemiological characteristics of *S. agalactiae* showed the lack of a program for mastitis control in the herds studied.

TABLE 3. Distribution of microorganisms by herds./ *Distribución de microorganismos, en los rebaños estudiados.*

Microorganisms	Machine milking	Hand milking
CNS	100.0 % [a]	90.9 % [a]
Corynebacterium bovis	100.0 % [a]	90.9 % [a]
Streptococcus agalactiae	88.9 % [a]	18.2 % [b]
CPS	66.7 % [a]	45.5 % [a]
Staphylococcus aureus	77.8 % [a]	45.5 % [a]
Streptococcus sp.	44.4 % [a]	27.3 % [a]
Corynebacterium sp.	44.4 % [a]	18.2 % [a]

Proportions with different superscripts in the same row are significantly different ($p<0.05$).

S. agalactiae is not present in the cow environment, it needs to be in the mammary gland to survive; otherwise this pathogen lives in the galactoforous ducts and can be easily eliminated by antibiotics (23). Also it is very sensitive to penicillin and simple and routinely control measures can eliminate it from herds (24). Actually, from the beginning of the use of antibiotics in dairy farms, *S. agalactiae* has been taken place for *S. aureus* as a major cause of bovine mastitis (6).

The more frequently isolated microorganisms are shown in Table 4. In this case, just isolates in pure culture from the original sample were recorded. The frequency of mixed infections was 6.7% and 9.2% for machine and hand milking, respectively. In machine milking, the more frequent combination of mixed infection was *S. agalactiae* - *C. bovis*, whereas CNS - *C. bovis* in hand milking.

TABLE 4. Frequency of microorganisms, pure isolates on quarters sampled./ *Frecuencia de microorganismos, aislamientos en solitario sobre cuartos muestreados.*

Microorganism	Machine milking	Hand milking
CNS	0.330[a]	0.133[b]
Corynebacterium bovis	0.158[a]	0.244[b]
Streptococcus agalactiae	0.074[a]	0.011[b]
CPS	0.043[a]	0.018[a]
Staphylococcus aureus	0.045[a]	0.066[a]
Streptococcus sp.	0.029[a]	0.015[a]
Corynebacterium sp.	0.019[a]	0.011[a]

Proportions with different superscripts in the same row are significantly different ($p<0.05$).

It was found that there was not a preponderance of contagious pathogens in machine milking, but they were present in hand milking. Regarding this, *Karimuribo et al*. (25) expressed that the predominance of contagious pathogens in developing countries could be correlated with hand milking and/or with a poor hygiene.

When both milking systems were compared by the frequency of pathogens, it was possible to find a significant difference only in some species, with high number of isolates. That was the case of CNS, *C. bovis* and *S. agalactiae* (Table 4), the first two microorganisms were considered as minor pathogens (26). These results are very different to others, i.e. Rodríguez (6), who found *S. agalactiae* as the most prevalent in hand milking and *S. aureus* in machine milking. In Venezuela, the following pathogens were found in machine milking: CPS, CNS and *Streptococcus* spp. in that frequency order (8). However, in Brazil, the results concerning machine milking were similar to our results; with: *Staphylococcus* spp., *Corynebacterium* spp., *Micrococcus* spp. and *Streptococcus* spp. as more frequent species (27).

According to these results, CNS could be the most prevalent pathogen in Cuba. This is a phenomenon recently found in many countries with a developed dairy production (24, 28). CNS infections might play a major role in udder health and milk quality (29).

Concerning hand milking, our results were different to those obtained in Venezuela, where CNS, CPS and *Corynebacterium* sp. were more prevalent (8). Studies in Brazil were closer to our results; *Corynebacterium* spp., *Staphylococcus* spp., *Streptococcus* spp. and *Micrococcus* spp. were more prevalent in that order (27).

The lack of basic control measures justified the presence of *C. bovis* as the most isolated species in hand milking system. This species must have a prevalence of 1% or less when there was a teat disinfection before and after milking (30).

It was found that SCC values for pathogens in machine milking were higher (Table 5), such as the results obtained by Faría *et al.* (18). CNS became an important pathogen in our country; showing high values of SCC and SCS for this group. The infections by CNS were two or three time SCC of non-infected quarters. CNS could cause persistent infections, resulting in an increased milk SCC which affected milk quality, and may have been related to a decreased milk production (31). In Venezuela, higher values were found in herds using machine milking (5 550 000 cells/ml); regarding hand milking, the results were similar to ours (410 000 cells/ml) (18). Some authors reported values inferior to 500 000 cells/mL for CNS (26) and some others values higher than 600 000 cells/mL (32).

