Journal of Agricultural Sciences Research

ISSN 2764-0973 vol. 5, n. 8, 2025

ARTICLE 10

Data de Aceite: 04/12/2025

PREVALENCE OF SUBCLINICAL MASTITIS AND ASSOCIATED RISK FACTORS IN DOUBLE-PURPOSE COWS OF SMALL-SCALE PRODUCERS IN THE MEXICAN TROPICS

Horacio León-Velasco
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0009-0001-1759-3856.

Flavio Ramírez-Albores
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0000-0001-5685-509X.

Hubercein Ramírez-Barrios
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0009-0000-5921-5223.

Gilberto Yong-Ángel
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0000-0002-6275-396X.

Gerardo Uriel Bautista-Trujillo
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0000-0001-5685-509X.

Humberto León-Velasco
Benemérita Universidad Autónoma de Chiapas, Facultad de Ciencias Agronómicas, Campus V. Villaflores, Chis.
ORCID 0009-0000-4202-3081.

Oscar León-Velasco*
Benemérita Universidad Autónoma de Chiapas, Escuela de Estudios Agropecuarios Mezcalapa, Campus XI. Copainalá, Chis.
ORCID 0009-0000-4060-2784.
*Corresponding Author.

Horacio Ruiz-Hernández
Benemérita Universidad Autónoma de Chiapas, Facultad de Medicina Veterinaria y Zootecnia, Campus II. Terán, Chis.
ORCID 0000-0003-3861-0093.

ABSTRACT. The objective was to determine the prevalence of subclinical mastitis and associated risk factors in dual-purpose cows on small farms. Twenty farms were studied, of which 50% (10) used manual milking and the remainder used mechanical milking, with between eight and 63 cows per producer. The California Mastitis Test (CMT) was applied once per quarter just before milking to identify healthy and diseased animals. The CMT was performed on 501 cows to assess prevalence. The results were: negative, 1, 2, and 3. A 2×2 contingency table was used, using the Chi-square test (P≤0.05). Mastitis positivity was taken as the independent variable and the following as the dependent variables: ranch, udder quarters, type, number of milkings, and breed. Simultaneously, the odds ratio was calculated with a 95% confidence interval. A prevalence of 9.5% was detected in manually milked cows compared to 31.4% in mechanically milked cows (P≤0.05). Risk factors associated with subclinical mastitis were number and type of mechanical milkings per day (odds ratio: OR= 2.8 to 4.3), right anterior udder quarter (OR=1.46), farms with more than 60 milking cows (OR=1.9), and Holstein cows (OR=2.8). Therefore, good management practices should be taken into account as control and prevention measures.

KEYWORDS: risk factors, mastitis, cows.

INTRODUCTION

In the state of Chiapas, cattle farming continues to be one of the most important socioeconomic activities. The state has a livestock inventory of 2.5 million head, predominantly dual-purpose (80%) beef and dairy cows, resulting from crosses between European breeds such as Brown Swiss, Holstein, and Simmental, and their crosses with Zebu breeds such as Gyr, Guzerat, Black Sardinian, and Brahman. The majority (80%) of production units are small land areas ≤ 50 ha with fewer than 30 cows. Of these small producers, more than 97% milk manually with the help of the calf, and the remaining percentage of producers use mechanical milking. It should be noted that milk represents between 50 and 60% of the ranch's annual income (León *et al.*, 2023). However, there are limitations in good management practices during milking and in basic infrastructure such as the roof and firm floor of the milking parlor, as only 50% of producers have such infrastructure. Facilities definitely play a very important role in milk quality and quantity, as well as in the welfare of animals and workers in their daily tasks.

One of the most frequent problems in this small-scale production system is subclinical mastitis, which causes economic losses due to decreased milk production, animal culling, and treatment costs.

Therefore, the objective of this study was to determine the prevalence of subclinical mastitis and associated risk factors in dual-purpose cows on small farms.

MATERIALS AND METHODS

Study area

The study was conducted in the Frailesca region, located in the Central Depression of the state of Chiapas, Mexico, which is located between coordinates 15° 41' – 16° 21' north latitude and 92° 37' – 93° 16' west longitude; altitudes range from 539 to 1220 m; the predominant climate in the

region is warm subhumid with spring-summer rains, followed by a semi-warm humid climate with abundant summer rains; the average annual temperature in the region varies from 22 to 26°C in warm areas, in semi-warm areas from 18 to 22°C, and in small areas of the high mountains above 2000 m from 16 to 18°C, as well as average annual rainfall from 1000 to 2600 mm (CEIEG, N/A; INEGI, 2021; 2024).

