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Factors associated with the prevalence of subclinical mastitis in double-purpose cattle

Factores asociados a la prevalencia de mastitis subclínica en ganado bovino de doble propósito

Pérez-Morales Rosalva*[1ID], Padilla-Ramírez Francisco[2ID], González-Ríos Humberto[3ID], De-la-Cruz-Leyva María[4ID], Castañeda-Vázquez Hugo[2ID], Hernández-Moreno María[5ID]**

[1]Departamento de Ciencias de los Alimentos, Ecología Química, Centro de Investigación en Alimentación y Desarrollo, A. C. Carretera Gustavo Enrique Astiazarán Rosas No. 46, Col. La Victoria CP 833040. Hermosillo, Sonora, México. [2]Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km. 15.5, Carretera a Nogales, Predio las Agujas, Zapopan, Jalisco. México. [3]Departamento de Tecnología de Alimentos de Origen Animal, Centro de Investigación en Alimentación y Desarrollo, A. C. Carretera Gustavo Enrique Astiazarán Rosas No. 46, Col. La Victoria, Hermosillo, Sonora, México. CP 83304. [4]División Académica Multidisciplinaria de los Ríos. Universidad Juárez Autónoma de Tabasco. Km 1, carretera Tenosique-Estapilla. Col. Solidaria. Tenosique, Tabasco, México. CP. 86901. [5]Departamento de Desarrollo Regional, Centro de Investigación en Alimentación y Desarrollo, A. C. Carretera Gustavo Enrique Astiazarán Rosas No. 46, Col. La Victoria, Hermosillo, Sonora, México. CP 83304. *Responsible author: Pérez-Morales Rosalva. **Author for correspondence: Padilla-Ramírez Francisco. Centro Universitario de Ciencias Biológicas y Agropecuarias, Universidad de Guadalajara, Km. 15.5, Carretera a Nogales, Predio las Agujas, Zapopan, Jalisco. México. E-mail: rperez@ciad.mx, fjavier.padilla@academicos.udg.mx, hugory@ciad.mx, concepción97@hotmail.com, hcastañe59@yahoo.com.mx, mar@ciad.mx

ABSTRACT

Factors associated with the prevalence of mastitis subclinical in dual-purpose cattle were evaluated in Sonora, Mexico. 350 representative milking cows from the region were sampled. Prevalence of mastitis was determined with the California test and microbiological plate growth. The effects of: season of the year, type of milking, calving number, days in lactation and presence/absence of calf on prevalence of mastitis were evaluated. The Chi^2 method and the test of two independent proportions were used to determine statistical differences. The prevalence of subclinical mastitis was similar between seasons (summer 64.9% vs winter 65.7%). The type of milking did not affect the prevalence of mastitis, while the cows with more than 7 calvings showed higher (P≤0.05) prevalence of mastitis 78.2%, compared with the first calving heifers 49.5%, and between 2-6 calvings 67.5% respectively. The days in lactation affected the prevalence of mastitis being higher (P≤0.05) in the cows of ≥141: (74.0%) vs the groups of 7-70 days: (64.2%), 71-140: (58.8%) respectively. The prevalence of mastitis was lower (P≤0.05) in the cows that were milked with the presence of the calf compared to without a calf (63.9% vs 77.9% respectively). The main factors associated with prevalence of mastitis in dual-purpose cattle were determined.

Keywords: bovine mastitis, dual-purpose cattle, risk factors.

RESUMEN

Se evaluaron los factores asociados a la prevalencia de mastitis subclínica en bovinos doble propósito en Sonora, México. Se muestrearon 350 vacas en ordeña representativas de la región. Se determinó la prevalencia de mastitis con la prueba de California y crecimiento microbiológico en placa. Se evaluaron los efectos de: época del año, tipo de ordeño, número de parto, días en lactancia y presencia/ausencia de becerro sobre la prevalencia de mastitis. Se utilizó el método de Chi^2 y prueba de dos proporciones independientes para determinar diferencias estadísticas. La prevalencia de mastitis subclínica fue similar entre épocas (verano 64.9% vs invierno 65.7%). El tipo de ordeño no afectó la prevalencia de mastitis, mientras que las vacas con más de 7 partos mostraron mayor (P≤0.05) prevalencia de mastitis 78.2%, comparadas con las vaquillas de primer parto 49.5%, y entre 2-6 partos 67.5% respectivamente. Los días en lactancia afectaron la prevalencia de mastitis siendo mayor (P≤0.05) en las vacas de ≥141: (74.0%) vs los grupos de 7-70 días: (64.2%), 71-140: (58.8%) respectivamente. La prevalencia de mastitis fue menor (P≤0.05) en las vacas que se ordeñaron con presencia del becerro comparado sin becerro (63.9% vs 77.9% respectivamente). Se determinaron los principales factores asociados a la prevalencia de mastitis en ganado bovino de doble propósito.

