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Case Report

Mastitis Accompanied by Lymphadenitis in a Dog Caused by *Staphylococcus hyicus*

Marina R. Araújo[1], Ingred S. Preis[1], Sílvia A. França[1], Juliana G. Paniago[1], Mirella C. Costa[1], Juliana S. V. Oliveira[1], Roselene Ecco[1]

[1]Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, Universidade Federal de Minas Gerais, UFMG.
Corresponding author: Roselene Ecco. Departamento de Clínica e Cirurgia Veterinárias, Escola de Veterinária, UFMG, 30123-970, Belo Horizonte, MG, Brasil.
E-mail: ecco@vet.ufmg.br

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Abstract

Canine mastitis is an infrequent condition that occurs most commonly in the postpartum period due to ascending bacterial infection. This report aims to describe clinical, pathological and bacteriological aspects of mastitis caused by *Staphylococcus hyicus* accompanied by disseminated superficial lymphadenitis in a dog. In the postpartum period the animal showed clinical signs of prostration, dyspneia, and unresponsivess to environment. At physical examination, hypothermia, dehydration and subcutaneous edema of the limbs were detected. Gross and histologic features were consistent with marked purulent and necro-hemorrhagic mastitis accompanied by lymphangitis, lymphadenitis and thromboembolic pneumonia. Isolation and biochemical identification confirmed the infection by *Staphylococcus hyicus*. This study shows that one must be attentive not only of the risk of uterine infection postpartum, but also of the possibility of mammary gland infection.

Key Words: dog, mastitis, lymphadenitis, *Staphylococcus hyicus.*

Description

Canine mastitis occurs mainly in the postpartum period, but it may occur late in pregnancy and pseudopregnancy of the female dog (6, 4, 26). The main bacterial agents involved are *Escherichia coli*, *Staphylococcus* sp and *Streptococcus* sp (4, 24, 26). Ascending infection is the major cause of canine mastitis, however, traumatic lesions and hematogenous spread of bacteria from other sites of infection may also be involved in the pathogenesis of this disease (6, 24). The acutely affected mammary gland becomes enlarged, firm and edematous and purulent exudate is observed (21). Lymphadenitis can be acute or chronic, suppurative, caseous or granulomatous. It usually occurs when an infectious agent is drained to the lymph node of a distant inflammatory process. Some agents specifically invade both the lymph nodes and vessels. The inflammatory reaction will be determined by the type of the infectious agent. Thus, when the inflammation is due to a pyogenic agent, abscesses may occur (23). Mastitis with disseminated bacterial lymphadenitis and lymphangitis has not been reported in dogs. This report aims to describe clinical, pathological and bacteriological aspects of mastitis caused by *S. hyicus* accompanied by lymphangitis and lymphadenitis in a dog.

A three year old female Pit bull dog presented for examination. The owner reported that it had given birth to seven live puppies and a week after that two dead puppies were expelled. Since the dog gave birth, its appetite became selective. Fetid vaginal discharge was also observed by the owner. The animal was examined and medicated by the veterinarian. On the following day, a large amount of blood was observed in the kennel where the dog spent the night. The animal was unresponsive to medication and died one day later. Necropsy was performed and samples of most organs were harvested and fixed in 10% buffered formalin. Five micron-thick sections stained with hematoxilin and

eosin were prepared from paraffin-embedded tissue blocks using standard methods. Selected tissues (lymph node, mammary gland and lung) were prepared for MacCllum- Good pasture Gram stain. Tissue sections were examined by light microscopy. Mammary glands and lymph nodes were cultured for evidence of bacterial pathogens. Both samples were macerated with 0.85% saline solution and autoclaved sand. Using a sterile platinum loop, the homogenized fluid was collected and properly seeded on blood and MacConkey plates by the exhaustion technique. The plates were incubated aerobically, both at 36 ± 1ºC for 24h and 48 h.

According to the veterinarian, the dog was prostrated when presented to the clinic. In addition, the clinical examination showed loss of skin elasticity and the right thoracic and pelvic limbs were enlarged due to subcutaneous edema. The veterinarian suspected postpartum uterine infection and prescribed a tablet (15m/kg) of sulfa + trimethoprim (Bactrim®) every 8 hours and fluid therapy using Ringer lactate® was done. On the following day the clinical picture worsened. The dog vomited a dark red fluid and a dark red, viscous and fetid secretion flowed from the vulva. The animal did not respond to any external stimuli. At physical examination, all limbs presented subcutaneous edema, there was marked retraction of ocular bulbs, weak pulse and low body temperature. The animal presented with dyspnea, was intubated but died on the following morning.

