Bacteriology & Parasitology

Motta, et al., J Bacteriol Parasitol 2014, 5:3
http://dx.doi.org/10.4172/2155-9597.1000186

Research Article Open Access

Microbiologic Characterization of Equine Mastitis

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Rec date: Mar 22, 2014, **Acc date:** Apr 18, 2014 **Pub date:** Apr 21, 2014

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Abstract

Mastitis occurrence in mares is low if compared to other livestock species. The microorganisms often isolated and detected in milk and mammary gland secretions of mares are *Streptococcus beta-haemolytica, Staphylococcus spp., Pseudomonas aeruginosa, Actinobacillus spp*., and enterobacter. The present experiment was designed to evaluate the main microorganisms present in the milk of healthy mares and having a mammary infection. One hundred and ten mammary glands from 55 lactating mares were analyzed, ranging from 15 to 150 d post-partum. The mastitis diagnostic was performed through analysis of the milk via the screened test of the mug with dark background (Tamis), mammary gland inflammation and/or systemic signs. The subclinical mammary gland infection was characterized via the California Mastitis Test (CMT). From the 55 lactating mares, 2 (3.64%) had clinical mastitis. Following the CMT, the mares presented: 13 (23.60 %), 7 (12.72%), and 12 (21.88%) scores from 1+, 2+, and 3+, respectively. From the 110 mamary glands were analysed, in 47 (85.45%) of these samples strains of microorganisms were isolated. In summary, results from our experiment suggest a low occurrence of clinical mastitis in lactating mares.

Keywords: Mares; Mastitis; Microorganisms

Introduction

In South America, equine milk is not frequently used for human consumption. However, in other countries, such as Germany and France, the equine milk is often offered in hospitals for the feeding of premature born children [1]. Among the mammalian species, the milk from mares has the highest similarity to the human's, due to the high digestibility, low protein content, high lactose content, and the albumin: globulin ratio [1,2]. In general, the milk produced by the mare is the only nutrient source for the foal in the earlier weeks of life and this production is enough to nurture the newborn foal, being an essential factor affecting the survival of the offspring [3].

Mastitis occurrence in mares is relatively low when compared to domestic ruminants [4]. Mastitis occurs primarily during lactation and secondarily in the dry period, usually as a consequence of injuries in the breasts or teats [5]. The microorganisms usually isolated from milk and mammary gland secretions of mares are *Streptococcus* beta-haemolytic, *Staphylococcus spp.* [4], *Pseudomonas aeruginosa, Actinobacillus spp*., and enterobacteria [2]. Furthermore, if mastitis is suspected, microbiologic analysis is required for positive diagnosis [6].

However, to the best of our knowledge, little data are available evaluating the milk microbiology of mares in Brazil. Therefore, the present study was designed to evaluate the main microorganisms present in the milk of healthy mares, but with breast infection.

Materials and Methods

The present study was conducted in Botucatu, Sao Paulo, Brazil, from June to December 2010. All mares were originated from the same ranch and managed similarly throughout the period data were being collected.

Animals

Fifty-five lactating mares, aging from 4 to 15 yr, primiparous and multiparous (1 to 9 births), from different breeds and crosses (Quarter horses, Mangalarga, Creole, Appalousa, Paint Horse, and Crossbreds) were used in the present study.

Sampling and analysis

The mammary glands of all mares were evaluated and the clinical mastitis diagnosis was confirmed in animals that had alterations in the milk (presence of lumps, pus, dessora, or streaks of blood) through the screened test of the mug with dark background (Tamis), inflammation in the mammary gland (pain, edema, redness, or lumps), and/or systemic symptoms [4]. Subclinical inflammation was detected via the California Mastitis Test (CMT; scores from 1+ to 3+). After cleaning and disinfection of the mammary gland with cotton soaked with 2% iodine alcohol, samples of milk from all mares were collected (5 to 10 mL) in the form of pool of both breasts of each mare. Following the discard of the first milk ejections, samples were collected in sterile vials, identified with the data of each animal, immediately sealed, packed in coolers containing ice, transported to the laboratory, and kept refrigerated (4 to 8°C) up to 24 h before processing. All milk samples with cultured in defribinated bovine blood agar (5%), Mac Conkey agar and Saboraund agar and incubated aerobically at 37°C for 72 h. Isolated microorganisms colonies were identified based on

morphological, staining, and biochemical features, as well as cultivation [7].

Statistical analysis

As previously mentioned, all data were obtained from mares reared in the same ranch and from similar management. The major question in this study was to investigate mastitis occurrence in healthy lactating mares. Parity was introduced as a categorical variable with 3 classes, first, second, and third or higher. Day's post-partum was introduced as categorical variable. According to the microbiologic exam, the Somatic Cell Count (SCC) was performed using the Friedman or Kruskall-Wallis test. The means comparison from milk constituents and microbiological exam were analyzed using the Student's t-test. For all analysis performed, significance was set at $P<0.05$.