Animals

The main characteristic of this production is a semi-intensive system (85%) with dual-purpose livestock, predominantly crossbreeds of Zebu × Swiss (63%) and Gyr × Holstein (7%). Other crossbreeds include American Swiss × Holstein and Holstein (30%).

With regard to animal feed, the vast majority of producers use rotational grazing systems with tropical grasses such as African star grass (*Cynodon plectostachyus*), Señal (*Brachiaria decumbens*), insurgente (*Brachiaria brizantha*), jaragua (*Hyparrhenia rufa*), llanero (*Andropogon gallanus*), and cubano (*Pennisetum purpureum* × *Pennisetum glaucum*). In addition, 60% of producers provide permanent supplementation of mineral salts, corn silage, ground corn, ground sorghum, distillers dried grains with solubles (DDGs), palm kernel meal, poultry manure, soybean meal, and cane molasses, while only 3% use commercial concentrates. In this area, agricultural residues (corn and sorghum stubble) are traditionally used directly or ground for animal feed, especially during the dry season (December to May).

On the other hand, the vast majority of farms perform manual milking (97%) with the support of calf suckling for milk release, and a low percentage of producers practice mechanical milking (3%) and oxytocin applications (20 IU) prior to milking, normally performing one milking per day in the morning from 5:00 to 7:00 a.m. Furthermore, 48% of milking parlors have solid floors (concrete, cobblestone), as well as covered galleries in 66% of cases, with the remaining percentage having dirt floors and no roof. This infrastructure is undoubtedly very important both for milk quality and for the welfare of the animals and workers, protecting them from wind, rain, and sun during their daily tasks.

Methodology

Twenty livestock production units were studied, of which 50% of the farms (10) used manual milking and the remaining 50% (10) used mechanical milking, with a number of cows being milked ranging from eight to 63 animals per producer, with an average milk production of 7.8 ± 5.4 liters per day/cow. Of the farms that practiced mechanical milking, 60% (six) used double milking per day and the remaining 40% (four) used only one milking per day. The study was prospective, observational, and cross-sectional (Hernández et al., 1991); thus, 501 animals were tested using the California Mastitis Test (CMT), with the following results: negative, 1, 2, and 3. The test was performed only once per quarter, just before milking, to identify healthy and sick animals, using the technique described by Bedolla *et al.* (2007). Animals with a positive reaction to the test with a grade of 1, 2, or 3 were considered positive, and the prevalence of this disease was calculated (Rodríguez, 2020).

Estimation of associated risk factors. A record sheet was designed for data collection, which included questions related to the cattle production system, such as: name of the ranch, breed, management practices, type of milking, number of cows, and knowledge of the mastitis test.

Statistical analysis

The sample size was determined using Fernández's formula (1996). A hypothetical proportion (p) of subclinical mastitis of 50% was considered, with a confidence level (z^2) of 95% and a permissible error (d^2) of 5%. Based on the above, a total of 384 animals were obtained. However, due to the cooperation of the producers and to obtain better accuracy and prevalence estimates, 501 cows were studied, corresponding to 211 cows milked manually and 290 cows milked mechanically.

A 2×2 contingency table was used to calculate statistical significance, using the chi-square test (P≤0.05). Mastitis positivity was taken as the independent variable and the following as the dependent variables: ranch, udder quarters, type and number of milkings, and breed. Simultaneously, the odds ratio (OR) was calculated with a 95% confidence interval. The bivariate analysis was performed using SPSS software (2017).

RESULTS AND DISCUSSION

Table 1 shows the overall results of subclinical mastitis prevalence in dual-purpose herds. A lower prevalence was detected in manually milked cows (9.5%) compared to mechanically milked cows (31.4%) (P≤0.05). This difference in results may be due to management, the presence and suckling of residual milk from the calf after manual milking, cow stimulation, breed, a greater number of cows in mechanical milking, teat cleaning, and mechanical milking equipment management. However, Pérez Morales *et al.* (2022), who worked with dual-purpose cattle in northern Mexico, did not observe significant differences in the prevalence of subclinical mastitis (P>0.05) between cows that were milked manually compared to those that were milked mechanically, with results of 63.8% and 69.3%, respectively. Medrano-Galarza *et al.* (2021), when evaluating the prevalence of subclinical mastitis in small producers specializing in milk production with Holstein cattle in Colombia, identified an overall prevalence of 35 to 55%, and the factors associated with the highest risk of developing this condition were cows with more than three lactations, production exceeding 180 days, and management practices during milking. However, the results of this study can be considered a moderate index, which could perhaps be significantly reduced and herd productivity improved with good pre- and milking practices. The prevalence found in this study may seem low; however, it is a source of continuous infection for other cows through pre- and milking management, so measures to prevent and control this disease must be taken.