At necropsy the animal was in good corporal condition. There were linear chronic ulcers on the cranial, ventral and dorsal surfaces of the tongue, measuring approximately 1.0 cm of diameter. Marked subcutaneous edema was observed in the cervical ventral region and all limbs. All mammary glands were firm and diffusely increased in volume. The cut surface was dark red, intercalated with multifocal to coalescing white, soft and necrotic friable areas mixed with purulent exudate (Figure 1). In the right left abdominal and right inguinal mammary glands the lesions were more severe. The skin on these glands had a 10.0 cm locally extensive dark red area. The cut surface revealed multiple 3.0 cm foci of liquefactive necrosis with abundant purulent exudate. The superficial lymph nodes were moderately enlarged. Dark red areas intercalated with liquefactive necrosis and abundant purulent exudate were observed on the cut surface (Figure 2). Loss of normal architecture was observed in some areas. The cervical, axillary and popliteal lymph nodes were the most affected. The medial iliac and retromammary lymph nodes were enlarged with dark red cut surfaces. The lymphatic vessels adjacent to the popliteal and axillary lymph nodes were markedly distended, tortuous and flowed moderate amount of purulent exudate (Figure 3). The uterine horns were extensively distended. The uterine mucosa was diffusely and intensely brown. There were also multifocal, irregular and friable brown red areas indicating recent placental attachment. In the lungs, multifocal, slight firm and prominent dark red areas measuring 0.2 to 0.3 cm of diameter were observed. The kidneys were diffusely yellowish white and slightly firm. On the gastric mucosa there were multifocal erosions and ulcers measuring approximately 0.2 to 0.5 cm of diameter.

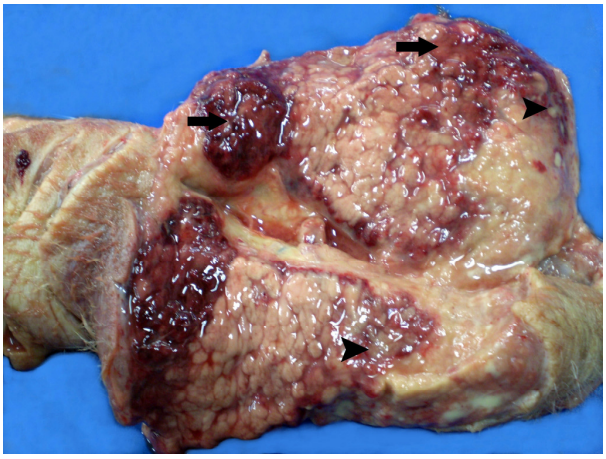

Figure 1 – Mammary gland, dog. Extensive areas of hemorrhagic necrosis (arrows) and liquefactive necrosis (arrow heads).

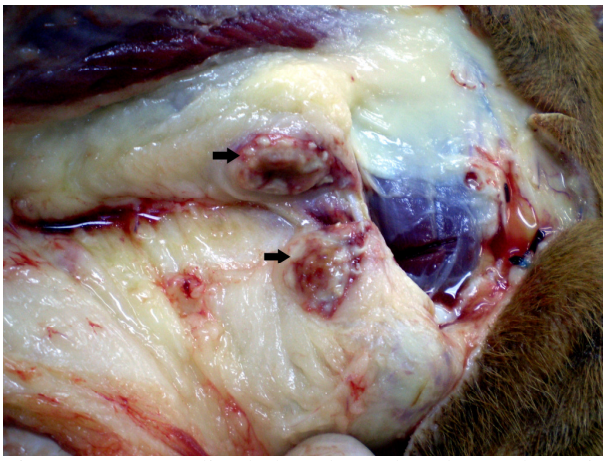

Figure 2 – Popliteal lymph node, dog. There is moderate increase of lymph node volume. At cut surface, multifocal white areas (liquefactive necrosis) oulined by hyperemic line in the cortical and capsular region (arrows) are observed.

