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Diagnosis of clinical and subclinical mastitis in a rural property in Carambeí, State of Paraná

Diagnostico da mastite clínica e subclínica, em uma propriedade rural em Carambeí, Estado do Paraná

Diagnóstico de mastitis clínica y subclínica en una propiedad rural de Carambeí, Estado de Paraná

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Abstract
The aim of the present study was to diagnose clinical and subclinical bovine mastitis in a property in the municipality of Carambeí-PR. 86 dairy cows were diagnosed in two evaluations carried out on July 23 and November 4, 2020. The detection of clinical mastitis was performed by testing the black-bottomed mug, whereas the detection of animals with sub-clinical mastitis by the California Mastitis Test (CMT) and laboratory analysis of bacteria and fungi present. In the first evaluation, 9.4% of the animals had mastitis, while in the second 5.16% of the herd. The milk of the animals that tested positive were submitted to laboratory analysis. In the first evaluation, they were diagnosed with an incidence of 15% *Escherichia coli*, 15% *Prothoteca* / yeast, 23% *Staphlococcus aureus*, 46% *Streptococcus agalactiae*. In the second evaluation, there was an incidence of 25% *Enterococcus* sp. and 25% *S. agalactiae*, the other animals showed 50% *Staphlococcus* non aureus. There was a higher occurrence of subclinical mastitis in the property, in both evaluations. There was a reduction from the first to the second evaluation in the incidence of cases of subclinical mastitis and the cases of clinical mastitis were resolved. Contributing to the lower disposal of milk caused by the high somatic cell count.
Keywords: Antimicrobials; Economy; *Staphylococcus* spp.

Resumo
O objetivo do presente trabalho foi realizar o diagnóstico da mastite bovina clínica e subclínica em uma propriedade no município de Carambeí-PR. Foram diagnosticadas 86 vacas de aptidão leiteira em duas avaliações realizadas em 23 de julho e 4 de novembro de 2020. A detecção de mastite clinica foi realizada através do teste da caneca de fundo preto, já a detecção de animais com mastite sub clinica pelo teste Califórnia Mastite Test (CMT) e análise laboratorial das bactérias e fungos presentes. Na primeira avaliação, 9,4% dos animais apresentaram mastite, já na segunda 5,16% do rebanho. O leite dos animais que deram positivo foram submetidos a análise laboratorial. Na primeira avaliação, forram diagnosticados com a incidência de 15% de *Escherichia coli*, 15% *Prothoteca/levedura*, 23% *Staphlococcus aureus*, 46% *Streptococcus agalactiae*. Na segunda avaliação, houve incidência de 25% *Enterococcus* sp e 25% *S. agalactiae*, os outros animais apresentaram 50% *Staphlococcus não aureus*. Houve maior ocorrência de mastite subclínica na propriedade, nas duas avaliações. Ocorreu redução da primeira para a segunda avaliação na incidência de casos de mastite subclínica e os casos de mastite clinica foram sanados. Contribuindo para o menor descarte de leite ocasionado pela elevada contagem de células somáticas.
Palavras-chave: Antimicrobianos; Economia; *Staphylococcus* spp.

Resumen
El objetivo del presente estudio fue diagnosticar mastitis bovina clínica y subclínica en un predio del municipio de Carambeí-PR. 86 vacas lecheras fueron diagnosticadas en dos evaluaciones realizadas el 23 de julio y el 4 de noviembre de 2020. La detección de mastitis clínica se realizó mediante la prueba de la taza de fondo negro, mientras que la detección de animales con mastitis subclínica mediante la prueba de California Mastitis Test (CMT) y análisis de laboratorio de bacterias y hongos presentes. En la primera evaluación, el 9,4% de los animales presentaba mastitis, mientras que en la segunda el 5,16% del rebaño. La leche de los animales que dieron positivo se sometió a análisis de laboratorio. En la primera evaluación se les diagnosticó una incidencia de 15% *Escherichia coli*, 15% *Prothoteca* / levadura, 23% *Staphlococcus aureus*, 46% *Streptococcus agalactiae*. En la segunda evaluación, hubo una incidencia de 25% de *Enterococcus* sp y 25% de *S. agalactiae*, los otros animales mostraron 50% de *Staphlococcus* non aureus. Hubo mayor ocurrencia de mastitis subclínica en la propiedad, en ambas evaluaciones. Hubo una reducción de la primera a la segunda evaluación en la incidencia de casos de mastitis subclínica y se resolvieron los casos de mastitis clínica. Contribuye a la menor eliminación de la leche provocada por el alto recuento de células somáticas.
Palabras clave: Antimicrobianos; Economía; *Staphylococcus* spp.

