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Rev Med Vet Zoot. 66(3), septiembre-diciembre 2019: 208-218 ________________________ Investigación

Bacterial and fungal infectious etiology causing mastitis in dairy cows in the highlands of Boyacá (Colombia)

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ABSTRACT

Mastitis is one of the most important diseases for the dairy industry worldwide due to the great economic losses it causes, and the bacterial agents that cause it vary from one herd to another. The objective of the investigation was to determine the infectious agents involved in the development of mastitis in specialized dairies herds in the Highlands of Boyacá, Colombia. Was performed CMT tests in 4432 teats to determine the positive results for inflammation (CMT + 2), sampling in aseptic material of positives quarters, identification and differentiation through microbiological cultures, Gram staining, and biochemical tests of bacterial and fungal agents present in milk samples. It was determined that the total prevalence varied in the three years of the study as follows: in 2016, 45.19%; in 2017, 51.06% and in 2018, 39.76%. The months of April, May, and September, October presented the highest number of teats affected with mastitis during the three years of study. *Streptococcus agalactiae* and *Staphylococcus aureus* were the most prevalent microorganisms that cause mastitis in the three study years. There was an increase in the prevalence of infected teats corresponding to the months (April, May, September, and October) due to rainfall increases. The contagious pathogens were most prevalent that environmental pathogens.

Keywords: California Mastitis Test, etiological agents, inflammation, udder.

Etiología bacteriana y micótica infecciosa causante de mastitis en vacas lecheras en el altiplano boyacense (Colombia)

RESUMEN

La mastitis es una de las enfermedades más importantes para la industria lechera a nivel mundial debido a las grandes pérdidas económicas que genera; además, los agentes que la generan varían de un rebaño a otro. El objetivo de esta investigación fue determinar los agentes infecciosos involucrados en el desarrollo de mastitis en lecherías especializadas del departamento de Boyacá (Colombia). Se realizaron pruebas de CMT a 4432 cuartos para determinar los positivos a inflamación (CMT+2), toma de muestras en material aséptico de cuartos positivos, identificación y diferenciación a través de cultivos microbiológicos, tinción de Gram y pruebas bioquímicas de los agentes bacterianos y micóticos presentes en las muestras de leche. Se determinó que la prevalencia total varió en los tres años de

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estudio así: en el año 2016, 45,19%; en el 2017, 51,06%, y en el 2018, 39,76%. Los meses de abril-mayo y septiembre-octubre presentaron la mayor cantidad de cuartos afectados con mastitis a lo largo de los tres años de estudio. Finalmente, se determinó que *Streptococcus agalactiae* y *Staphylococcus aureus*, microorganismos que generan mastitis, fueron los de mayor prevalencia durante los tres años de estudio debido a un aumento en las lluvias. Los patógenos contagiosos causantes de mastitis fueron los más prevalentes, por encima de los medioambientales.

Palabras clave: California Mastitis Test, agentes etiológicos, inflamación, ubre.

INTRODUCTION

Mastitis is the inflammation of the mammary gland, caused by the invasion and destruction of the mammary glandular tissue due to the pathogenic action of microorganisms. It is a major problem in specialized dairies herds due to the direct economic losses that it causes through the decrease of milk production, the poor quality of raw milk, increase in culling rate of animals and additional expense for treatment and labor costs. In addition to the milk that is lost due to antibiotic treatment. (Andrade-Becerra *et al.* 2012; Taponen *et al.* 2016; Haubert *et al.* 2017; Petersson-Wolfe *et al.* 2018).

Mastitis has special economic importance, it is estimated that the losses in countries like India are $1.1 billion, the United States $ 2 billion, United Kingdom $ 371 million and worldwide $ 35 billion. In Colombia, the estimated annual losses are from $ 1,250–25,000 dollars, caused by the prevalence of mastitis in cows and dairy farms, respectively. (Haubert *et al.* 2017; Ortíz-Duran *et al.* 2017).

Mastitis can be differentiated into clinical and subclinical mastitis. The first form produces noticeable inflammation of the mammary gland and alterations occur in the milk due to the presence of bacteria and cells participating in the inflammatory process. In subclinical mastitis, there are no visible changes in the mammary gland, but milk production decreases, there is the presence of bacteria in the milk and the milk properties change due to the enzymatic processes (Ferrero *et al.* 2014; Caggiano *et al.* 2016; Andrade-Becerra *et al.* 2018).