Table 1. Prevalence results for subclinical mastitis by milking type.

Type of milking	No. of cows	Negative cows	Positive cows*	Prevalence (%)
Manual	211	191	20	9.5[a]
Mechanical	290	199	91	31.4 [b]

Figures with different letters are statistically different (P≤0.05).

* At least a quarter of the mammary gland was positive, with a grade 1, 2, or 3 test reaction.

It was also determined that the number of milkings per day (two) and the type of milking (mechanical) present an odds ratio (OR) of 2.8 to 4.3 times, respectively, of manifesting subclinical mastitis; contrary to a single milking per day and manual milking (P≤0.01). There is a correlation between the presence of subclinical mastitis and the type of milking. Farías *et al*. (2005) described a 29% higher infection rate in quarters of animals milked mechanically, in contrast to cows milked manually. This is possibly due to the handling and operation of the equipment, poor staff training, and ineffective cleaning and disinfection of the milking equipment and the cows' mammary glands.

In addition, the right front quarter had a 1.46 times higher risk (P≤0.05) of subclinical mastitis compared to the other quarters of the mammary gland. Calderón and Rodríguez (2008), when evaluating 11,416 mammary quarters in Holstein cows in the highlands of Colombia, determined a higher prevalence of subclinical mastitis in the right and left rear mammary quarters of 33.1% and 32%, respectively, compared to the right (30.6%) and left (29.9%) front quarters. It should be noted that 92.5% of the production units in this study used mechanical milking and double milking per day with specialized cows. These results are controversial, as other studies have found higher positive reactions in the front quarters, with the front right quarter being the most affected, with a 48.5% prevalence of subclinical mastitis (Torrez and Duartes, 2006).

With regard to the epidemiological situation of subclinical mastitis per ranch, it was observed that ranches with more than 60 cows in mechanical milking, representing 20% of the herds in the study, showed a significant risk ratio (P≤0.05) ranging from 1.9 times more risk of subclinical mastitis, respectively, compared to other ranches with 11 to 45 cows in milking (P>0.05). Cuenca-Condoy *et al*. (2021) detected results of 10.9 times the incidence of this disease in mechanical milking (P≤0.01) due to some bad practices, such as drying teats with a non-individual towel in Holstein cows and their crosses.

One of the factors associated with the manifestation of subclinical mastitis is the breed and zootechnical purpose of the cows. This study found that Holstein cows had a 2.8 times higher risk of developing subclinical mastitis compared to other crossbreeds of Zebu × Swiss and American Swiss (P≤0.05). It should be noted that these cows had an average milk yield of more than 18 L per cow per day. Similar results were published by Santivañez-Ballón *et al*. (2013), who found a 2.1 times higher risk of subclinical mastitis in Holstein cows compared to Criollo or Jersey breeds. For their part, Saidani *et al*. (2018) pointed out that Holstein cows are 9.97 times more likely to develop mastitis than Fleckvieh cows. Undoubtedly, there is a genetic predisposition, but it can also be attributed to the fact that these animals have higher milk productivity and, in many cases, good pre-milking and milking management practices are not followed. Unfortunately, in the present study, more than 25% of producers were unaware of the mastitis test (CMT) and ignored the udder health of their animals. Furthermore, among the remaining producers, this activity is not carried out on a daily basis; only cases of clinical mastitis in cows are treated with antibiotics. Therefore, it is necessary to work more with small producers in a technical assistance program to improve milk quality and quantity, animal welfare, and family finances.

REFERENCES

Bedolla, C.C., V.H. Castañeda, and W. Wolter. 2007. Methods of detection of the bovine mastitis. EDVET. Revista Electrónica de Veterinaria.VIII (9). http://www.produccion- animal.com.ar/sanidad_intoxicaciones_metabolicos/infecciosas/bovinos_leche/12- mastitis (Mayo 2025).