Palabras clave: mastitis bovina, ganado doble propósito, factores de riesgo.

INTRODUCTION

Worldwide, mastitis represents large productive and economic losses in dairy cattle (DANE, 2014; Vissio *et al.*, 2015; Gómez, 2015; Addis *et al*., 2016; Ruiz *et al.*, 2016) being no exception Mexico where these losses are reported in dual purpose cattle (Pech *et al.*, 2007), as well as in specialized dairy cattle (Gerlach *et al.*, 2009). Cows with mastitis can have clinical signs (clinical mastitis: CM) or no clinical signs (subclinical mastitis: SM). The latter is considered the most common form of this condition and the one that generates more costs, since, as it goes unnoticed by the producer, the animal not only lowers its milk production, but also becomes a focus of infection for the entire herd (Gómez, 2015; Addis *et al*., 2016). The causes of its high prevalence are multifactorial, where its incidence depends on exposure to pathogens, effectiveness of udder defense mechanisms and the presence of environmental risk (Huijps *et al*., 2009; Zaror *et al*., 2011; Alonso *et al*. 2018; Saidani *et al*., 2018; Bedolla *et al*., 2019). Breed has also been described as one of the influential factors. The Holstein breed seems to be among the most susceptible (Ramírez *et al*., 2011; Santivañez *et al*., 2013; Saidani *et al*., 2018), type of milking and teat drying (Saidani *et al*., 2018; Guevara *et al.*, 2020), lactation number (Saidani *et al*., 2018), as well as the interaction present among all factors.

Among the most accepted techniques for diagnosis field is the California test (CMT). It has been employed for decades and continues to be the most widely used test at the field level for the diagnosis of subclinical mastitis in dairy cattle, as well as the traditional plate culture method for positive samples (Castañeda *et al*., 2013; Sánchez & Gutiérrez 2015;

Alonso *et al*., 2018; Sánchez *et al*., 2018). The isolation of microorganisms allow evaluating the sanitary quality of milk and thus the conditions in cattle management (Moreira *et al*., 2020).

The last census reported with 16 220 and 15 410 cows from La Colorada and Ures municipalities respectively, within which the Ejido Cobachi and Pueblo de Alamos community are located with 2 188 and 5 031 cows respectively, where the samples for this study were collected (SAGARHPA, 2020). The level of technology and investment for the management and production of milk in dual-purpose cattle in the entity is low; milking is manual and some producers' milk mechanically with portable equipment. Generally, cattle are free in large areas where they feed on endemic forage species such as grasses of the Bouteloua genus, or introduced species such as buffel grass, "*Cenchrus ciliaris*". Animals under these systems show low production levels, averaging around 6 L cow per day. In addition to the above, a marked fluctuation in production has been observed due to the effect of the year time, reaching its highest milk production in the rainy season during the summer, when the pasture has a greater abundance of green feed with higher nutritional content. Some producers support the feeding of milking cattle with some agricultural waste or a grain derivative such as bran.

Milk production in this type of system is used mainly for the production of artisanal cheese, which is one of the main economic activities. Creole and European zebu cattle (Hernández, 2012) mainly represent the genotypes used for milk production. Given the socioeconomic importance of the dual-purpose dairy production system in central Sonora and the scarce information on the mastitis prevalence, the aim of this study was to determine the factors associated with the prevalence of bovine mastitis in dual-purpose cattle in two municipalities of Sonora, Mexico. The hypothesis of the work was the prevalence of mastitis in dual-purpose cattle in central Sonora differs by effect of season of the year (SY), type of milking (TM), days of lactation (DL), calving number (CN) and presence/absence of the calf at milking (PC/AC).