According to its infectious etiology, mastitis can also be divided into contagious and environmental forms. Contagious mastitis is caused by microorganisms such as *Staphylococcus aureus, Streptococcus agalactiae, Arcanobacterium pyogenes* and *Mycoplasma* spp.; and its reservoirs are the mammary gland and the milk of infected cows. Its transmission can occur at the time of milking by poor practices such as the sharing of towels to wash and dry teats; by the contaminated hands of farmworkers or by the sharing of non-disinfected teat liners between cows in the milking (Blowey and Edmondson 2010; Andrade-Becerra *et al.* 2014).

Environmental mastitis is caused by Gram-negative germs, normally found in the environment such as *Escherichia coli, Klebsiella* spp., *Enterobacter* spp., *Serratia* spp., *Pseudomonas* spp., and *Proteus* spp. and some Gram-positive bacteria such as *Streptococcus uberis* and *Streptococcus dysgalactiae,* which cause mild and moderate forms of mastitis and within the rare germs are yeasts and molds (Haubert *et al.* 2017). Also, mastitis can be due yeast and molds, but this presentation in dairy herds is low (Williamson and Di Menna 2007).

The objective of this study was to determine the bacterial etiological agents and infectious fungal causes of subclinical mastitis, and its prevalence, in dairy farms in the Highlands of Boyacá.

MATERIALS AND METHODS

Determination and characteristics of the farms included

By means of a convenient probabilistic sampling of and by means of a retrospective longitudinal descriptive study during the years 2016–2018 (Martin *et al*. 2010), 25 dairy farms were chosen, located between 2300 and 2800 meters above sea level, with average annual temperatures of 13 °C and rainfall from 800–1200 mm/year (Institute of Hydrology, Meteorology and Environmental Studies, 2018). All the farms had mechanical milking twice daily. The genetic basis of the cattle was 100% Holstein Friesian. The main grass was the Kikuyu (*Cenchrus clandestinum*). 1108 animals were involved, cows with 2–4 calving, with a total of 4432 teats. The average milk production was 4300 L /cow/year and the number of somatic cells in the milk tank on average was 330,000 cel / mL.

Sampling and transport

From January 2016 to December 2018, California Mastitis Test (CMT) tests were performed in all quarters. Previous to this test, a complete clinical analysis was performed in every mammary gland, and no one apparent changes were discovered on its. The CMT was performed following this: the first stream of milk was discarded and then a few streams of milk (5 mL) were collected in corresponding paddle wells, then an equal amount of commercial reagent was added to each cup. A gentle circular movement was applied in a horizontal plane for 20 seconds to mix milk with reagent. The results were scored and interpreted as either 0 (negative or no thickening of the mixture), Trace (slight thickening), 1 (or +, distinct thickening but no tendency to form a gel), 2 (++, or immediate thickening, with a slight gel formation), or 3(+++, or gel is formed and surface of the mixture becomes elevated) to asses the inflammatory response based on the viscosity of the gel formed by mixing reagent with milk (Tolosa *et al.* 2013; Godden *et al.* 2017; Mendoza *et al*. 2017).

In all the teats that were positive (CMT > o 2+) milk samples were stored in new and sterile plastic bags (Whirl-pack), after cleaning and disinfecting the tip of the teat, the procedure described by the National Mastitis Council was followed (NMC 2009).

Teats were washed, dried and disinfected with a soap solution, then rinsed with sterile water and dried with sterile gauze, disinfected with iodine solution. Once asepsis was completed, the sample was collected in Whirl-pack bags. The collected samples were kept and transported in refrigeration until they arrived at the laboratory for analysis.

Bacteriological tests and bacterial identification

The samples were taken by a veterinarian and transported in refrigeration to the laboratory of milk quality analysis and mastitis control, as well as to the veterinary microbiology laboratory of the Universidad Pedagógica y Tecnológica de Colombia in Tunja. The laboratory of analysis of milk quality and mastitis control, as well as the Veterinary Microbiology lab, proceed in a routine diagnosis, as well as with the isolation of non-common microorganisms following the methodology proposed in the Laboratory Handbook on Bovine Mastitis, by the National Council of Mastitis of the United States (NMC 2017).

