SHORT COMMUNICATION

Yeast mastitis outbreak in a Brazilian dairy herd

Surto de mastite causado por leveduras em um rebanho brasileiro

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Abstract

This study aimed to describe an outbreak of bovine mastitis caused by yeast in a dairy herd located in the state of Minas Gerais, Brazil. Microbiological analysis of milk samples (106) of the infected mammary quarters of lactating cows showed that *Corynebacterium bovis* (43.48%) and yeasts from the genus *Candida* (29.35%) were the main agents isolated in the herd. *C. albicans*(33.34%), *C. catenulate* (22.23%) and *C. glabrata* (18.52%) were the species most commonly isolated. The high prevalence of yeast mastitis was associated with lack of training formilkers, repetitive intramammary treatment and poor teat hygiene prior to the intramammary infusion. Appropriate management measures, specific treatment with antifungal drugs and removal of chronically infected animals were effective for the control of mastitis outbreak.

Keywords: *Candida* spp.. Yeast mastitis. Treatment. Milking hygiene. Mastitis outbreak.

Resumo

O objetivo deste estudo foi descrever um surto de mastite bovina causada por leveduras em um rebanho leiteiro localizado no Estado de Minas Gerais, Brasil. Análises microbiológicas de amostras de leite (106) dos quartos mamários infectados de vacas em lactação demonstraram que *Corynebacterium bovis* (43,48%) e leveduras do gênero *Candida* (29,35%) foram os principais agentes isolados no rebanho. *C. albicans* (33,34%), *C. catenulata* (22,23%) e *C. glabrata* (18,52%) foram as espécies mais comumente isoladas. A elevada prevalência da mastite por leveduras foi associada à falta de treinamento dos ordenhadores, ao tratamento intramamário repetitivo e a falhas na higienização de tetas antes da infusão intramamária. Medidas adequadas de manejo, tratamento específico com drogas antifúngicas e descarte de animais cronicamente infectados foram eficazes para o controle do surto.

Palavras-chave: *Candida* spp. Mastite fúngica. Tratamento. Higiene de ordenha. Surto de mastite.

Bovine mastitis is a multifactorial disease involving interrelationships between the host, the environment and infectious agents. It is regarded as the most prevalent and economically important infectious disease of dairy cattle on all continents, with annual losses estimated at $35 billion in the dairy industry worldwide[1]. Several microorganisms are involved in the etiology of intramammary infections in cattle, represented mainly by bacteria, viruses, mycoplasma, algae and yeasts[2].

Yeasts are found in moist places that are rich in organic matter, and are easily isolated from teats and milking equipment[3]. Although the incidence of mastitis due to yeast has generally been low, outbreaks are occasionally reported[4,5]. Mastitis outbreaks caused by yeast have particularly been reported in intensively managed herds in which there were failures in environmental hygiene or in association with repetitive intramammary treatment[6,7].

Several species of yeast from the genera *Candida*, *Cryptococcus*, *Rhodotorula* and *Trichosporum* have

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been associated with mastitis in dairy cattle. *Candida* is usually the most frequently isolated genus, with great variations in prevalence among herds and identified species[6,8,9]. Here, we present a descriptive study of one mastitis outbreak, which occurred in 2009, caused by yeast infection in a dairy herd in the state of Minas Gerais, Brazil, addressing the species isolated and the factors determining its occurrence.

The outbreak occurred in a herd composed of 220 Holstein cows, which were kept in *Brachiaria* pasture, supplemented by concentrates and corn silage. Mastitis incidence was monitored monthly using a strip cup test and bulk milk somatic cell count (BMSCC). Milking hygiene included washing all teats in warm water before milking, and teat disinfection before and after milking using commercial solutions of chlorine and iodine. The milking machine received regular maintenance and was kept in good condition for use. No problems with the protocols for washing the milking equipment were experienced, and the hygiene conditions of the milking room were considered satisfactory. There was an excess of mud and feces near the feeding places, which promoted excessive dirt in the udders of the animals. The water used for washing the milking equipment and teats of the animals was not contaminated with coliforms.

Lactating cows were submitted daily to a strip cup test before milking. All mammary glands for which the strip cup test had a positive result were submitted to intramammary treatment, consisting of commercial products composed of cefquimone and a combination of tetracycline, neomycin, bacitracin and prednisolone. Treatment generally consisting of two daily applications was continued for 48 hours after the disappearance of clinical symptoms.

Milkers incorrectly identified several animals as positive in the strip cup test. They were not properly trained in milking procedures and confused dirt with grumous in the strip cup test, resulting in a large number of cows being unnecessarily submitted to intramammary antibiotic treatment. At the peak of the outbreak, a group of 42 cows, representing 19.1% of the milking cows, either had clinical mastitis or was waiting to be reincorporated into the milk production lots following intramammary antibiotic treatment. About 50% of the animals in this group were submitted to intramammary treatment for a period longer than ten consecutive days.

In order to understand the dimension of the outbreak and its etiology, all lactating cows in the herd were submitted to the California Mastitis Test (CMT) and a strip cup test, and milk samples from mammary positive quarters were collected for microbiological analysis. Individual milk samples (n=106) were collected and submitted to culture, comprising 18 samples from mammary quarters with clinical mastitis and 88 samples from mammary quarters with subclinical mastitis. This sampling included all mammary glands under treatment that either had recently been submitted to intramammary treatment or showed a positive reaction in CMT. Samples of bulk milk were also collected to determine its somatic cell count (BMSCC).