MATERIAL AND METHODS

The study was conducted in two rural development districts in the center of Sonora State, Mexico where dairy activity is carried out with dual-purpose cattle. It is characterized by the use of biotypes where crosses of Creole cattle with pure breeds such as: Angus, Charolais, Hereford, and Beefmaster and Brangus, Limousin, Gyr, Simbrah, Gelbvieh, Brown Swiss, Romagnola, Salers, Simmental, Brahman, Holstein and New Zealander (Hernández, 2012). A statistically representative sample (n=350) was selected from the

total number of milking cows in the districts of Ures and Mazatán, Sonora, and the same sampling was performed in winter (December 2017-February 2018) and summer (June-September, 2017) with the same number of animals for each season. Ures is located between parallels 29° 00' and 29° 43' north latitude; meridians 109° 57' and 110° 37' west longitude; altitude between 10 and 1 600 m and Mazatán is located between parallels 28° 45' and 29° 07' north latitude; meridians 109° 55' and 110° 26' west longitude; altitude between 95%. *E*, estimation error or desired precision; *p*, the expected proportion of observation units with the characteristic sought; and *q*, the expected proportion of observation units without the characteristic sought (q = 1-p).

The prevalence of mastitis per cow and per mammary gland (referring per cow to the sum of cows with at least one infected gland and per gland to the sum of individual infected glands) was recorded. In addition to the management and production information, the following independent variables were recorded: genotype, milking type, 300 and 1,300m.

A total n of 350 cows was estimated for each station based on the following equation from Mendenhall *et al*., 2006.

$$n = \frac{Z^2 p*q*N}{Ne^2 + Z^2*p*q} \qquad n = \frac{(1.96)^2(0.5)(0.5)(3850)}{3850(0.05)^2 + (1.96)^2(0.5)(0.5)} = 349.3 = 350$$

Where *n*, is the selected sample size; *N*, is the total population; *Z*, the confidence level, age, weeks of lactation, calving number, type of milking. For the diagnosis of mastitis, the California Mastitis Test (CMT) and bacterial growth were performed.

Diagnosis of subclinical mastitis. First, the teat orifice was cleaned to eliminate the bacterial load. The CMT test is a qualitative test and consisted of mixing approximately 2.5 mL of milk from each of the glands with 2.5 mL of California reagent (aryl alkyl sulfonate of sodium) in a 1:1 ratio and mixing. The positive test was given by the formation of a gel, visible to the naked eye, the test yields 5 readings: negative where there is no change, traces where a slight and transitory precipitate forms at the base of the paddle. Degree 1 where there is a greater precipitate without gel formation, degree 2 where a dense precipitate forms in the center of the base of the paddle and degree 3 where a very dense gel forms and adheres to the paddle, (Bedolla *et al*., 2007; Echeverría *et al*., 2010).

The mammary glands were disinfected and then milk samples were collected in sterile *Whirl pak* bags for subsequent microbiological analysis. Recovered samples were transported to the Molecular Microbiology Laboratory of CIAD, (Center for Food and Development Research), A C. Unidad Hermosillo, Carretera Gustavo Enrique Astiazarán Rosas No. 46, Col. La Victoria CP 83304. The milk sample was kept at a temperature of 4-10 ºC under the procedures of the OIE Manual (OIE, 2004; NOM-109-SSA1-1994). Traditional plate culture was used for microbiological analysis. Subsequently, each milk sample was inoculated on 5% blood agar plates and Blood-Scum Agar plates with the aid of sterile swabs. The inoculated plates were incubated at 37 °C for 24 h. (Castañeda *et al*., 2013). Plates showing growth of three or more different colonies were discarded.

Statistical analysis. For data analysis, the effect of time of year (SY) considering summer and winter, type of milking (TM), calving number (CN), and days in lactation (DL) on the prevalence of subclinical mastitis was evaluated. When TM was evaluated, two groups were considered: mechanical milking group (MMG) and hand milking group (OMA). Three groups were considered to evaluate in CN: first calving heifers (CH); cows between 2 to 6 calvings (C 2-6), and cows of seven or more calvings (C7). Five groups were made to evaluate the effect of DL on the prevalence of subclinical mastitis: From 7 to 90 days in lactation (D7-90), from 91 to 180 days in lactation (D91-180), from 181 days onwards (D>181) days in lactation. Chi2 test was used to evaluate the effects of the independent variables CN, DL, on the prevalence of subclinical mastitis and to estimate the relationship of the factors SY, TM and presence or absence of the calf, with the prevalence of subclinical mastitis, hypothesis tests were performed for two independent proportions.