1. Introduction

The milk production chain has great importance for the Brazilian economy, generates jobs, income and, in many places, still maintains it social function, due to family production. Dairy production is considered a commodity for producing milk 365 days a year (Benini & Bonoto, 2019; Benatti et al., 2020). In Brazil, according to the Brazilian Institute of Geography and Statistics (IBGE), it estimates that in the second quarter of 2020, Brazilian production reached 5,758,935 liters of milk.

The southern region of Brazil is the largest producer of milk. Among the states, Paraná has great importance, especially the municipality of Carambeí, in the Campos Gerais region, nationally recognized for its high productivity (Silva et al., 2008; Costa et al., 2013).

However, dairy production has a major problem, mastitis, which consists of an infection of the mammary gland. It is responsible for major economic losses, for causing a reduction in the production and quality of milk in the affected mammary quarters, in addition to interfering in the industrial dairy process (França et al., 2017; Terra et al., 2020). The loss of production can be drastic, especially when a significant percentage of the herd is infected, which can reduce milk production by up to 50% in addition to decreasing the productive life of the cow (Mostert et al., 2018).

The origin of mastitis, which is quite diverse, is usually caused by microorganisms (*Staphylococcus* sp., *Streptococcus agalactiae* sp. and *Corynebacterium bovis* sp.) Disseminated mainly during milking and environmental microorganisms (*Streptococcus uberis* sp., *Enterobacteraceae* and fungi) (Acosta et al., 2016; Breser et al., 2018).

Mastitis can be classified into clinical or subclinical (Pantoja et al., 2009). The clinical form of the disease shows visible signs; while, the subclinical form shows an increase in somatic cell count (CCS) (Oliver et al., 2004). The frequencies of clinical and subclinical mastitis are established parameters in the evaluation of the health of the mammary gland (Defiltro et al., 2020). Therefore, they are the first to be considered for the implementation of a mastitis control program. In addition, microbiological analyzes are complementary and indispensable in a program to control this disease, as they allow the isolation and identification of its etiological agent (Oliveira et al., 2020).

Due to technological advances, the producer needs to seek competitiveness and productivity, reduce and control costs and guarantee quality standards, however for this, it is essential to carry out the diagnosis of herd health (Cha et al., 2016; Terra et al. 2020).

In this context, the aim of the present study was to diagnose the occurrence of clinical and subclinical mastitis, as well as the identification of the etiological agents in a dairy herd, of Holstein cows on a rural property in the municipality of Carambeí-PR.

2. Material and Methods

The work was carried out on a private property in the municipality of Carambeí, state of Paraná, which performs two mechanized milking a day. The breeding system used is semi-extensive in tifton 85 pastures, providing a total diet divided into two daily treatments, with water and bicarbonate at will.

86 dairy cows (Hpb and Hvb) were evaluated at different stages of lactation. Animals at the end of lactation did not have the milk collected for testing. The other cows were evaluated for the prevalence of cows without mastitis (healthy) and chronic, with mastitis (clinical and subclinical), by collecting milk from each animal. Two evaluations were carried out, one in July and the other in November 2020.

Before the collections and evaluations were carried out, the ceiling was previously washed with soap and water, dried with paper towels in aseptic conditions. For the accomplishment of each experiment, the milk collection was performed in the mammary quarters of each animal (previously identified). The milk collected from each mammary quarter was subjected to the clinical mastitis detection test, carried out by testing the black-bottomed or screened mug. At the time of milking, the first three jets of milk were removed from each teat in a black-bottomed mug, the procedure was performed on all milked cows, observing the appearance of the milk, if changes were observed, such as the presence of lumps, yellow or watery pus is a diagnostic indicator of clinical mastitis.

After testing the black-bottomed mug negative, the CMT (California Mastitis Test) test was performed next, which estimates the CCS count in milk, identifying cases of subclinical mastites (Bhutto et al., 2012). The CMT reagent is a detergent that has a pH indicator, so its color is purple. When milk is mixed with CMT in equal amounts, this reagent dissolves or breaks the membrane of the defense cells (leukocytes) and the genetic material (DNA) of the cell is released. The DNA will form a mass or gel. The greater the number of leukocytes, the greater the amount of gel formed.

After identifying the animals with mastitis, milk samples from the sick animals were collected individually from the mammary quarters for analysis in the property's laboratory. The samples (4 ml of milk) were collected in sterile test tubes, properly identified, packed in isothermal boxes with ice and shipped under refrigeration in the laboratory located on the property.

