Mastitis Acuta in a Pure Breed Cane Corso female. A Case Report

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Abstract

Data regarding bitch mastitis is fairly scarce compared to the literature regarding ruminant mastitis. Neglecting clinical and subclinical cases of mastitis can be life threatening for both dam and puppies. The aim of this report is to present a case of *Mastitis acuta* complicated with a case of neonatal septicaemia in a Cane Corso (*Canis lupus familiaris* L.) pure breed female. Laboratory assays showed a milk pH value of 7.5, milk cytology revealed the presence of segmented neutrophils, while foamy cells, phagocytosis and highly pathogenic bacteria (i.e. *Escherichia coli* and *Pseudomonas aeruginosa*) were isolated from milk and puppy. To the author's knowledge, this is the first case of bitch mastitis linked to neonatal septicaemia with mixed *E. coli* and *P. aeruginosa* infection.

Keywords: Bitch, cytology, *Escherichia coli*, mastitis, *Pseudomonas aeruginosa*, septicaemia.

INTRODUCTION

In *Mastitis acuta* episodes, both local (hot, red, engorged, painful mammary glands) and systemic, (depression, lethargy, anorexia, fever, failure to care for pups) signs of illness may be found (Johnston *et al.*, 2001). Local signs of illness may vary from classical signs of inflammation to gangrene lesions (Trasch and Wehrend, 2008).

So far, bacterial infections are held responsible for bitch acute mammary gland infections. *Proteus mirabilis* (Lee *et al.*, 2009), *Staph. hyicus* (Aráujo *et al.*, 2011) and *Staph. intermedius* (Ververidis *et al.*, 2007) have been reported to cause acute mammary infections.

Bitch vaginal microflora is thought to be one of the causes of neonatal infections in pups (Milani

et al., 2012). From uterine infections (Martí, 2009) such as acute metritis (Fontaine *et al.*, 2007) via the blood stream (Martí, 2009), bacteria can embolize during the postpartum period into the mammary glands (Fontaine *et al.*, 2007).

Milk cytology is used to determine the mammary glands' health status (Olson and Olson, 1984; Sangha *et al.*, 2010). Because milk antibiotic therapeutic levels must be reached whenever dealing with mammary gland infections, milk pH levels should always be considered before starting treatment (Martí, 2009).

Subclinical mastitis can lead to neonatal infections after milk intake, because feeding on the infected mammary glands can lead to septicaemia or fatal gastroenteritis (Sager and Remmers, 1990;

Schäfer-Somi *et al.*, 2003). The aim of this paper is to highlight the important role of early diagnose and treatment of mammary gland infections, in order to limit or even avoid neonatal mortality by describing a postpartum *Mastitis acuta* episode complicated with mortinatality in a Cane Corso breed.

MATERIAL AND METHODES

A 45 kg, 5-year-old multiparous, Cane Corso female, gave birth to thirteen pups, after a normal pregnancy, out of which 4 were stillborn. During the third week after whelping, the remaining puppies started to lose weight and one puppy died. The owner arrived at the hospital of the Veterinary Faculty at the University of Agricultural Science and Veterinary Medicine in Cluj-Napoca, with the dam and the dead puppy, a day after the pup died.

During the consult, the bitch presented inflamed, red, painful, engorged inguinal mammary glands that expressed a brown milk secretion. All other clinical parameters (i.e. temperature, respiration, heart rate) were within normal limits. After the consult, the bitch was diagnosed with *Mastitis acuta*. A 14-day course of amoxicillin/clavulanic acid (15mg/kg b.w., per os, BID) (Novartis, Switzerland) antibiotic treatment was prescribed for the bitch, along with hot packs of water every 8 to 12 h on all the glands. The puppies were allowed to nurse until further notice, while keeping track of which pup had sucked from the clinically affected glands.

Before treatment, even though the bitch continued to feed the litter, puppies showed weight loss. The puppy presented to the hospital showed before death (a night before consult), progressive weakness, ataxia and generalized tremor.

For complete blood count (CBC), from the dam, a blood sample was collected from the vena cefalica in an EDTA tube (Vacutest Kimas r.l., Piove di Sacco PD, Italia). Complete blood count was determined with Abacus Junior Vet analyzer (Diatron Messtechnik, Budapest, Hungary) whilst differential blood count was counted on blood film stained with Dia-Quick Panoptic dye kit (Reag-Fix Panoptic, ReagensKft., Budapest Hungary).

From the inguinal mammary glands, two separate milk samples were collected into sterile tubes (Nunc TM, Waltham, USA), by manual expression (the first milk drop was discarded),

using sterile gloves, after a thorough disinfection of the inguinal mammary glands using 70% alcohol (Martín *et al.*, 2010). The samples were kept on ice and immediately rushed to the Infectious Disease Department for microbiology assays.

Milk samples were collected for, microbiology, pH and cytology evaluation. The other mammary glands as well as milk secretions, posed no overt signs of illness. The non-surviving puppy's necropsy and milk sensitivity were performed *pro bono*.

Standard microbiological tests were used to isolate and identify the bacteria species (Schäfer-Somi *et al.*, 2003). Biochemical assays were performed using the commercial API 20 NE and API 20 E systems (BioMérieux, Marcy l'Etoile, France), according to the manufacturer's instructions (Vela *et al.*, 2006). For susceptibility, antimicrobial disc diffusion testing was performed. The following antimicrobial discs were used: 25 µg trimethoprim-sulfamethoxazole, 5 µg marbofloxacin, 10 µg gentamicin, 30 µg amoxicillin-clavulanate, 30 µg amoxicillin, 30 µg kanamycin, 21 µg enrofloxacin and 26 µg cefquinome (Wedley *et al.*, 2011).