All analyses were performed at a significance level of P≤0.05 at Type I error, in the statistical package NCSS versión 2007.

RESULTS

Table 1 shows the prevalence of subclinical mastitis due to the effect of SY. Negative glands were defined as those that were negative to the tests used (CMT and microbiological examination) for the diagnosis of mastitis. Positive glands, on the other hand, were those that presented microbiological growth and/or were positive for CMT (degree 1, 2 and 3). Blind (dysfunctional) glands are those glands that are unproductive and therefore not milked.

Table 1. Prevalence of clinical and subclinical mastitis (%) at two periods of the year in mammary glands in dual-purpose cattle

	Year season		
	SUMMER (n=1400)	WINTER (n=1400)	VALUE P
CLINICAL MASTITIS	1.07	0.29	P<0.05
SUBCLINICAL MASTITIS	46.50	47.57	P>0.05
BLIND GLANDS	1.86	1.93	P>0.05
NEGATIVES	50.57	50.21	P>0.05

The prevalence rates of subclinical mastitis per cow for the summer and winter seasons were similar 64.9 and 65.7 %, respectively, showing no significant differences (P≥0.05). When the prevalence rate of subclinical mastitis at the gland level was evaluated (Table 1), similar values were observed between seasons (P>0.05) showing values of 46.50% and 47.57% for summer and winter, respectively.

The season of the year did not affect (P>0.05) the prevalence of subclinical mastitis, so the 350 summer cows and the 350 winter cows were added, giving 700 animals for the subsequent analyses. However, for some of these, the total number of animals was not included for two reasons: those that showed clinical mastitis and some for which reliable information was not available.

The prevalence of subclinical mastitis was similar P>0.05) in cows that milked manually 63.8% compared to those that milked mechanically 69.3% respectively.

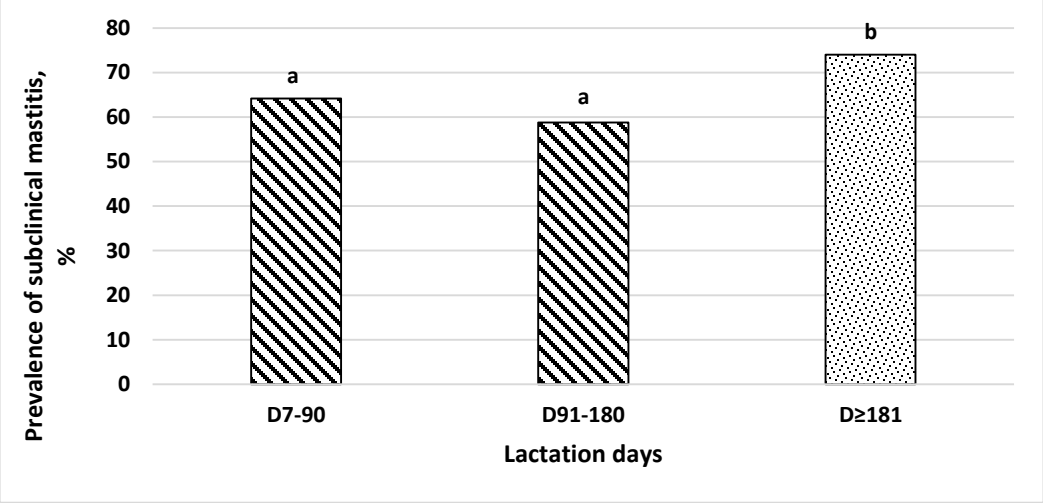
When the effect of calving number was evaluated, cows calving 7 and more showed the highest values (P≤0.05) of subclinical mastitis prevalence (78.2%) compared to 67.5 and

50.0% for V2-7 and CH animals respectively (Figure 1). An increase in the prevalence of subclinical mastitis was observed as calving number advanced.