The milk samples were cultivated in a blood agar base (ovine blood), to determine the type of hemolysis. Also, MacConkey agar was used to cultivate the samples. These two agars were incubated between 24–48 hours. After this time, Gram coloration was performed, to classify samples into Gram-positive and Gram-negative and cocci or bacilli. The Gram-positive cocci were tested using catalase and differentiated as positive and negative coagulase. Then the colony was cultivated in ID32 STAPH, to identify and confirm gender and species, thus *Staphylococcus aureus* differed from other Positive Coagulase Staphylococcus. For *Streptococcus,* CAMP, hydrolysis of esculin, hippurate and inulin, and growth in NaCl was used. For Gram-negative bacilli: oxidase, indole, triple sugar, Methyl Red-Voges Proskauer (RM-VP), Lys Agar decarboxylase (LIA), urea and citrate tests were used. All samples were cultivated in Saboureaud agar for yeast and molds.

Statistical analysis

The data obtained were recorded in the database using dBASE 5.0 and were analyzed statistically using SPSS Windows 7. Descriptive statistics were used. The results of the statistical analysis were interpreted with a confidence level of 95%.

RESULTS

The results of the positive teats during the study are recorded in Figure 1. The results show an increase in positive teats in the months of April-May and September-October (months with the highest rainfall) during the 3 years of the study, for 2016 the number of positive quarters in April and May was 138 and 149 respectively, for 2017 the results was 135 and 142, and 2018 115 for April and 128 for May.

On the other hand, generally, the months with the lowest prevalence in the three years were January, June, July, November and December (lowest rainfall). In 2016, the prevalence of positive quarters for January was 112, June 100, November 106 and December 108. For 2017, in January the number of positive quarters 108, June 105, July 102, November and December 110. For 2018, in January and February the results were 102 and 104 respectively, in June and July 102 and 108, and November and December 108 and 107 positive quarters.

The Table 1 shows the microbiological results for each sample analyzed during the study. For the three years the major percentage of the samples did not show microbiological growth. In 2016 this percentage was 54.75%, in 2017 48.88% and in 2018 60.20%. The *Streptococcus agalactiae* was the most prevalent microorganism in all three years of sampling, in 2016 it had a prevalence of 17.37%, in 2017 13.77% and in 2018 12.28%. Followed by *Staphylococcus aureus* which has a prevalence of 10.16% in 2016, 11.55% in 2017 and 11.59% in 2018. The next microorganisms in the list was the others *Streptococcus* which had a prevalence of 3.93% in 2016, 6.22% in 2017 and 3.11% in 2018. The other microorganisms like: others *Staphylococcus CP*, *Staphylococcus CN*, *Arcanobacterium pyogenes*, mixed infection with *Staphylococcus aureus* and *Streptococcus agalactiae*, *E. coli, Corynebacterium bovis* and *yeast* had prevalence lower than 6%.

FIGURE 1. Prevalence of positive quarters to mastitis for each month in each year of the study.

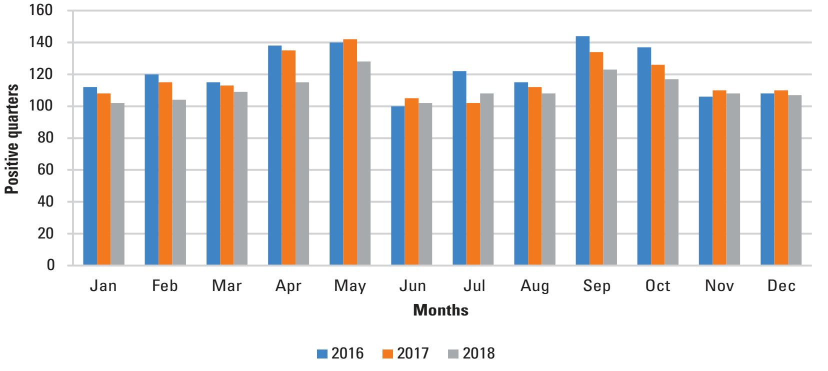

TABLE 1. Shows the microbiological results for each of the samples analyzed during the study.