In the laboratory, the milk samples were “sown” in Petri dishes containing 5% defibrinated sheep blood agar and Mac-Conkey agar and incubated at 37ºC in aerobic conditions. The readings were performed at 24, 48, 72 and 96 hours, observing the cultural characteristics of the colonies (morphology, pigment production and hemolysis) and morpho-tinting, through Gram stain, which allowed a preliminary view of the bacteria involved in the process.

The data obtained were submitted to descriptive statistical analysis (Pereira et al., 2018). The number of cases of clinical and subclinical mastitis and isolated microorganisms was calculated. The number of healthy cows was obtained by the difference in the number of animals (in total) of the sick animals (with clinical and subclinical mastitis).

3. Results and Discussion

In the first assessment, held on July 23, 2020, a total of eleven cases of mastitis were identified on the property, among them four were clinical mastitis and seven of subclinical mastitis. Subclinical mastitis was identified in the CMT test, which shows a high concentration of CCS (Figure 1).

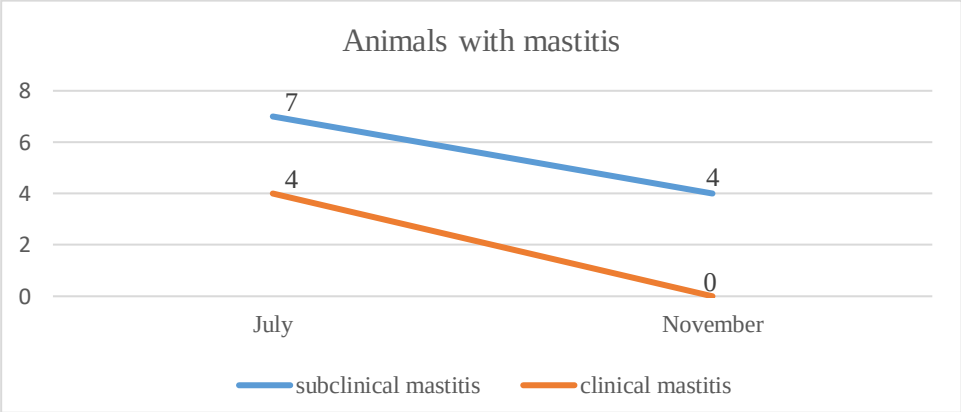
After this first diagnosis of the animals, some measures were adopted, such as the disposal of an infected animal and the treatment and monitoring of sick animals, with the aim of solving their problem. Good milking practices (GMP) were also intensified among employees so that cases of mastitis did not increase on the farm.

Among the GMP, Guerreiro et al. (2005), describes the disregard of the first three milk jets, direct pre-dipping with 750 ppm chlorinated solution in a non-refluxing mug, vigorous manual cleaning of buckets, cans and coolers with chlorinated alkaline detergent, inversion of the cans and buckets and inclination of refrigerators for draining waste water.

The cleaning of the equipment is as important as the handling and hygiene during milking, the purpose of this practice is to remove organic and mineral residues that may be attached to the surfaces of the equipment (Cunha et al., 2008). The implantation of BPO results in a reduction in the number of microorganisms in the milk leading to a better microbiological quality and a longer shelf life of the final product (Guerreiro et al., 2005; Lima et al., 2020).

To verify whether GMP practices were improving milk quality, after a five-month period, the second assessment was carried out (November 4, 2020). In this evaluation, there were no cases of clinical mastitis, and 4 animals reacted only in the CMT test, proving once again the incidence of subclinical mastitis. There was a reduction in cases of subclinical mastitis (from 7 to 4), and cases of clinical mastitis were completely resolved (4 to 0 animals), from the first to the second evaluation, respectively (Figure 1).

Figure 1- Number of cases of sick animals, with subclinical and clinical mastitis in two evaluations (first performed in July and second in November), of a dairy herd of 86 Holstein cows. Carambeí, Paraná, 2020.



Fonte: Djalma Cesar Clock.