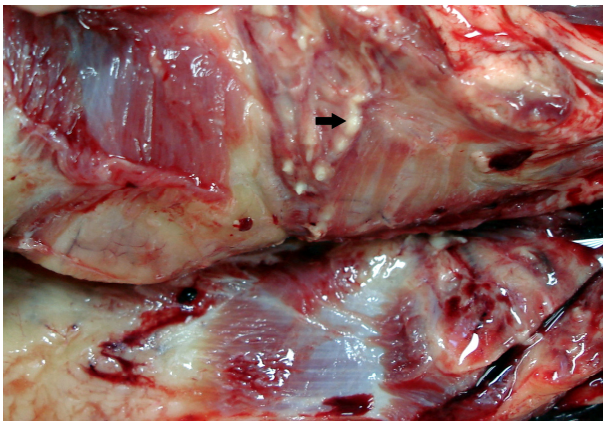

Figure 3 – Axilar region, dog. The lymphatic vessels are tortuous and engorged by purulent exudate (arrow).

At histopathology there was marked neutrophilic and histiocytic infiltrate within luminal ducts and acini of mammary glands. Epithelial necrosis was also observed. Most vessels showed vascular thrombosis (Figure 4). Liquefactive necrosis (Figure 5) was observed in some areas associated with numerous debris and bacterial colonies. Lymph node tissue architecture was distorted by liquefactive necrosis (Figure 6) aligned by debris and neutrophilic infiltration. Numerous bacterial colonies were observed. These lesions characterized marked acute necropurulent lymphadenitis observed in all superficial lymph nodes. Lymphatic vessels corresponding to these lymph nodes showed dilatation by marked infiltrate of neutrophils (purulent lymphangitis). Thrombosis was observed in the lymph nodes and adjacent vessels. Many vessels showed neutrophilic infiltration and bacterial colonies associated with the thrombi. The inflammatory reaction invaded the vessel walls (purulent vasculitis with thrombosis). In the lungs there were multifocal thrombosis and necrosis associated with edema, neutrophilic and histiocytic infiltration. These changes are associated with thromboembolic pneumonia (Figure 7). The MacCllum- Good pasture Gram stain showed numerous intralesional Gram positive cocci in the mammary glands, lymph nodes, lymphatic and blood vessels and in the lungs thrombi. In the kidneys many urinary corpuscles showed increased thickness of the glomerular basement membrane, mesangial matrix and urinary capsule. Interstitial and periglomerular lymphoplasmacytic infiltrate was also observed. Many kidney and liver vessels presented thrombosis.

Primary culture revealed white and medium sized colonies, non-hemolytic on blood agar plates. Gram staining showed GPC (Gram Positive Cocci). The colonies were selected and subcultured on blood agar in order to produce a large number of bacterial colonies for the biochemical tests. These tests indicated catalase (+), Dnase (+), coagulase (+), maltose (-) and mannitol (-) colonies. Based on these characteristics, the cultured agent was identified as *S. hyicus* coagulase-positive from mammary glands and lymph nodes samples.

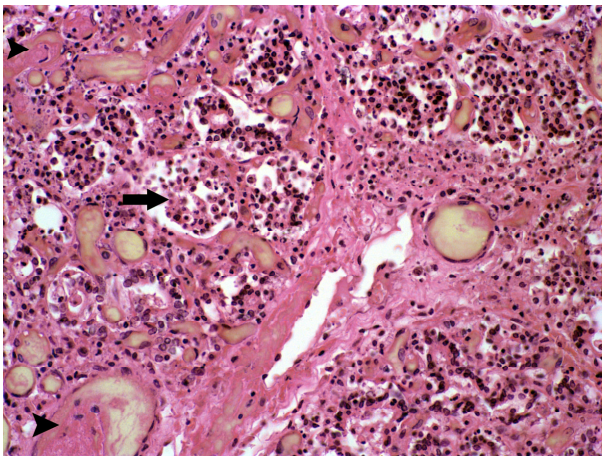

Figure 4. Mammary gland, dog. Epithelial necrosis (arrow) associated with vascular thrombosis (arrow heads). HE. 40x.

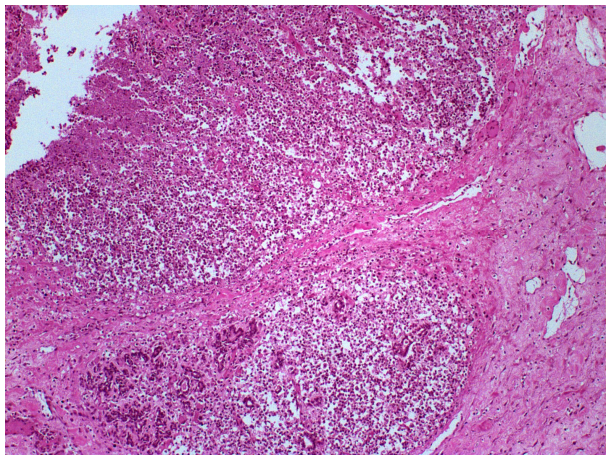

Figure 5. Mammary gland, dog. Most of glandular parenchyma is replaced by extensive necrosis and cellular debris. HE. 4x.