Years of sampling	2016		2017		2018	
Total number of test	305		225		578	
	Number	Percentage	Number	Percentage	Number	Percentage
No Growth	167	54.75%	110	48.88%	348	60.20%
Arcanobacterium pyogenes	6	1.96%	7	3.11%	10	1.73%
S. agalactiae	53	17.37%	31	13.77%	71	12.28%
Others Streptococcus	12	3.93%	14	6.22%	18	3.11%
Staphylococcus aureus	31	10.16%	26	11.55%	67	11.59%
Others Staphylococcus CP	8	2.62%	1	0.44%	9	1.55%
Staphylococcus CN	6	1.96%	4	1.77%	19	3.29%
S. aureus +S. agalactiae	5	1.63%	9	4%	11	1.90%
E. coli	3	0.98%	6	2.66%	9	1.55%
Corynebacterium bovis	6	1.96%	13	5.77%	10	1.73%
Yeast	8	2.62%	4	1.77%	6	1.03%
Total growth	138	45.19%	115	51.06%	230	39.76%

DISCUSSION

Many factors are associated with the presence of mastitis, even in the absence of bacterial growth, these are usually traumatic damages (lacerations, cuts, injuries), chemical irritants, external conditions, where the teats or the udder, in general, are affected, thus increasing the somatic cell count, (Bhutto *et al.* 2010; Andrade-Becerra *et al.* 2018), this could explain the presence of a CMT +2 but without growth of bacterium colonies.

The months when the prevalence of mastitis increased were between April and May; and September and October, due to the rainy season which can cause problems with contamination of the udder (Andrade-Becerra *et al.* 2012), these data are similar to those reported by Andrade-Becerra *et al.* (2014) in the Highland of Boyacá, evaluating farms under similar conditions.

The total prevalence of mastitis in the three years of study is low concerning the value reported by Santivañez *et al.* (2013) which was 72.25% in the Peruvian Andes, also using CMT for the diagnosis of intramammary infection. Similarly, Ruíz *et al*. (2011) in Brazil isolated pathogenic microorganisms in 61% of the samples evaluated, a value that is higher than reported in this study with 15.81% for 2016, 9.94% for 2017 and 21.24% for 2018. In Uruguay, Gianneechini *et al.* (2014), in similar productive conditions, with an average prevalence of 54.2%, using somatic cell count (SCC) (> 300,000 cells / mL), which is similar to those reported in this study for the year 2017, and less than the years 2016 and 2018.

In the North Coast of Colombia, Calderón *et al*. (2011) reported a prevalence of 92.21% using CMT, a value that is higher than that reported in this study for any of the three years. In Antioquia, Trujillo *et al.* (2011) determined a prevalence of 42.4% using CMT and SCC, which is similar to what was reported in this study for 2016 and 2018, but less than was reported for 2017.

Calderón and Rodríguez (2008) in Boyacá reported prevalence, using CMT, of 34.40%, a value that is lower than that reported in this study for any of the three years analyzed. Andrade-Becerra *et al.* (2014) determined an average prevalence for three years of that study (2010-2011-2012) of 36.7%, a value that is lower than that reported in this study for any of the three years evaluated.

Bacteria are the most important microorganisms that generate mastitis in dairy herds, and can act like an opportunistic pathogen or contagious pathogen. Due to its presence in the skin and mucous membranes, *Trueperella (Arcanobacterium) pyogenes* is an opportunistic pathogen that can contaminate the mammary gland and produce mastitis (Rodríguez *et al.* 2015), in this study, the CMT value from de samples with growth of these microorganisms was +2 and +3. However, this pathogen is seldom found in samples from mastitis cows, Gianneechini *et al.* (2014) only reported it at 0.3%, while for this study the results were higher. Concerning the reported by Andrade-Becerra *et al.* (2014), it found a value for the year 2010 of 1.1%; for 2011 of 2.2% and 2012 of 0.5% with similar values for CMT; values that are very similar but lower than those found in this study even with farms in similar conditions of milking practices.

Streptococcus agalactiae is a highly contagious pathogen in cows with mastitis, having a common transmission in herds that allow the microorganism to colonize, invade and it replicates in the udder

(Carvalho-Castro *et al.* 2017). In Brazil, Oliveira *et al.* (2015), reported a presence of 5% in cows with mastitis, a value similar to that reported by Mekonnen *et al.* (2017) in Ethiopia of 4%, and both are less than reported in this study. Both studies have been realized in dairy herds with mechanical milking like in this study. Generally, this pathogen can be transited between cows trough milking machine, so, differences between results can be due to differences in cleaning practices. The results of CMT in both studies indicates subclinical mastitis, similar to reported here.