The pH values were measured from the obtained milk samples (N=2) with indicator strips (E. Merk, Darmstadt, Germany), (Delucchi *et al.*, 2008) by using one drop of milk secretion collected with a sterile bacteriological loop.

For milk cytology interpretation, one drop of milk was used to prepare smears from lacto sediments obtained after a 10 minute centrifugation at 2500 rpm. The smears were stained by using the May-Grünwald Giemsa (MGG), (E. Merk, Darmstadt, Germany) technique, followed by optic microscopic interpretation (Ververidis *et al.*, 2007).

The puppy's necropsy showed acute bilateral greenish purulent ventriculitis, and *Toxocara canis* infestation. Under aseptic conditions, samples from the purulent exudates were collected and submitted to the above mentioned laboratory for further microbiology test.

RESULTS AND DISCUSSIONS

Haematology evaluation showed presence of normal range white blood cells with normocytic normochromic anemia, lymphopenia and reactive thrombocytosis.

Pseudomonas aeruginosa and *E. coli* were isolated from bitch milk. The susceptibility test

Fig. 1. Presence of multiple neutrophils (black arrows), and a foamy cell (red arrow) indicating an acute inflammation process in a milk smear of a postpartum lactating bitch inguinal mammary gland (MGG, x100).

Fig. 2. Presence of engulfed bacteria in an activated macrophage in a milk smear of a postpartum lactating bitch inguinal mammary gland (black arrow) (MGG, x100).

Fig. 3. Presence of erythrocytes (red arrows), evidence of blood traces in the milk, degenerated neutrophils (black arrow), and foamy cells in a milk smear of a postpartum lactating bitch inguinal mammary gland (MGG, x 100).

Fig. 4. Presence of foamy cells (black arrows), erythrocytes, and neutrophils in a milk smear of a postpartum lactating bitch inguinal mammary gland (MGG, x100).

showed resistance only to trimethoprim-sulfamethoxazole, while showing a marked sensitivity to all the other tested drugs, especially to amoxicillin/clavulanic acid. The bitch antibiotic treatment was carried out with the same drug.

Milk pH for the left and the right mammary inguinal glands was 7 and 7.5 respectively. Milk cytology showed the presence of a large number of degenerate neutrophils (Fig. 1 and Fig. 3), erythrocytes (Fig. 2 and Fig. 4), foamy cells (Fig. 3), and activated macrophages with phagocytosis (Fig. 2), in both glands. The laboratory findings were consistent with the clinical signs.

Necropsy and microbiological tests confirmed that the puppy died from systemic infection with central nervous system and lung involvement of

E. coli and *P. aeruginosa* mixed infection, which might have been transferred from the milk to the puppy.

Unfortunately, because the owner ceased to give any more feed-back, we were not able to keep track of the dam's health status.

Milk cytology is an important criterion in establishing the mastitis diagnosis in bitch. Presence of phagocytosis, degenerated neutrophils, macrophage-foamy cells, bacteria and erythrocytes on milk smears is consistent with a positive diagnostic for *Mastitis acuta* (Olson and Olson, 1984; Manuali *et al.*, 2004; Sangha *et al.*, 2011).

However, there are few reports where pH assays are taken into consideration when diagnosing a

mammary gland inflammation episode (Hasegawa *et al.*, 1993; Dziecioł *et al.*, 2006; Akhtardanesh *et al.*, 2013). Dziecioł *et al.*, (2006) findings suggest that mastitis milk has an alkaline mean value of 7, while pseudocyesis scenarios present a mean pH value of 6.7 (Dziecioł *et al.*, 2006).

It is of great importance to keep in mind the bacteriological findings of both dam and pups when trying to establish the cause of death in neonatal mortality cases. In one study, from bitches presenting puppies with neonatal mortality, *E. coli* was isolated in pure culture, in 41.7% of bitch's vagina (Bjurström, 1993).

An important source for infections in newborns (Vela *et al.*, 2006) are potentially pathogenic bacteria such as *Staph. pseudintermedius*, *Staph. aureus*, *Streptococcus canis* and *E. coli*, that are considered to be part of the normal flora in dam's vagina and milk (Milani *et al.*, 2012). However, bitch subclinical mastitis has also been incriminated to cause septicaemia in puppies (Schäfer-Somi *et al.*, 2003). *Streptococcus dysgalactiae* subsp. *dysgalactiae* was isolated from two Great Dane puppies that died from neonatal septicaemia (Vela *et al.*, 2006). In the case described here, presence of *E. coli* and *P. aeruginosa* in both bitch milk and pup, highlights the important role of milk pathogens in neonatal septicaemia.

The present case suggests that *E. coli* and *P. aeruginosa* mixed infection should be considered as differential diagnosis in the cases of *Mastitis acuta* in bitch. Sanitary conditions and close monitoring of neonates are very important factors in the prophylactic management of the postpartum dam. Nosocomial infections or transmission of dog bacterial pathogens to owners have also been reported (Münnich and Lübke, 2004; Rota *et al.*, 2011).

This report presents a comprehensive summary of the clinical signs and diagnostic strategy in a case of *Mastitis acuta* due to *E. coli* and *P. aeruginosa* co-infection in bitch, followed by mammary transmitted infection and mortinatalità in pups. To limit pathogen transmission from bitch to newborn through milk and to prevent neonatal mortality, the use of laboratory assays (i.e. pH, milk cytology and microbiology) can be of great benefit to breeders worldwide.

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