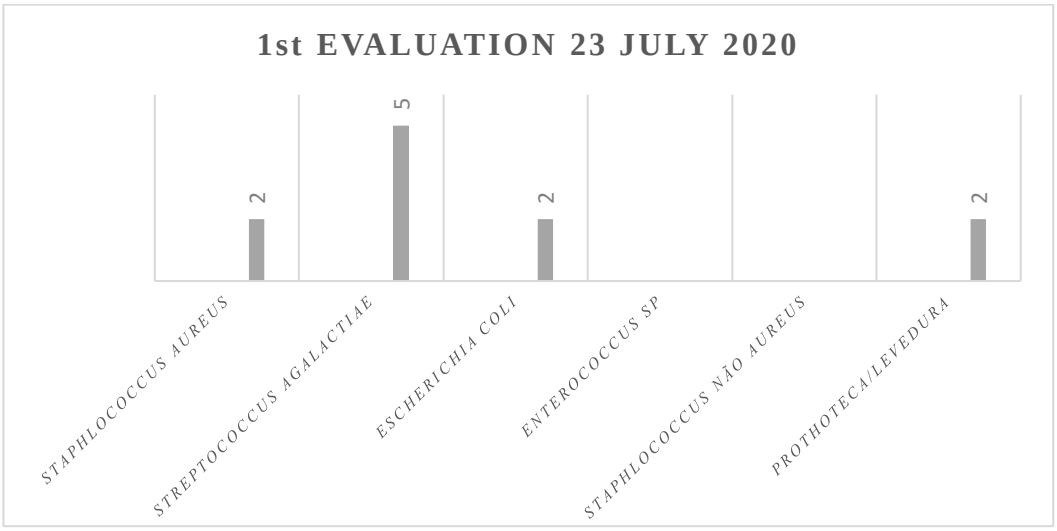
The repetition of the tests was used as an indicator, to verify whether the measures had an effect or not, in addition to checking whether there was a decrease in the sick animals and better quality of the milk, as the producer receives a bonus when the milk has a lower amount of CCS, lower incidence of mastitis in the herd (Lima et al., 2020; Defiltro et al., 2020).

When there is some type of inflammatory process that is reaching the mammary gland, there is a drastic increase in the composition of the milk of CCS (Silva et al., 2018), which are composed of white blood cells, which are the main cells responsible for the defense of the organism, and also by flaking cells on the internal surfaces of the mammary gland in order to protect it from the aggression it is suffering (Cunha et al., 2008).

CCS is one of the main ways to check the quality of the milk that arrives in the dairy, it is usually a parameter used to make the payment to producers, because the lower the value of this count, the better the quality of the milk produced (Silva and Antunes, 2018).

Milk samples from animals that tested positive for clinical mastitis were subjected to laboratory analysis in order to diagnose the causative agent (pathogen) of the disease. In the first evaluation, there was an incidence of 15% of *Escherichia coli*, 15% *Prothoteca / yeast*, 23% *Staphlococcus aureus*, 46% *Streptococcus agalactiae*, with a prevalence of GRAM group 31% negative and 69% positive, with this and it is possible to observe the greatest incidence of cases of subclinical mastitis in this evaluation (Figure 2).

Figure 2 - Incidence of mastitis-causing pathogens, first assessment carried out on July 23, 2020, in a dairy herd of 86 Holstein cows. Carambeí, Paraná.

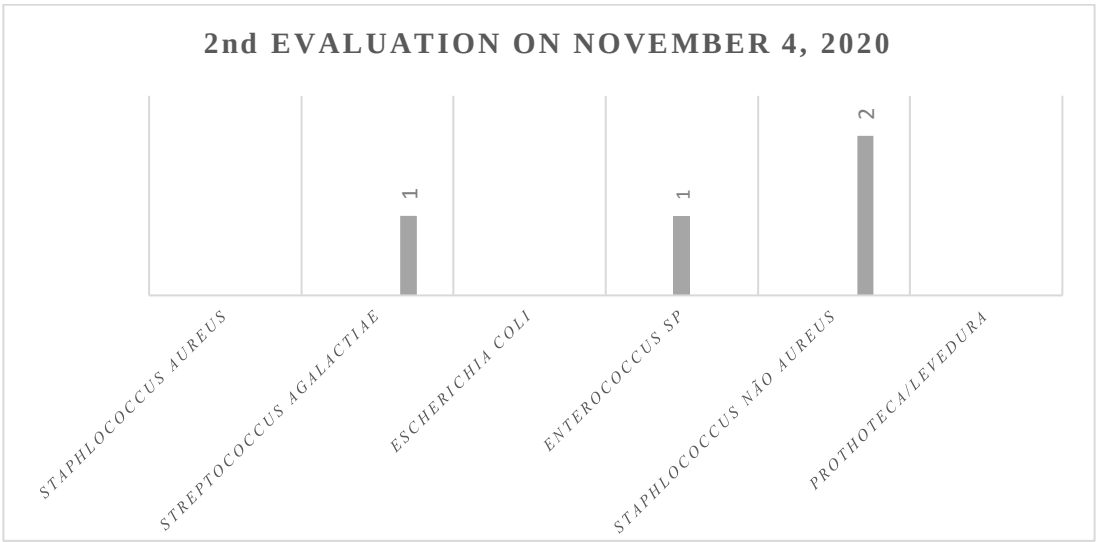


Fonte: Djalma Cesar Clock.