The values reported for this pathogen are lower than found by Ramírez *et al.* (2011) who reported a prevalence of 34%, and also was the pathogen mostly isolated as in the present study. Calderón *et al.* (2011) in Montería-Colombia, found 2.1%, which was one of the lowest values in this study. In Boyacá, Calderón and Rodríguez (2008) found a prevalence of 6.84% and Andrade-Becerra *et al.* (2014) in 2010 a prevalence of 9.7%, 9.1% in 2011 and 10.4% in 2012, all this value is lower than reported in this study for all years. In Uruguay, Gianneechini *et al.* (2014) reported a prevalence of only 0.6%, a value lower than that reported in this study. Ruíz *et al.* (2011) in Brazil found a prevalence of 12.9%; in India, Ranjaan *et al.* (2011) found a prevalence of 5.9%, in China, Yuan *et al.* (2012) found a prevalence of 15.5% all these studies were performed in herds with mechanical milking, and shows similar epidemiology, which can be due similar practices of cleaning of milking machines.

Staphylococcus aureus is one of the most commonly reported pathogens worldwide as a cause of mastitis, due to its pathogenic characteristics (exotoxins) and it is easily transmitted to the teat and is especially important due to the generation of resistance to antibiotics (Haubert *et al.* 2017). Generally, mastitis due to this microorganism is clinical; however, due to the absence of clinical changes in mammary glands evaluated here the mastitis that it caused here was subclinical due the CMT result was +2.

Gianneechini *et al.* (2014), in Uruguay, determined that this microorganism was the one that was most isolated from samples from cows with clinical mastitis, with a prevalence of 23.1%; double what was reported in this study. In India, Ranjaan *et al.* (2011) determined a prevalence of 27.37%, a value that is more than double that reported in this study for any of the three years evaluated. In Brazil, Ruíz *et al.* (2011), found it in 36.4% in mechanical milking, results that are higher than reported in this study, and the form of mastitis also was different for any study.

In Colombia, Trujillo *et al.* (2011) in Antioquia determined a prevalence of 10.3%, a result that is very similar to that reported in this study for any of the three years. Calderón *et al.* (2011) determined it in 87.56% of the samples evaluated in Monteria, a value that is higher than that reported in this study for any of the three years, possibly due to the differences between routines of milking.

In Boyacá, Calderón and Rodríguez (2008), found a prevalence of 29.09%. On the other hand, Trujillo *et al.* (2011) found it in 61.76% CMT +2 and in 29.41% CMT +3, results that are higher than those reported in this study, and with similar results of CMT; for the last three investigations, it was determined that this was the pathogen with the highest presentation, contrary to that reported in this study. In Boyacá in 2012, Andrade-Becerra *et al.* (2014) found a prevalence of 10.7%, a result that is lower than what was determined in this study for 2017 and 2018, but similar to 2016.

In the case of other Positive Coagulase *Staphylococcus*, which due to its presence in the skin of the teats and the udder it facilitates the contamination of the teats canal and the generation of mastitis, generally subclinical. In Boyacá, Calderón and Rodríguez (2008) found a prevalence of 4.04%, a value higher than that reported in this study; for 2016 this value is above 1.42%; 3.6% in 2017 and 2.49% for 2018. Likewise, Andrade-Becerra *et al.* (2014) determined it at 0.6% for 2010, 0.5% for 2011 and 0.0% in 2012, values that are below what was found in this study, even when production conditions were similar.

Negative Coagulase *Staphylococcus* are environmental pathogens found in sites such as the apex of the teats and are frequently isolated from quarters with subclinical mastitis worldwide (Mekonen *et al.* 2017), and its epidemiology can vary due the cleaning process at the milking time, also due cleaning of the milking equipment. Calderón and Rodríguez (2008), in the same region, found a prevalence of 11.75%, a value that is greater than that reported in this study; however, the same author in 2011, only isolated it in 0.3% of the samples in the Northern Colombian zone. On the other hand, Trujillo *et al.* (2011), in Antioquia isolated these microorganisms in 23%, and Ramírez *et al.* (2011), reported prevalence 10.2%. In Uruguay, Gianneechini *et al.* (2014) found a prevalence of 5% for clinical mastitis and 6% for subclinical mastitis, data greater than those in this study. Similarly, in India, Ranjaan *et al.* (2011) found a prevalence of 12.63%. Like was mention before cleaning practices are the most determinant factor for the presentation of these microorganisms in dairy herds.