CH, calving heifer n=105; C2-6= cows with 2 to 6 calvings n=456; C7= cows with 7 or more calvings n=110. [ab] Different literals indicate significant differences between groups (P<0.05)

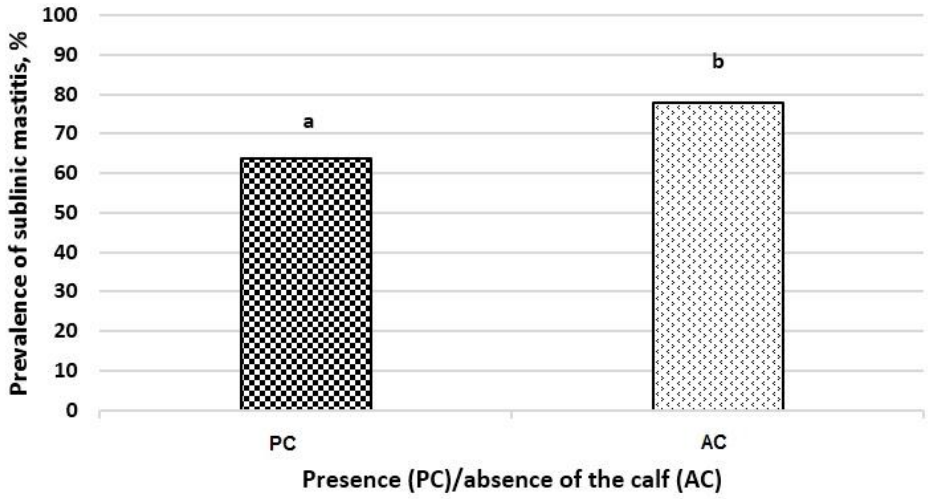
Figure 1. Effect of cow calving number on the prevalence of subclinical mastitis

Days in lactation significantly affected the prevalence of subclinical mastitis. Cows in the D>181 days in lactation group showed the highest value (P<0.05) 74.0% prevalence of subclinical mastitis compared to cows in the other two groups where values of 64.2 % and 58.8% were observed for the D7-90 and D91-180 groups, respectively.



D7-90, 7 to 90 days of lactation *n*=302; D91-180, 91 to 180 days of lactation *n*=160; D>181, Equal to or greater than 181 days of lactation n=96. [ab] Different literals indicate significant differences (P<0.05) between groups indicated

Figure 2. Effect of the number of days in lactation on the prevalence of subclinical mastitis



PC, calf presence *n*=549, AC, calf absence *n*=122. [ab] Different literals indicate significant differences (P<0.05) between groups indicated

Figure 3. Effect of calf presence (PC)/absence (AC) on the prevalence of subclinical mastitis

A lower (P<0.05) prevalence of subclinical mastitis was observed in cows that had calf at the time of milking at foot (PC) compared to cows that were milked without calf at foot (AC) 63.9 vs 77.9% respectively, (Figure 3).

DISCUSSION

YS was not significantly related to the prevalence of subclinical mastitis both per cow and per gland. These results coincide with the information obtained by Pinzón *et al*., (2009) who found no significant differences when they evaluated the prevalence of gland mastitis in specialized Holstein cattle in two different seasons (spring and winter). In the present work, the prevailing management systems are the so-called extensive systems, speculating that in these production systems there is not the same management pressure on the animals as in intensive systems where stress could be greater and in low stress conditions, the prevalence of pathologies is reduced. It has been documented that mainly in summer, a time when environmental infections influenced by the heat and humidity of the region are more prevalent, a higher risk (7.67 times more) of clinical mastitis infection is observed in cows in intensive systems compared to semi-extensive systems (Bedolla *et al*., 2019; Saidani *et al.,* (2018). The values observed for prevalence of subclinical mastitis in this study in both seasons (64.9% in summer and 65.71% in winter) are similar to those reported by Alonso *et al*., (2018) where they referred a 66% prevalence of mastitis under tropical climate conditions. However, they are higher than those found by other authors (Pech *et al*., 2007), where they found a 53% prevalence of mastitis in dual purpose cattle in Yucatan as well as by other authors such as Muñoz *et al.,* (2012). The prevalence of subclinical mastitis was 45.9 %, in dual purpose cattle, in Guerrero state, Mexico; in another study by Lazo *et al*., (2018) in dairy cows as well in extensive system they found a moderate rate (29.32%) of subclinical mastitis per gland while Pinzón *et al.,* (2009) found values higher than 50%. All these differences with these authors may be due to climatic conditions, deficiency in milking practices, breed of cattle, as well as cattle management in general in this study group. Although a slightly higher trend of subclinical mastitis prevalence was observed in cows that were milked mechanically compared to cows under manual milking, no statistical differences were detected ($P \geq 0.05$). In contrast, other authors have reported higher prevalence of subclinical mastitis under mechanical milking (Ruiz *et al*., 2011; Guevara *et al.,* 2020). In the present study was observed that in barns where manual milking is used, hygiene practices during milking are minimal. They do not wash or dry the teats before milking, the same person, ties the cow and (in most cases the rope is impregnated with manure), places the calf before and/or at the end of milking, among other activities, without washing their hands. It is important to note that the production units sampled are classified as small family units (5-30 milking animals), whose activities and customs have been inherited through generations and are generally carried out by a single person, which makes it difficult to carry out good hygienic milking practices.

