

Occurrence of pathogens causing subclinical mastitis in Jaguapitã county, state of Paraná - Brazil

Ocorrência de patógenos causadores de mastite subclínica no município de Jaguapitã, estado do Paraná - Brasil

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Abstract

The aim of this research was to identify the occurrence of pathogens causing subclinical mastitis in grade B milk farms of the Jaguapitã county, state of Paraná, Brazil. California Mastitis Test (CMT) were carried out in 400 milk samples from 100 animals and 157 teats from 55 animals (55%) were positive, showed score two or higher to CMT. When these 157 positive samples to CMT were transported for bacterial culture in blood agar, 25.48% (40/157) samples showed no bacterial growth or more than two types of bacterial colonies grew, 28.03% (44/157) were Coagulase-negative staphylococci (CNS), 8.28% (13/157) were *Streptococcus uberis*, 7.64% (12/157) were *Staphylococcus aureus*, 7.64% (12/157) were *Corynebacterium* spp, 7.01% (11/157) were *Staphylococcus intermedius*, 4.46% (7/157) were *Staphylococcus hyicus*, 3.82% (6/157) were *Bacillus* spp., 2.55% (4/157) were *Streptococcus dysgalactiae*, Enterobacteria and Yeasts. We conclude that CNS is the most relevant subclinical mastitis causative agent.

Key words: CMT, Coagulase-negative staphylococci, dairy cattle, Grade B milk

Resumo

O objetivo desta pesquisa foi identificar a ocorrência dos patógenos causadores de mastite subclínica em um rebanho leiteiro tipo B no município de Jaguapitã, estado do Paraná, Brasil. Foram realizados 400

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testes de Califórnia Mastite Teste (CMT) em amostras de leite de 100 animais, totalizando 400 tetos. Dentre os animais testados 55% reagiram ao CMT apresentando grau dois ou superior, com 157 tetos positivos. Após as amostras de leite dos 157 tetos serem submetidos à cultura em ágar sangue, 25,48% (40/157) não apresentaram crescimento ou houve crescimento de mais de duas colônias bacterianas, 28,03% (44/157) foram observadas *Staphylococcus coagulase negativa* (CNS), 8,28% (13/157) *Streptococcus uberis*, 7,64% (12/157) *Staphylococcus aureus*, 7,64% (12/157) *Corynebacterium* spp, 7,01% (11/157) *Staphylococcus intermedius*, 4,46% (7/157) *Staphylococcus hyicus*, 3,82% (6/157) *Bacillus* spp., 2,55% (4/157) para *Streptococcus dysgalacteae*, Enterobactéria e Leveduras. Conclui-se que a CNS é o mais relevante agente causador de mastite subclínica.

Palavras-chave: CMT, *Staphylococcus coagulase-negativa*, bovinos leiteiros, leite tipo B

According to Harmon (1994) mastitis is defined as an inflammation of the mammary gland usually caused by bacterial infection and the mastitis may lead changes on milk quality, veterinarian expenses and require adequate management to avoid antibiotics residues in milk.

Subclinical mastitis is the biggest problem affecting dairy cattle worldwide (MEMON et al., 2013), usually cause a rise in somatic cell counts (SCC) and affect the daily milk production between 15-45% without visible changes to milk itself or the udder (MALEK DOS REIS et al., 2011) furthermore, may occurs expenses with treatment expenses, death or culling of animals.

Within the epidemiological context, its important know the mainly etiological agent, which can be detected by different diagnostic methods including the California Mastitis Test (CMT), the Modified White Side Test (MWT), SCC, pH and, in addition, chloride and catalase tests. They are easy to perform and have rapid diagnosis (LESLIE et al., 2002).

The aim of this study was to evaluate the occurrence and predominant bacterial agents involved in subclinical mastitis on a dairy property in Jaguapitã county - Northern Paraná - that produces grade B pasteurized milk.

Research was carried out in March 2011 in a dairy property, pasteurized milk grade B, in the northern region of Paraná, Brazil, in Jaguapitã County (23°06'46"S and 51°31'55"W). Milk samples were collected from 100 lactating Girolando

cows. Milking was mechanically performed with a herringbone milking machine and the presence of subclinical mastitis was detected using CMT. All milk samples (n = 400) were subjected to CMT, which was assigned a score ranging from zero to five, according to Leslie et al. (2002), and those with scores equal or above two were considered positive (coagulated milk or slightly viscous mixture).

Positive teats were washed and dried prior to the sample collection. Each teat sphincter and teat was disinfected with cotton soaked in ethyl alcohol 70° GL. The first four milk streams were discarded in a pitcher with disinfectant (quaternary ammonium and glutaraldehyde). The teat was positioned horizontally and it was milked directing the milk jet to a sterile flask, which was opened and closed immediately before and after collection. A 10 mL sample was collected from each teat, reacting to CMT. The sample was identified according the number of the animal's ear tag and the respective teat, being: RAT (right anterior teat), LAT (left anterior teat), RPT (right posterior teat) and LPT (left posterior teat). The samples were stored in isothermal box with recycled ice.

The samples were submitted to and processed in the laboratory of Veterinary Microbiology of Unopar, Campus Araçongas - Paraná. Samples were dispersed in a fume hood (Fanen) and with the aid of a Bunsen burner and of a platinum loop they were put on agar plates with 5% defibrinated sheep blood, incubated at 37°C and observed for 24 to 72 hours in order to check bacterial growth.

The colony morphology, the presence or absence and type of hemolysis were recorded in an appropriate form. The colonies were subjected to the standard technique of gram staining and catalase and coagulase tests. We performed, in accordance with Winn Júnior et al. (2008), biochemical tests for identification as proof of aerobic and non-aerobic mannitol, TSI, LIA, SIM.