The only mixed infection found in this study was *Staphylococcus aureus* and *Streptococcus agalactiae.* Both are major pathogens causing mastitis, generally with extensive damage to the glandular tissue. Similarly, in Uruguay, Gianneechini *et al.* (2014), determined it in 0.3% of the samples of clinical mastitis, and in 0.7% of subclinical mastitis, results much lower than those of results much lower than those determined in this investigation. Likewise, Calderón and Rodríguez (2008) determined that this type of mixed infection was the most frequent in dairy herds of the department of Boyacá, determining the prevalence of 1.2%. Andrade-Becerra *et al.* (2014) in their study, determined a 0.9% prevalence for this mixed infection in 2010 and 2011, and 0.5% in 2012; values that are lower than those reported in this study even when the herds had similar conditions, which show differences in milking practices.

Mastitis due *E. coli* is reported worldwide and is clinically important due to the possibility of endotoxic shock in the cow if there is a large bacterial presence. Its pathogenicity factors (endotoxins) are responsible for this problem (Yangliang *et al.* 2016; Zhang *et al.* 2017). Generally, mastitis due *E. coli* is clinical, but this can vary due the number of microorganisms in the mammary gland, also the number of endotoxins presents (Andrade-Becerra *et al.* 2012). Its presentation is related with poor cleaning practices at the milking time.

In Uruguay, Gianneechini *et al.* (2014) reported it in 1.5% of clinical mastitis samples *E. coli* but did not find *E. coli* in subclinical mastitis, which is similar to what was reported in this study for 2018; lower than for 2017 and higher than in 2016. In Antioquia, Trujillo *et al.* (2011) reported it in 1.3% for clinical mastitis, similar to reported in 2018. In Montería, (Calderón *et al.* 2011), reported it in 0.60% in double purpose farms, a result inferior to the one reported here. In Antioquia (Ramírez *et al.* 2011) reported E. coli at 0.9% similar to what was reported in 2016, but lower in 2017 and 2018.

Among the rare germs isolated from mastitis are the yeasts, which enter the teat canal due to anthropogenic factors (introduction of a contaminated cannula) and are very rare worldwide (Andrade-Becerra *et al.* 2012). In the current study, they were determined at 2.62% in 2015; 1.77% in 2016 and 1.03% in 2017 of samples; values that are inferior to that reported by Ranjaan *et al.* (2011) who in India reported mastitis due to yeasts in 3.15% of the herds. Gianneechini *et al.* (2014), reported clinical yeast mastitis in 0.6% of the animals sampled, and none in subclinical mastitis, thus showing the aggressiveness of yeast mastitis. Andrade-Becerra *et al.* (2014) report it in a maximum of 0.9% of the samples, in a similar study. Ramírez *et al.* (2011) reported *Candida* spp. in 0.6% of cows with clinical mastitis.

Corynebacterium bovis generates moderate forms of mastitis; it is easily transmitted when there are deficient milking techniques and poor cleaning of the milking equipment. In 2016 its prevalence was 1.96%; 5.77% in 2017 and 1.73 in 2018; however, these results are lower than reported in Boyacá by Calderón and Rodríguez (2008), who reported it in 8.44% of the milk samples. In Brazil, Ruíz *et al.* 2011 reported this pathogen in 45% of clinical mastitis. In Antioquia, Ramírez *et al.* 2011 reported it in 8.6% of the teats affected a result higher than the one given in this study. In Montería, Calderón *et al.* 2011 found a prevalence of 2.13% in dual-purpose farms, higher than in 2016 and 2018, bur lower than in 2017. In Antioquia Trujillo *et al.* (2011) found a prevalence of 1.3% a result lower than all those found in all the years evaluated.

CONCLUSIONS

There was an increase in the prevalence of infected teats corresponding to the months (April, May, September, and October) of increased rainfall in the Highlands of Boyacá. The infectious pathogens like *Staphylococcus aureus* and *Streptococcus agalactiae* causing mastitis were the most prevalent, more than the environmental ones. The etiological agents vary from one farm to others, even in the same geographical region and the same milking practices.