Milk samples were cultivated on nutrient agar supplemented with 5% ovine blood and Sabouraud dextrose agar supplemented with chloramphenicol (0.4g/L) at 37°C for 24–96 hours. The biochemical tests and identification keys proposed by Yarrow[10] were used for identifying yeasts and algae. Other agents were identified according to Quinn et al.[11].

The incidence of clinical and subclinical mastitis was 6.8% and 30.2%, respectively. Twenty-two milk samples showed no growth of microorganisms, including ten samples collected from mammary glands that had recently been treated or were under treatment. Ninety-two microorganisms were isolated, with the main identified pathogens being *Corynebacterium bovis* (43.48%), yeasts (29.35%), *Staphylococcus* spp. (8.70%), coliforms (5.43%) and *Prototheca**zopfii* (4.34%) (Table 1). All yeasts were identified as

Table 1 - Microorganisms associated with an outbreak of mastitis caused by yeasts in a dairy herd from the state of Minas Gerais, Brazil, 2009

Total Microorganisms	**Number of Isolates (%)**
Corynebacterium bovis	40 (43.48)
Yeasts	27 (29.35)
Staphylococcus spp.	8 (8.70)
Coliforms	5 (5.43)
Protothecazopfii	4 (4.34)
Enterococcus spp.	4 (4.34)
Other agents	4 (4.34)
Total of isolates	92 (100)
Milk samples without growth	22
Milk samples cultured	106
Yeasts	**Number of Isolates (%)**
Candida albicans	9 (33.34)
Candida catenulata	6 (22.23)
Candida glabrata	5 (18.52)
Candida parapsilosis	3 (11.11)
Candida krusei	2 (7.40)
Candida tropicalis	1 (3.70)
Candida sp.	1 (3.70)
Total	27 (100)

C*andida* (Table 1), including *C. albicans* (33.34%), *C. catenulata* (22.23%), *C. glabrata* (18.52%), *C. parapsilosis* (11.11%), *C. krusei* (7.40%), *C. tropicalis* (3.70%) and *Candida* sp. (3.70%).

The BMSCC result was 2.01 x 10^5 cells/mL, which was indicative that major contagious pathogens, e.g., *Staphylococcus aureus* or *Streptococcus agalactiae*, were not important mastitis agents in this herd, as was demonstrated by the microbiological results (Table 1). Microbiological analysis showed the predominance of *Corynebaterium bovis* as an etiological agent of mastitis in this herd justified the low BMSCC observed, although there was a relatively high rate of subclinical mastitis (30.92%). Infections caused by this agent are generally associated with low scores for BMSCC[12].

Yeasts are considered to be uncommon pathogens of bovine mastitis[13]. Yeast infections tend to be chronic and the infected animals eliminate the agent continuously over long periods[4]. Previous studies on several herds also showed that *Candida* was the yeast most frequently isolated from bovine mastitis and a great diversity of species[8,9,14,15]. However, in contrast to our results, previous outbreaks[4,5] were usually associated with only one or a few species of yeasts.

The recommended 30 second period for the action of the pre-dipping solution to be effective was not observed, and milkers used a commercial solution based on iodine with a low concentration of the active ingredient in post-dipping. This practice could be associated with the high prevalence of *Corynebacterium bovis* in

the etiology of subclinical mastitis in the herd, as reported by Watts, Lowery and Tell[16]. Faults in predipping were associated with infections caused by environmental microorganisms[12]. Although there are few studies concerning the *in vitro* and *in vivo* efficiency of antiseptic solutions against yeasts[17], absence of or inefficient teat disinfection may contribute to mastitis occurrence caused by these environmental pathogens.

As soon as the fungal etiology of the outbreak was established, specific treatment with nystatin was implemented. Mammary quarters of 27 animals infected by yeast were submitted to treatment with an intramammary infusion composed of nystatin (26.05 mg), sulfadiazine (500 mg) and prednisolone (5 mg), two times daily, for a single period of seven consecutive days. The objective of treatment was to recover the infected animals and to evaluate the efficacy of the specific medication. One observed inefficiency of the treatment of animals from this group and 18 cows infected by yeasts and four infected by algae (not treated) were discarded because they did not respond to medication or because of the presence of atrophy and fibrosis of infected quarters, these being alterations associated with chronic mastitis. An important aspect of mastitis caused by yeasts is that there are few commercial products for treating these infections, aggravated by the occurrence of multidrug resistance[18]. Such limitations in the treatment of these infections can cause the lost of the infected quarters or the discard of infected animals in order to resolve the cases as was observed in this study.

This yeast outbreak could be associated with repetitive intramammary treatment and failures in disinfection of teats before intramammary infusion. Several animals had teats that were extremely dirty prior to the application of the intramammary infusion. Environmental agents such as yeasts and *Prototheca*zopfii are commonly isolated from the environment, milking equipment and cow teats. These microorganisms can easily be introduced into mammary glands as a result of repetitive intramammary treatment, especially when teats are not disinfected properly[5,13,14,19], as was observed in this study.

The resolution of the outbreak occurred approximately two months after the beginning of the implementation of control measures, based on a redefinition of the criteria for interpreting the strip cup test and improved teat end disinfection prior to application of the intramammary infusion.

The epidemiological and microbiological data indicated that inadequate interpretation of the strip cup test, unqualified milking personnel and repetitive intramammary treatment without the observance of basic principles of teat disinfection were the determining factors for occurrence of the outbreak.

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