Results and Discussion

Of the 55 lactating mares, only 2 had clinical mastitis (3.64%), with painful tenderness to palpation of the gland, nodules, abscesses isolates, viscous pus, and milk with lumps as shown in the proof of Tamis. Furthermore, the milk secretion was purulent with unpleasant odor in one of the samples. Following the CMT, results showed 13 (23.60%), 7 (12.72%), and 12 (21.88%) scores of 1+, 2+, or 3+, respectively. In 23 of the 55 mares (41.80%), no reaction to the CMT was observed. Figure 1 illustrates one of the mares having clinical mastitis.



Figure 1: Clinical mastitis illustration in one of the mares analyzed in the present study.

In 47 (85.45%) of the 55 milk samples analyzed, strains of microorganisms were isolated (Table 1), whereas no microbial isolation was observed in the remaining 8 milk samples (14.55%). The presence of microorganisms isolated in the milk of mares, with CMT scores of 1+, 2+, or 3+, was observed in 12 (25.53%), 6 (12.76%), and 12 (25.53%) animals, respectively, while 17 (36.17%) of the 47 animals were detected with microbial isolation, but not reactive to the CMT.

Microorganisms	Relative Frequency	Absolute Frequency (%)
Streptococcus sp.	11	20.00
Staphylococcus aureus	7	12.73
Streptococcus equi	5	9.09
Staphylococcus sp.	3	5.45
Nocardia sp.	3	5.45
Corynebacterium sp.	2	3.63
Staphylococcus hyicus	1	1.81
Arcanobacterium pyogenes	1	1.81
Enterobacter cloacae	1	1.81
Staphylococcus aureus + Enterobacter cloacae	2	3.63
Staphylococcus sp. + Escherichia coli	2	3.63
Streptococcus sp. + Enterobacter cloacae	2	3.63
Streptococcus equi + Citrobacter freundi	1	1.81
Streptococus sp. + Corynebacterium spp	1	1.81
Streptococcus sp. + Nocardia spp	1	1.81
Staphylococcus intermedius + Corynebacterium spp	1	1.81
Staphylococcus sp. + Streptococcus spp. + Enterobacter cloacae	2	3.63

Enterobacter cloacae + Bacillus sp. + Candida sp.	1	1.81

Table 1: Isolated microorganisms in a culture pure or in association in the milk of 55 mares

The average and median milk cellularity from the 8 mares with microorganism isolation and not reactive to the CMT were 247.57 and 259.81 × 10^3 CS/mL, respectively. On the other hand, SCC from the 47 animals with microorganism isolation resulted in 1,621.86 and 1,397.97 × 10^3 CS/ml for average and median, respectively, ranging from 110 to 9,589 × 10^3 CS/ml. The mares diagnosed with clinical mastitis infected with *A. pyrogenes* and *S. aureus* had, respectively, 9,589 and 6,320 × 10^3 CS/ml.

In a study from Canada, Welsh (1989) observed the occurrence of clinical mastitis in 2 mares during the 4th month of lactation that presented purulent exudate in both breasts [8]. Milk cytology demonstrated a greater amount of neutrophils and the isolation of *Streptococcus zooepidemicus* in a pure culture. Another study performed in Germany from 1985 to 1988, using 33 mares from different ages (4 to 14 yrs), breeds, and having acute mastitis identified predominantly the following microorganisms: *Streptococcus spp., Staphylococcus aureus, Escherichia coli, and Klebsiella spp.* [9]. In a review of 28 cases of clinical mastitis, McCue and Wilson isolated *Streptococcus spp.* (36.8%) [5], *Staphylococcus spp.* (14.5%), and *Actinobacillus suis* (10.5%).

In a study conducted in Brazil, Prestes et al. [2] evaluated 38 mares milk samples and identified 51 strains of microorganisms distributed as follows: 17 *Staphylococcus spp.* (33.3%), 15 *Streptococcus spp.* (29.4%), 10 *Corynebacterium spp.* (19.6%), 5 *Bacillus spp.* (9.8%), 4 *Pasteurella spp.* (7.9%), 4 *Candida spp.* (7.9%), 4 *Enterobacter cloacae* (7.9%), and 4 *Shigella spp.* (7.9%).

In agreement with domestic ruminants, we observed a high prevalence of microorganisms from the *Staphylococcus, Streptococcus,* and *Corynebacterium* strains, which inhabit the skin and mucous membranes of animals, further causing opportunistic infections and trauma in the breast or even by the suckling of the foals [4]. Only 2 of the 55 mares had signs of clinical mastitis, with isolation of *Staphylococcus aureus* and *Arcanobacterium pyogenes.* These findings support other data, in which there was a low occurrence of clinical cases of infectious mastitis in mares [3].

The poor correlation between the CMT reacting animals and the microorganisms isolated from the milk supports the idea that this technique is limited for mares, which was standardized for cattle, as an indication of breast infection.

Conclusions

In conclusion, our findings allow inferring the low occurrence of clinical mastitis in mares, the prevalence of infectious microorganisms even in animals without signs of clinical mastitis and the low application value of the CMT technique as an indirect method in diagnosing mammary infections in mares.

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