In barns where mechanical milking is used, it was observed that approximately half of the cows were previously disinfected, which could help to reduce the effect of machine milking on the mastitis prevalence. Therefore, poor hand and milking equipment hygiene are risk factors for infection, as well as other poor milking practices (Pinzón *et al*., 2009; Santibáñez *et al*., 2013; Aguilar *et al*., 2019). A significant relationship was detected between CN and the prevalence of subclinical mastitis that was manifested in an increase in the same as calving number advances. The C7 group showed high values of up to 78.2%. These results coincide with some authors who mention that the increase in calving number, increases the probability of getting mastitis (Ramírez *et al.,* 2011; Santibáñez *et al.,* 2013; Mora *et al.,* 2015; Vidales *et al*., 2017; Saidani *et al*., 2018). This is also explained by the fact that the older the cow is, the more flaccid the tissue around the teat is and the more open the sphincter and lactiferous ducts are. This increases the risk of introduction of bacteria through the teat and teat canal into the gland. On the other hand, the older the cow's immune system is more deficient and this is another important factor to take into account for getting the infection (Aguilar *et al*., 2019). The trend of the relationship between cow DL and mastitis prevalence is similar to that cited by some authors who found significant differences (P≤0.05) (Ramírez *et al.,* 2011; Santibáñez *et al.,* 2013). In the first months of lactation the highest rates are observed, a decrease in the fourth month and a marked increase of mastitis from the sixth month onwards. Biffa *et al*., (2005) found the highest prevalence of mastitis in late lactation. Differences in the prevalence of subclinical mastitis related to PC or AC could be explained by the fact that the calf after milking finishes sucking residual milk in the mammary glands. In addition, cows remain standing longer, an attitude that somehow prevents the entry of pathogenic microorganisms (Calderón *et al.,* 2009; Valero *et al*., 2010). The results of the present study contribute to the knowledge of risk factors in the dual-purpose milk production system under the study area conditions. Although no significant statistical differences were detected in several factors, a high prevalence of subclinical mastitis was found regardless of the year period. In addition, some of the most important risk factors associated with the prevalence of subclinical mastitis were determined, among which the following stand out: older cows, keeping the calf in lactation for many days, and cows that are milked mechanically. This suggests taking into account the necessary measures and emphasizing these issues to contribute to the control of such a serious problem as the prevalence of subclinical mastitis in dual-purpose bovine milk production systems.

CONCLUSION

No effect of year season was observed on the prevalence of mastitis per cow or per mammary gland. Hand milking versus mechanical one did not represent a risk factor for the prevalence of subclinical mastitis as they showed similar values. The number of calving significantly influenced the prevalence of subclinical mastitis as values were increased in animals with higher number of calving. Days in lactation significantly affected the values of subclinical mastitis prevalence, showing the highest values in the last stage of lactation in dual-purpose cows. The presence or absence of the calf at the time of milking was a determining factor for the prevalence of subclinical mastitis, with a tendency to be higher in cows without the presence of the calf.

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Errata Erratum
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