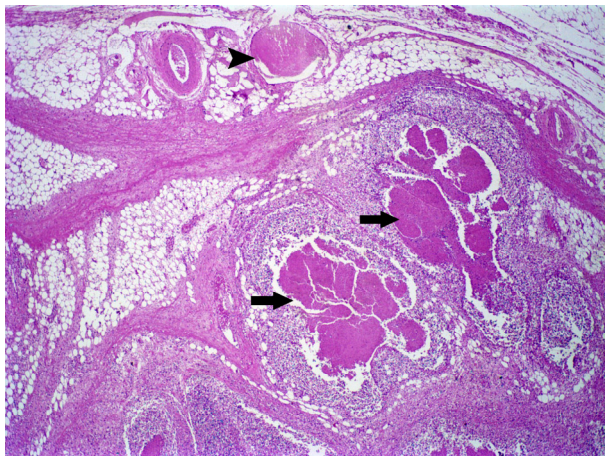

Figure 6. Popliteal lymph nodes, dog. Areas of liquefactive necrosis (arrows) in the cortical areas with loss of lymphoid follicles can be observed. There is also vascular thrombosis (arrow head) in the capsule vessel. HE. 4x.

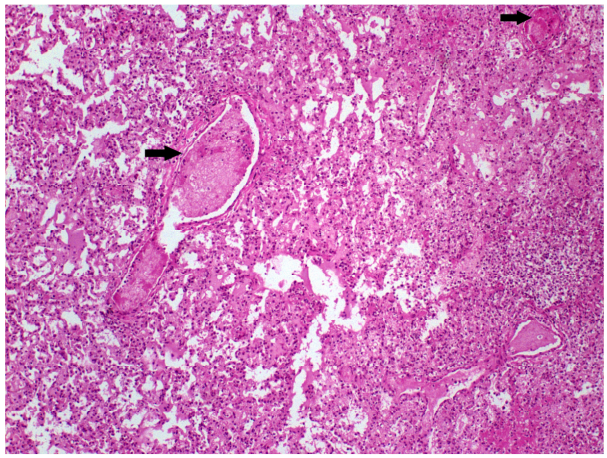

Figure 7. Lung, dog. Thromboembolic pneumonia characterized by thrombosis associated to edema, necrosis and neutrophilic infiltration surrounding thrombotic vessels. HE. 10x.

The gross lesions were compatible with acute hemorrhagic and necropurulent mastitis accompanied

by marked necropurulent lymphadenitis, lymphangitis and thromboembolic pneumonia. The histologic changes, isolation and biochemical tests allowed association of these lesions with *S. hyicus* infection.

The mammary gland infection in dogs generally occurs postpartum and can be classified in subclinical or clinical mastitis. Animals with clinical mastitis present changes in milk aspect, increased mammary gland volume and temperature, pain at palpation and erythema on the skin around mammary glands (24, 26). In the present study, information about health of mammary glands was not found in the clinical history. Systemic signals like depression, fever, anorexia, vomit and dehydration can be detected on more severe cases of mastitis (4). The dog in this study presented similar signs a few days postpartum, indicating systemic involvement and severity of its condition. The clinical mastitis can be focal or diffuse in one or involving multiple mammary glands (4). In this report, the inflammatory reaction involved all mammary glands but the right cranial abdominal and inguinal glands were more severely involved. Marked inflammation can be related to several factors, as the type of microorganism, time of infection, milk stasis and immunologic depletion of the host (12). Moreover, acute mastitis can result in gangrenous mastitis and sepsis (10). In the subclinical mastitis the appearance of milk is normal, however the undergrowth of puppies is an important aspect to be considered (26). The diagnosis is made by cytological and bacteriological examination of milk (20).