In the second evaluation (November 4, 2020), there was an incidence of 25% *Enterococcus* sp. and 25% *Streptococcus agalactiae*, with a GRAM group negative 50% and 50% positive, the other animals showed 50% *Staphlococcus* non aureus (Figure 3).

Damasceno and Silva (2020), classify the etiologic agents of bovine mastitis into contagious pathogens (*Staphylococcus aureus*, *Streptococcus agalactiae*, *Corynebacterium bovis* and *Mycoplasma bovis*), environmental pathogens (*Streptococcus uberis*, *Streptococcus dysgalactiae*, *Streptococcus equinus*, *Escherichia coli*, *Klebsiella* spp., *Citrobacter* spp., *Enterobacter* spp. and *Pseudomonas* spp.), Secondary or minor pathogens (*Staphylococcus* spp. Coagulasenegative) and uncommon pathogens (*Arcanobacterium pyogenes*, *Nocardia* spp., *Pasteurella* spp., *Mycobacterium bovis*, *Bacillus cereus*, some species, *Serratia* anaerobic bacteria, fungi and yeasts).

Figure 3 - Incidence of mastitis-causing pathogens, second assessment carried out, on November 4, 2020, in a dairy herd of 86 Holstein cows. Carambeí, Paraná.



Fonte: Djalma Cesar Clock.

Although there is great diversity among the pathogens that cause mastitis in cattle, *S. aureus*, *S. agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis* and *Escherichia coli* are responsible for about 80% of the cases (Ranjan et al., 2006). Individually, *S. aureus* stands out as one of the microorganisms most frequently found in intramammary infections of cattle on all continents, and is also the one that alone determines the greatest losses in dairy farming (Schlegelová et al., 2003; Vasudevan et al., 2003). In Brazil, *S. aureus* is considered to be the main causal agent of bovine mastitis, with isolation rates between herds that vary between 8.3% and 49.23% (Brito et al. 1999; Laffranchi et al., 2001).

In the property, there was a greater number of cases of subclinical mastitis (Figure 1). With a higher incidence of the pathogen *S. agalactiae* (Figure 2), bacteria is highly contagious, and has a high CCS count, impacting the quality of milk in the tank.

S. aureus and *S. agalactiae* are frequently isolated from Brazilian herds. Transmission of *S. agalactiae* occurs mainly from cow to cow through milking units, contaminated hands or towels in common use (Souza et al., 2009). *S. aureus*, in addition to being responsible for great damage to dairy farming, is resistant to several antibiotics used routinely in the treatment of this disease (Costa et al., 2013). Thus, the isolation and identification of this agent in laboratories and the in vitro analysis of antimicrobial sensitivity is of great importance for better control through appropriate therapy.

According to Brito et al. (2001), several studies dealing with the susceptibility to antimicrobials of bovine mastitis pathogens in Brazil point to an increasing increase in the resistance pattern, mainly for *S. aureus*, the most frequently isolated agent, causing losses of milk production, treatment costs of clinical cases, disposal and premature death of animals, added to the industry's losses due to reduced quality and yield in the manufacture of derivatives (Demeu et al., 2015).

Magalhães et al. (2006), concluded that 70% of the expenses with mastitis are due to the decrease in milk production in cows that present the subclinical picture of the disease, 14% of the damage is due to devaluation, early disposal and death of the animals, 8% are services performed by the veterinarian and expenses with medicines, and 8% corresponds to the disposal of milk from animals being treated.

Zafalon et al. (2007), evaluated the cost-benefit ratio of the treatment of bovine subclinical mastitis caused by *S. aureus*, the authors concluded that the treatment of bovine subclinical mastitis caused by *S. aureus*, during lactation is economically unfeasible.

4. Conclusions

There was a higher occurrence of subclinical mastitis in the property, in both evaluations. There was a reduction in the incidence of cases of clinical and subclinical mastitis, from the first to the second evaluation. Contributing to the lower disposal of milk caused by the high somatic cell count.

In the first evaluation, they were diagnosed with an incidence of 15% *Escherichia coli*, 15% *Prothoteca / yeast*, 23% *Staphlococcus aureus*, 46% *Streptococcus agalactiae*. In the second evaluation, there was an incidence of 25% *Enterococcus* sp. and 25% *Streptococcus agalactiae*, the other animals showed 50% *Staphlococcus non aureus*.

Future work is needed to reduce problems with mastitis and to direct treatment according to the causative microorganism. Contributing to the improvement of milk quality and the maintenance of families in the dairy industry.

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