Conflict of interest

The authors declare they have no conflicts of interest with regard to the work presented in this report.

REFERENCES

Andrade-Becerra RJ, Pulido MO, Rodríguez CE. 2012. Sanidad de ubre, calidad de leche. 1d ed. Editorial Universidad Pedagógica y Tecnológica de Colombia. 169 p.

Andrade-Becerra RJ, Caro-Carvajal ZE, Dallos-Baez AE. 2014. Prevalencia de mastitis subclínica bovina y su etiología infecciosa en fincas lecheras del altiplano boyacense (Colombia). Rev Cient. 24: 305-310.

Andrade-Becerra RJ, Vargas JC, Caro ZE. 2018. Importancia del conteo de células somáticas en la calidad de la leche de vaca. 1d. Editorial Universidad Pedagógica y Tecnológica de Colombia. 139 p.

Bhutto AL, Murray RD, Woldehiwet Z. 2010. Udder shape and teat-end lesions as potential risk factors for high somatic cell counts and intra-mammary infections in dairy cows. Vet jour 183: 63-67. Doi: 10.1016/j.tvjl.2008.08.024.

Blowey R, Edmondson P. 2010. Mastitis control in dairy herds. 2° ed. CAB International editorial. 239 p.

Caggiano N, Bottini JM, Lorenzo A, De Simeone E, Chiappe M. 2016. Determinación de biomarcadores moleculares en leche y su posible aplicación en el diagnóstico y tratamiento de la mastitis bovina. InVet. 18 (2): 323-331.

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

Calderón A, Rodríguez VC, Arrieta GJ, Máttar S. 2011. Prevalence of mastitis in dual purpose cattle farms in Monteria (Colombia): etiology and antibacterial susceptibility. Rev Colomb Cienc Pecu. 24: 19-28.

Carvalho-Castro GA, Silva JR, Paiva LV, Custódio DA, Moreira RO, Mian GF, Prado IA, Chalfun A, Costa GM. 2017. Molecular epidemiology of *Streptococcus agalactiae* isolated from mastitis in Brazilian dairy herds. Braz Jour Microb. 48: 551-559. Doi: 10.1016/j.bjm.2017.02.004.

Ferrero MJ, Valledor M, Campo JC. 2014. Screening method for early detection of mastitis in cows. Measurement. 47: 855-860. Doi: 10.1016/j.measurement.2013.10.015.

Gianneechini R, Concha C, Delucci I, Gil J, Salvarrey L, Rivero R. 2014. Mastitis bovina, reconocimiento de los patógenos y su resistencia antimicrobiana en la Cuenca Lechera del Sur de Uruguay. Vet (Montevideo). 50: 111-132.

Godden SM, Royster E, Timmerman J, Rapnicki P, Green H. 2017. Evaluation of an automated milk leukocyte differential test and the California Mastitis Test for detecting intramammary infection in early- and late-lactation quarters and cows. J Dairy Sci. 100: 6527–6544. Doi: 10.3168/jds.2017-12548.

Hauber L, Schneid I, Almeida M. 2017. First report of the *Staphylococcus aureus* isolate from subclinical bovine mastitis in the South of Brazil harboring resistance gene dfrG and transposon family Tn916-1545. Mic Patho. 113: 242-247. Doi: 10.1016/j.micpath.2017.10.022.

Martin S, Meek A, Willeberg P. 2010. Epidemiología veterinaria. Principios y Métodos. 3° ed. Editorial Acribia. 341 p.

Mekonnen SA, Koop G, Melkie ST, Getahun CD, Hogeveen H, Lam TJ. 2017. Prevalence of subclinical mastitis and associated risk factors at cow and herd level in dairy farms in North-West Ehiopia. Pre Vet Med. 145: 23-31. Doi: 10.1016/j.prevetmed.2017.06.009.

Mendoza JA, Vera YA, Peña LC. 2017. Prevalencia de mastitis subclínica, microorganismos asociados y factores de riesgo identificados en hatos de la provincia de Pamplona, Norte de Santander. Rev Med Vet Zoot. 64: 11-24.

[NMC] National Mastitis Council. 2009. Procedures for the collection of milk samples. In: Microbiological procedures for the diagnosis of bovine udder infection. 3° ed. p.125-128.

[NMC] National Mastitis Council. Laboratory. 2017. Handbook on bovine mastitis. 3° ed. 148 p.

