A fatal case of mastitis caused by *Klebsiella pneumoniae* in bovine

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It’s reported the occurrence of fatal case of hyper-acute mastitis in Simental cow by Klebsiella pneumoniae in the third week of lactation. Described epidemiological, clinical symptoms, procedures for microbiological diagnosis, therapeutic management and control measures. The present study reinforce the need of rapid diagnosis of peracute coliform mastitis in etiology in cattle for establishment of effective control measures and treatment protocol, in order to avoid the toxemic and / or septicemic complications, Generally associated to poor prognosis. In addition, alerts also to importance of K. pneumoniae severe coliform pathogen in the etiology of bovine mastitis in Brazil peracute.

**Keys words:** Mastis, bovine, Klebsiella pneumoniae, Brazil

**INTRODUCTION**

The spread of multidrug-resistant microorganisms in health care facilities has resulted in increasing morbidity, mortality, and cost of care, with significant implication for public health. In this way, the prevention of the emergence of resistance and the dissemination of resistant microorganisms has become a major public health priority. Numerous reports have indicated that antimicrobial resistance is commonly encountered in long-term-care facilities (LTCFs), which may act as reservoirs and amplifiers of resistant organisms. Most isolation guidelines have targeted acute-care settings and may not be suitable or effective in LTCFs. LTCF residents remain in the facility for prolonged periods of time, share common eating and rehabilitation areas, and participate in common activities. Implementing infection-control measures that are identical to those in an acute-care setting may result in less effective rehabilitation as well as undesirable psychological and social consequences. Furthermore, data on the effectiveness of infection-control measures in LTCFs are limited (Ben-David et al., 2014).

The causal agents involved in bovine mastitis were conventionally classified into contagious and environmental pathogens. Fungal, algae and coliform are the most important environmental pathogens in the etiology of bovine mammary infections (Santos and Fonseca, 2007). *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter aerogenes* are recognized as more common coliform bacteria in bovine mastitis (Bannerman et al., 2003; Riet-Correa et al., 2001). Exposure of uninfected quarters to coliform pathogens occurs primarily between milking periods, and secondly during milking procedures and the dry period. Coliform pathogens are essentially opportunistic. The primary

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RESULTS AND DISCUSSION

The specific examination of the udder revealed a positive Tamis test (clinical mastitis) in the right posterior quarter, and a score of 3+ in the California Mastitis Test (subclinical mastitis) in the other three quarters. In the palpation, the quarters were cold, swollen, painful, warm, and exhibited enlargement of the mammary lymph node, with discolored areas (blue-blackish) in the right posterior quarter (Figure 1). The milk secretion showed watery in all quarters, containing flakes in the right posterior quarter.

Milk samples from the four quarters were aseptically obtained and submitted to microbiological culture on defibrinated bovine blood agar (5%), MacConkey agar, incubated aerobically, at 37°C for 72 h. The microorganisms isolated were classified on the basis of macro and microscopical morphology (Gram stain), culture characteristics and biochemical profile (Krieg and Holt, 1984; Quinn et al., 1994). Circular, non-hemolytic, grayish colonies were observed in the bovine blood agar after 24 h, while on MacConkey agar, very mucoid, brilliant, pink (lactose-positive) colonies were observed in pure cultures from the material collected from all quarters. Biochemical tests performed in lactose-positive colonies enabled the classification of the microorganism as K. pneumoniae. The microorganism was submitted to an in vitro antimicrobial susceptibility test using disk diffusion method (Bauer et al., 1966), and the strain showed susceptibility to cefalexin (30 µg), gentamicin (10 µg) and ceftriaxona (30 µg), and resistance to ampicillin (10 µg), tetracin (30 µg) and florfenicol (30 µg).

Because of the severe clinical signs, emergency treatment was attempted using commercial cephalosporin (30 mg/Kg, 8h, IV), flunixinmeglumin (1.1 mg/Kg, 24h, SC), and aggressive intravenous fluid therapy. In spite of therapy, 12 h after initiating the treatment, euthanasia was recommended due to the worsening of general conditions and the evolution of clinical signs. Pneumonia and edema pulmonar, renal and hepatic congestion, enlargement and congestion of mammary lymph nodes, congestion of mammary parenchyma, containing watery secretion was observed at necropsy. Fragments of lung, liver, kidney and mammary gland were submitted to microbiological culture. After 24 h of the microbiological exam, K. pneumonia was isolated in pure culture from fragments obtained from the mammary gland and lung, with the same antimicrobial profile of strain isolated from the milk.

E. coli, K. pneumonia and E. aerogenes are recognized worldwide as predominant coliform microorganisms involved in bovine mastitis (Radostits et al., 2007). In Brazil, E. coli is considered the most common bacteria with an environmental origin described in clinical bovine mastitis (Ribeiro et al., 2006; Santos and Fonseca, 2007), including in peracute and toxemic cases, with lethal evolution (Ribeiro et al., 2002). However, little attention has been given to the evaluation of the occurrence and severity of K. pneumonia in the etiology of toxemic and/or septicemic bovine mammary infections in Brazil.
Figure 1. Simental cow, with peracute clinical signs of mastitis caused by *Klebsiella pneumoniae*.

*K. pneumoniae* have been reported in different countries in serious outbreaks or in isolated cases of peracute or acute bovine mastitis, mainly in the first two weeks of lactation. The mammary infections frequently are associated with wood or sawdust contamination used in the environment of the animals (Wenz et al., 2001; Munoz et al., 2006; Sampimon et al., 2006; Radostits et al., 2007). In the present report, the animal had no history of contact with sawdust or wood products in the environment. However, severe clinical signs were observed 3 weeks prior to calving, after a prolonged rain period. The excess of rain and lack of removal of organic material certainly increased the humidity and fecal material between milking and in the milking environment from an animal that probably was the primary source of the microorganism for transmission by mammary gland. In a similar way, other studies have also hypothesized that fecal shedding of *K. pneumonia* and excess of organic material in the environment between milking will be associated with bovine mastitis by these agents (Munoz et al., 2006). Thus, the adoption of hygiene measures in order to avoid the accumulation of organic material in the environment between milking periods, in milking installations, and fecal contamination of wood or sawdust used where the dairy animals are raised tend to control the occurrence of peracute coliform mastitis, including that produced by *K. pneumoniae*.

It is well established in the literature that clinical signs of coliform mastitis occurs, in part, due to severe systemic inflammatory response, unregulated by endotoxins (LPS) liberation. The mammary gland from domestic animals is highly sensible to LPS. These lipopolysaccharide molecules present in the outer membrane of Gram-negative bacteria – including *K. pneumoniae* – are liberated during multiplication and/or death of the bacteria (Bannerman et al., 2003). In the present case, the peracute severe clinical signs observed in the animal certainly were determined by LPS liberation from the outer surface membrane of *K. pneumonia* isolated from the mammary gland that leads to exaggerated inflammatory response. The microbiological identification of *K. pneumonia* in the lung of the present case causing bacteremia, with consequent systemic complications (septicemia), also has been described in similar cases (Wenz et al., 2001), and probably contributed to the severity of clinical signs and a worsening of the corporal condition that leads to the euthanasia of the animal.

**Conclusion**

The present report reinforces the need of rapid diagnosis of etiology in peracute coliform mastitis in cattle for establishment of effective treatment protocol and control measures, in order to avoid the toxemic and/or septicemic complications, generally associated with a poor prognosis. In addition, it alerts also the importance of *K. pneumonia* as severe pathogen in the etiology of
peracute bovine mastitis in Brazil.

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