The incidence of subclinical mastitis in bitches in lactation appears to be high. In one study, bacteria were found at 10 to 50% of canine milk samples tested. Nevertheless, bacteria isolated from milk of bitches with subclinical mastitis do not always cause infection in puppies. In some cases, toxins or bacteria in milk may predispose neonates to bacterial or viral infections due to their lower level of immunity (20). The main cause of mastitis is bacterial infection and the main agents involved are *Escherichia coli*, *Staphylococcus sp.* and *Streptococcus sp*. (4, 24, 26). The genus *Staphylococcus* is characterized by Gram positive cocci, facultative anaerobic, catalase positive and belong to the Micrococcaceae family. It represents part of normal microbiota of skin and mucosa of respiratory, digestive and urogenital tracts of mammals and birds (25). Coagulase-positive strains of *S. hyicus* have been isolated from dogs with skin lesions (15), external otitis or infections of the urinary tract (5) and from dogs and cats with bone infections (9).

The pathogenicity of *S. hyicus* appears to be related with the production of exfoliative toxins. Recent cloning and sequencing demonstrated the existence of five toxins designated as ExhA, ExhB, ExhC, and ExhD(2). The ExhA toxin was also found in an isolate from a cow with mastitis in Japan (3).

Few studies reported the isolation of *S. hyicus* from mammary glands of domestic animals (3,19) but specific toxic effects of this bacteria in the epithelial cells of mammary glands have not been described. Acinar and ductal epithelial cell necrosis observed in the mammary glands of the animal in this study could be the result of the production and effect of these toxins.

The necropurulent bacterial lymphangitis and lymphadenitis probably occurred secondary to mammary infection. Isolation of the same bacteria (*S. hyicus*) was done in both organs (mammary gland and lymph nodes). The lymph node lesions possibly resulted in impairment of lymphatic drainage with marked subcutaneous edema in the limbs and in the ventral cervical region. In domestic animals, report of mastitis with lymphadenitis and lymphangitis has not yet been found. In dogs, lymphadenitis results from granulomatous infection and therefore has a chronic course. The agents involved included fungi such as *Histoplasma capsulatum* (23), *Cryptococcus neoformans* (1) and *Monocillium indicum* (13), bacteria like *Mycobacterium bovis*, *Mycobacterium tuberculosis* (22) and *Bartonella henselae* (16) and protozoans such as *Leishmania* sp. (23). Another cause of lymph node lesions is the pyogranulomatous lymphadenitis observed in the canine juvenile cellulitis, a rare disease characterized by pyogranulomatous inflammation of the skin and/or subcutaneous of face, in the ear and in the lymph nodes but the etiology is unknown (11). A similar condition to canine juvenile cellulitis has also been reported in adult animals (17).

The uterus gross examination revealed changes compatible with recent placental attachment (uterus postpartum). Apparently, uterine infection was not presented. However, histopathology evaluation of uterine mucosa was not made and, thus, it was not possible to rule out puerperal endometritis. Metritis is an acute uterine infection that occurs postpartum. It may be associated with abortion, fetal infection, placental or retained fetuses, dystocia, obstetric manipulation or ascending infection when the female inhabits an environment with poor sanitary conditions (26).

The gross and histologic changes in the lungs were consistent with thromboembolic pneumonia. This condition results of establishment of bacterial emboli from inflammatory reaction in other organs, which reach hematogenously the lungs (8). The thrombi observed in the mammary glands and lymphatic system probably was the cause of thromboembolic pneumonia in the animal in this study. Pulmonary samples were not harvested for culture, but MacCluum-Good pasture Gram stain of pulmonary tissue showed Gram positive cocci identical to those observed in the lymph nodes and mammary glands. The bacteremia and/or septicemia probably caused the clinical picture and determined the death of the dog.

Canine mastitis has favorable prognosis when there is early diagnosis and appropriate treatment is performed (4). The treatment of acute mastitis consists of systemic antibiotic therapy, hot water compresses and massage of mammary glands (10). Drainage and surgical debridement are needed when there are abscesses and gangrenous mastitis (4).

Mastitis studies are more frequent in the cow because this is one of the most common infectious

diseases in economically important livestock and dairy cattle worldwide (18). Canine mastitis is an infrequent condition (21) and it does not cause as much interest as mastitis in farm animals. Few reports (12, 7, 14, 19), an experimental study (24) and a retrospective study (20) of this disease in dogs were found. Mastitis can be a risk of death in bitches, underdevelopment of puppies and sometimes mortality due to neonatal sepsis. Thus, veterinarians should be aware not only of postpartum uterine infection but also of mammary gland risk infection.

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