Calderón, A y C. Rodríguez 2008. Prevalencia de mastitis bovina y su etiología infecciosa en sistemas especializados en producción de leche en el altiplano cundiboyacense. (Colombia) 21: 582-589.

CEIEG (Comité Estatal de Información Estadística y Geográfica). S/A. Región VI Frailesca. Gobierno del Estado de Chiapas. https://www.ceieg.chiapas.gob.mx/storage/posts/productos/MAPASTEMREG/REGION_VI_FRAILESCA_post.pdf (Octubre 2025).

Cuenca-Condoy, M., D. García-Bracho, L. Reinoso-García, J. González-Rojas y J. Torracchi-Carrasco. 2021. Detección de mastitis subclínica bovina y factores asociados en fincas lecheras de la Provincia del Cañar- Biblián, Ecuador. Revista Científica FCV-LUZ. XXXI 3: 93-97.

Farías R., J., A. García U., G. Pool D., K. Valero L., M. Alfara C. y G. Angelosante. 2005. Detección de mastitis subclínica en bovinos mestizos doble propósito ordeñados en forma manual o mecánica. Comparación de tres pruebas diagnósticas. Revista Científica FCV-LUZ XV: 2. 109-118.

Fernández S., P. 1996. Determinación del tamaño muestreal. Unidad de epidemiología clínica y bioestadística. 3. 138.

Hernández S., C., C. Fernández C. y P. Baptista L. 1991. Metodología de la investigación. Mc Graw-Hill. México.

INEGI (Instituto Nacional de Estadística y Geografía). 2021. Censo de Población y Vivienda 2020. México. Instituto Nacional de Estadística y Geografía. Aguascalientes, México. https://inegi.org.mx/programas/ccpv/2020/ (Julio 2025).

INEGI (Instituto Nacional de Estadística y Geografía). 2024. Aspectos geográficos de Chiapas. Compendio 2023 – 2024. Instituto Nacional de Estadística y Geografía. México. https://www.inegi.org.mx/contenidos/productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/889463915768.pdf (Octubre 2025).

León V., H., H. Ruiz H., H. Ramírez B., M. Abarca A y R. Mendoza P. 2023. La industria láctea en México. 25. años del concurso estatal de la vaca lechera. Ocozocoautla Chiapas.

Medrano-Galarza, C., D. Ahumada-Beltrán, J. Romero-Zúñiga y P. Donado-Godoy 2021. Prevalencia, incidencia y factores de riesgo de mastitis subclínica en lecherías especializadas en Colombia. Agronomía Mesoamericana. 32:2 487-507.

Pérez-Morales, R., F. Padilla-Ramírez, H. González-Ríos, M. de la Cruz-Leyva, H. Castañeda- Vázquez y M. Hernández-Moreno 2022. Factores asociados a la prevalencia de mastitis subclínica en ganado bovino de doble propósito. Abanico Veterinario 12: 1-16.

Rodríguez, L. 2020. Prevalencia de mastitis subclínica en tambo lechero en Paraguay. Rev. Med. Vet. 1: 40. 61-68.

Saidini K. C.López-Sández H.Ziam M.Hamiroune S.Righi P. Diez-Baños, R. Panadero-Fontán y G. Fernández-Rodríguez. 2018. La mastitis bovina clínica en el norte de Argelia: factores de riesgo y plan de control. Livestock Research for Rural Development 30: 8.

Santivañez-Ballón, C., O. Gómez-Quispe, L. Cárdenas-Villanueva, M. Escobedo-Enríquez, R. Bustinza-Cárdenas y J. Peña-Sánchez. 2013. Prevalencia y factores asociados a la mastitis subclínica bovina en los Andes Peruanos. Veterinaria y Zootecnia 7: 2. 92-104.

SPSS (Statistical Package for the Social Sciences). 2017. IBM Statistics version 25. (IBM Corp. © Copyright IBM Corporation version 25).

Torrez, F. y O. Duartes 2006. Estudio preliminar de la utilización de la manteca de armadillo (*Dasypus novemcinctus* L.) en el tratamiento de mastitis bovina en el municipio de Paiwas. Tesis de Médico Veterinario. Departamento de Región Autónoma del Atlántico Norte. Universidad Nacional Agraria. Managua, Nicaragua.