Approved by the Animal Experimentation Ethics Committee of Unopar. Collection of milk samples was done by following standard operating procedure on dairy farm.

Statistical analysis: Descriptive statistics was used to analyze the data.

Regarding the total teats analyzed (n = 400), 157 were positive to CMT of a total of 55% (55/100) reagent animals. Microbiological results from these positive samples to CMT were: 25.48% (40/157) samples showed no bacterial growth or more than two types of bacterial colonies grew, 28.03% (44/157) were Coagulase-negative staphylococci (CNS), 8.28% (13/157) were *Streptococcus uberis*, 7.64% (12/157) were *Staphylococcus aureus*, 7.64% (12/157) were *Corynebacterium* spp, 7.01% (11/157) were *Staphylococcus intermedius*, 4.46% (7/157) were *Staphylococcus hyicus*, 3.82% (6/157) were *Bacillus* spp., 2.55% (4/157) were *Streptococcus dysgalacteae*, Enterobacteria and Yeasts.

Harmon (1994) describe that when subclinical mastitis proportions are above 15%, means that there are errors in managing the property. The rate of 55% found in this study indicates the need to revise some points in the management of the studied property. Memon et al. (2013), in East China, describe rates of 52.3% of values close to our study. Martins et al. (2010), evaluating animals from the region of Cuiabá, found 65% of subclinical mastitis. Duguma et al. (2014) describe 75.3%, well above the levels of the current study.

Ferreira et al. (2007) observed a percentage of 41.1% of mammary quarters reagent to CMT

in Teresina - PI, Saab et al. (2014) in Nova Tebas (Paraná) describe 15.4%, rates lower than the one found in this study. In the current study, in 25.48% of the samples, results were not achieved due to the non-growth of colonies or to the growth of more than two types of colonies, which were considered contaminants. Abrahmsen et al. (2014) describe the percentage of 24.9% and Saab et al. (2014) 24.1%, as non-bacterial growth, which is close to the data of the current study. Duguma et al. (2014) report in their study 35.78%, Beloti et al. (1997) report in Londrina (Paraná) 27.8%, while Dieser et al. (2014) report 16.9%. Malek dos Reis et al. (2011) describe the percentage of 1.2% as contamination.

Dohoo et al. (2011) cite the factors that contribute to the non-isolation of mastitis causative agents: species of microorganism, the method of collection, sample storage, transportation, number of organisms in each sample, duration of infection, culture medium, standard number of cultured microorganisms, cultured milk volume and frequency of the sample. To avoid this type of situation, Andersen et al. (2010) recommend the triple culture of samples.

Beloti et al. (1997) in Londrina (Paraná) describe as the most prevalent agents: *Staphylococcus* (17.97%), CNS (12.54%), *Streptococcus* (21.69%) and *C. bovis* (18.98%). Saab et al. (2014) in Novas Tebas (Paraná) describe CSN (32.9%) and *Corynebacterium bovis* (12.3%). In the research conducted in Jaguapitã (Paraná) among the most frequent agents, there were: coagulase-negative staphylococci (CNS) (28.03%), *Streptococcus uberis* (8.28%), *Staphylococcus aureus* (7.64%), *Corynebacterium* spp. (7.64%). It can be estimated that, despite the knowledge of the most prevalent agents in northern Paraná, few effective hygiene measures were implanted in the milking parlor. There was an increase of CNS involvement in subclinical mastitis in the region.

Duguma et al. (2014) describe, in Central Ethiopia, that *Staphylococcus* spp was isolated from

47.2% of the cases, *Streptococcus* spp. (25%), and *Micrococcus* (17.2%). Dieser et al. (2014) isolated MSC (53.9%), CNS (52.1%), *Staphylococcus aureus* (21.3%), *Corynebacterium* spp. (5.2%), *Streptococcus agalactiae* (4.4%), *Streptococcus dysgalactiae* (4.4%) and Enterobacteriae (2.3%). Abrahmsen et al. (2014) describe in Uganda 54.7% of CNS. Martins et al. (2010) describe that *Corynebacterium* spp. (27.6%), *Staphylococcus aureus* (21.5%), *Corynebacterium* spp. + *S. aureus* (6.8%) and *Staphylococcus intermedius* (6.5%) were the most prevalent causative agents in subclinical manifestations. It shows a significant trend of the presence of *Staphylococcus* spp. as well as CNS in both this study and the studies by the other authors.

Ferreira et al. (2007) identified the genus *Staphylococcus* sp. in 74.60% of the cases. In the current study, there was a significant participation of *Corynebacterium pyogenes* (7.64%). However, *C. bovis* and CNS, traditionally considered less pathogenic microorganisms, have become more frequent in such herds. The *C. bovis* spreads among the animals during the milking process, if this operation is not hygienically conducted. Moreover, a high prevalence of intramammary infections by *C. bovis* indicates the absent or inefficient disinfection of the teats after milking, a process called post-dipping (HALTIA et al., 2006). In the current study, 28.03% of CNS and 7.64% of *Corynebacterium* spp. were isolated, indicating a possible failure in the hygienic process.

The post-dipping procedure effectively prevents cross infection among cows by eliminating from the surface and the distal ostium of the teat, the contagious pathogens that may have been transferred from infected to healthy animals during milking. The plan for the control of mastitis, called Five Point Plan or Five Points Program, was primarily responsible for the successful control of contagious mastitis in the UK. The plan includes the use of teat disinfection after milking, the adoption of dry cow therapy in the entire herd, the early

identification and treatment of clinical mastitis, the culling of chronically affected cows and the regular maintenance of the milking equipment (BRADLEY, 2002).

In this study, prevalence of subclinical mastitis as 55%. There was a high participation of minor pathogens. Prophylactic measures of proper attention to health of the mammary gland should be implemented in the property.

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