Figure shows the prevalence of subclinical mastitis for every microorganism isolated, depending on the milking system. There was a significant difference for the milking system in the case of negative quarters, CNS and *C. bovis*. In all cases, the disease prevalence was lower for hand milking. For the rest of

TABLE 5. SCC and SCS for main bacteriological results in both milking systems./ *Comparación entre ordeño manual y mecánico de la media de CCS y SCS para los principales diagnósticos bacteriológicos.*

Average ± Standard Error / IMI diagnose	SCC (10^3 cells/mL)		SCS	
	Machine milking	Hand milking	Machine milking	Hand milking
Negative	$343^a \pm 119$	$58^a \pm 23$	$3.01^a \pm 0.33$	$0.77^b \pm 0.15$
CNS	$1\,103^a \pm 130$	$508^b \pm 145$	$4.91^a \pm 0.23$	$3.38^b \pm 0.43$
Corynebacterium bovis	$878^a \pm 190$	$325^b \pm 111$	$4.31^a \pm 0.35$	$2.58^b \pm 0.27$
Streptococcus agalactiae	$1\,714^a \pm 467$	$1\,272^a \pm 316$	$6.42^a \pm 0.38$	$6.57^a \pm 0.41$
Staphylococcus aureus	$1\,272^a \pm 661$	$1\,259^a \pm 323$	$4.55^a \pm 0.70$	$5.76^a \pm 0.48$

Means with different superscripts in the same row for each variable are significantly different ($p<0.01$).

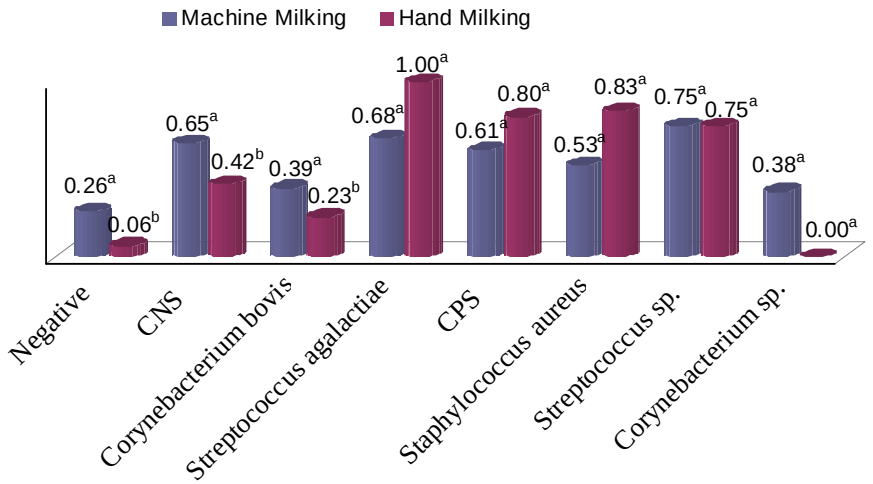

FIGURE. Prevalence according to SCS for each microorganism and negative quarters./ *Prevalencia según SCS para cada microorganismo y cuartos negativos.*
Proportions with different superscripts for the same pathogen are significantly different for $p<0.05$.

microorganisms, it was not possible to find significance because of the lowest number of isolates.

For negative quarters, there was a significant difference between hand and machine milking. The 26% of negative quarters (without IMI) had SCC values over 200 000 cells/mL on machine milking, while in hand milking, it was just 6 % (Figure). This was an evidence of inflammatory reaction without bacterial infection, explained by the insufficient maintenance, bad state and lack of spare parts of the milking machine.

CONCLUSIONS

Hand milking presented better mastitis epidemiological indicators than machine milking. The distribution of mastitis pathogens depended on the milking system, always with higher prevalence in machine milking and statistically different for *S. agalactiae*. Mastitis etiology had changed with minor pathogens as the most frequent mastitis causing microorganisms in both types of milking systems. To change the current bovine mastitis situation, it was necessary to improve the state, performance, hygiene and management of the milking machine.

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