Oliveira CS, Hogeveen H, Botelho AM, Maia PV, Coelho SG, Haddad JP. 2015. Cow-specific risk factors for clinical mastitis in Brazilian dairy cattle. Prev Vet Med. 121: 297-305. Doi: 10.1016/j.prevetmed.2015.08.001.

Ortíz-Duran EP, Pérez-Romero RA, Orozco-Sanabria CA. 2017. Identificación de agentes micóticos en muestras de leche obtenidas de tanques de enfriamiento. Rev Cienc Agric. 14: 99-106. Doi: 10.19053/01228420.v14.n2.2017.7176.

Petersson-Wolfe CS, Leslie KE, Swartz TH. 2018. An update on the effect of clinical mastitis on the welfare of dairy cows and potential therapies. Vet Clin North Amer: Food Anim 34: 525-535. Doi: 10.1016/j.cvfa.2018.07.006.

Ramírez N, Arroyave O, Cerón M, Jaramillo M. 2011. Factores asociados a mastitis en vacas de la microcuenca lechera del altiplano norte de Antioquia, Colombia. Rev Med Vet. 22: 31-42.

Ranjaan R, Gupta MK, Singh KK. 2011. Study of bovine mastitis in different climatic conditions in Jharkhand, India. Vet World. 4: 205-208.

Rodríguez V, Almario GA, Verjan N. 2015. *Trueperella pyogenes (Arcanobacterium pyogenes)*, un patógeno oportunista: una revisión. Rev Colom Cienc Animal. 8: 86-95.

Ruíz AK, Ponce P, Gomes G, Mota RA, Sampaio E, Lucena ER, Benone S. 2011. Prevalencia de mastitis bovina subclínica y microorganismos asociados: comparación entre ordeño manual y mecánico, en Pernambuco, Brasil. Rev Cienc Anim. 33: 57-64.

Santivañez CS, Gómez OE, Cárdenas LA, Escobedo MH, Bustinza RH, Peña J. 2013. Prevalencia y factores asociados a la mastitis subclínica bovina en los Andes peruanos. Vet Zoot. 7: 93-104.

Taponen S, Liski E, Heikkilä AM, Pyörälä S. 2017. Factors associated with intramammary infection in dairy cows caused by coagulase-negative staphylococci, *Staphylococcus aureus, Streptococcus uberis, Streptococcus dysgalactiae, Corynebacterium bovis*, or *Escherichia coli*. J Dairy Sci. 100: 493:503. Doi: 10.3168/jds.2016-11465.

Tolosa T, Verbeke J, Piepers S, Supré K, De Vliegher S. 2013. Risk factors associated with subclinical mastitis as detected by California Mastitis Test in smallholder dairy farms in Jimma, Ethiopia using multilevel modelling. Prev Vet Med. 112: 68-75. Doi: 10.1016/j.prevetmed.2013.06.009.

Trujillo CM, Gallego AF, Ramírez N, Palacio LG. 2011. Prevalencia de mastitis en siete hatos lecheros del oriente antioqueño. Rev Colomb Cienc Pecu. 24: 11-18.

Williamson JH, Di Menna ME. 2007. Fungi isolated from bovine udders, and their possible sources. N Z Vet J. 55 (4): 188-190. Doi: 10.1080/00480169.2007.36766.

Yangliang B, Wang YJ, Quin Y, Vallverdú R, Maldonado J, Sun W, Li S, Cao Z. 2016. Prevalence of Bovine Mastitis Pathogens in Bulk Tank Milk in China. PLoS ONE. 11: 1-13. Doi: 10.1371/journal.pone.0155621.

Yuan C, Tao Z, Liu WB, Cheng Q, Wang LG, Zhang NS. 2012. Prevalence and major pathogen causes of dairy cows subclinical mastitis in northeast China. J Anim Vet Advan. 11: 1278-1280. Doi: 10.3923/javaa.2012.1278.1280.

Zhang D, Zhang Z, Huang C, Gao X, Wang Z, Liu Y, Tian C, Hong W, Niu S, Liu M. 2017. The phylogenetic group, antimicrobial susceptibility, and virulence genes of *Escherichia coli* from clinical bovine mastitis. J Dairy Sci. 101: 572-580. Doi: 10.3168/jds.2017-13159.

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