



## Case report

# Acute Mastitis Caused by *Pasteurella multocida* in a Goat: Clinicopathological and Microbiological Findings

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## Abstract

Mastitis is still an important disease causing heavy and irreparable economic losses in the dairy industry. We describe the clinicopathological and microbiological features of an unusual acute septic mastitis caused by *P. multocida* in a 3-year-old dairy goat. At necropsy, the enlargement of the left udder half with hard consistency, the extensive areas of necrosis and the accumulations of pus (suppurative exudate) in the cut surface of the affected udder half were observed. Histopathological findings of the affected udder showed severe degeneration and necrosis of the alveolar epithelium and an extensive infiltration of neutrophils within the alveolar lumen. Most of the mammary secretory alveoli were devoid of milk and diffusely filled with basophilic bacterial colonies positively stained by Brown and Brenn method in red color. Microbiological examination of the affected udder revealed *P. multocida*. It is concluded that *P. multocida* should be considered as an etiological agent of acute septic mastitis in goats.

**Key Words:** Mastitis, Goat, *P. multocida*, Histopathology, Microbiology.

## Introduction

Mastitis is still one of the most important diseases with serious and heavy economic losses in the dairy industry worldwide because it can reduce milk quality, production and suitability for human consumption and eventually limit the profitability of dairy farmers (14, 16). Mastitis is mainly caused by bacteria in the dairy small ruminants that invade the udder and multiply in the mammary gland tissue (2). However, several researchers have reviewed the knowledge of mastitis such as the main causes, the role of intramammary pathogens, the epidemiological aspects and the approaches to control in dairy small ruminants (2, 3, 8, 9).

*P. multocida* is a non-spore forming, non-motile, small Gram-negative rods or coccobacilli of the family *Pasteurellaceae* (19). So far, it has been isolated from the respiratory tract and digestive system of human beings, birds, domestic pets, livestock, and many wild animals (19, 20). *P. multocida* is an important animal pathogen which causes mastitis (rarely), pneumonic pasteurellosis

(shipping fever) in cattle and enzootic pneumonia complex in calves (serogroup A), hemorrhagic septicemia (HS) in cattle (serogroup B & E), fowl cholera in poultry (serogroup A & F), atrophic rhinitis in swine (serogroup A & D) and snuffles in rabbits (serogroup A) (6, 17). It is also a commensal of both human and animal respiratory tracts (19).

The annual incidence of clinical mastitis in small ruminants is generally lower than 5%, but this incidence can increase sporadically (3, 8). On the other hand, no report of mastitis caused by *P. multocida* was found in the review of goat medicine literatures. Therefore, this report describes the clinicopathological and microbiological features of acute septic mastitis caused by *P. multocida* in a goat.

## Case report

A 3-year-old lactating doe with history of sudden death, kidding 2 months ago and suspicious of acute mastitis was referred from a locally raised herd. On gross

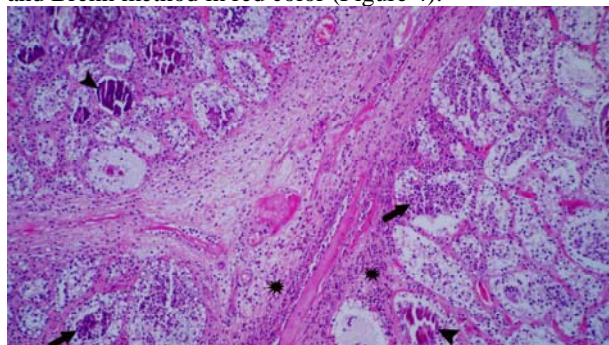
examination, the left udder half was enlarged with hard consistency and the cessation of the milk flow. The affected udder half had diffuse discoloration with sloughing of the skin and hemorrhage around the teat (Figure 1). There was no discharge, redness or any other gross lesions in the right teat. Further examination revealed extensive areas of necrosis, hemorrhage and accumulations of pus (suppurative exudate) in the cut surface of the affected udder half. Also, there were severe gross signs of septic shock such as severe petechial hemorrhages and congestion on the epicardium, lungs and serosal surfaces of the visceral organs.



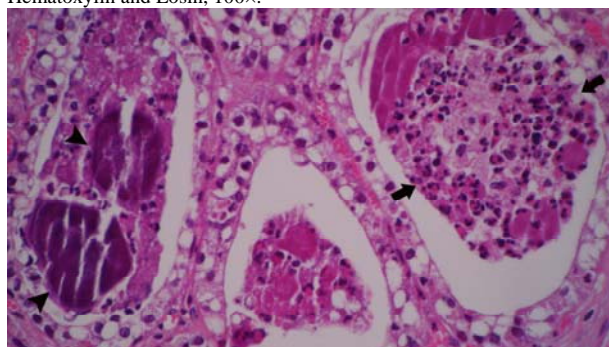
**Figure 1.** Goat, udder. Severe enlargement of the left half with diffuse discoloration, sloughing of the skin (thick arrow) and hemorrhage around the teat. There was no redness or any other gross lesions in the right teat (thin arrow).

An udder tissue sample was taken and divided into two parts. For histopathological studies, the first part was immediately fixed in 10% neutral buffered formalin (pH 6.8) and processed routinely, embedded in paraffin wax, sectioned in 5-6  $\mu$ m thickness, stained with hematoxylin and eosin (HE) and Brown and Brenn methods and finally examined using a light microscope. Histopathological examination of the mammary parenchymal tissue specimen revealed hyperemia, hemorrhage, severe cell swelling, vacuolar degeneration, necrosis of the alveolar epithelium and an extensive infiltration of neutrophils within the alveolar lumen, intralobular and interlobular connective tissue of the affected udder representing acute purulent necrotizing mastitis (Figure 2 and 3). Interlobular connective tissue of the mammary gland was mild to moderately expanded by edema fluid, fibrin exudates and thrombosis within the blood vessels (Figure 2). Most of the mammary secretory

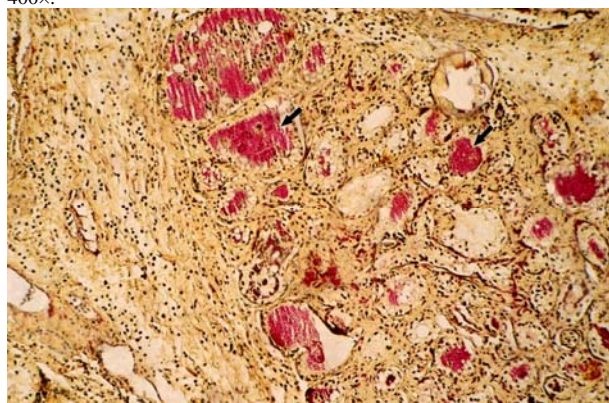
alveoli were devoid of milk and diffusely filled with basophilic bacterial colonies positively stained by Brown and Brenn method in red color (Figure 4).



**Figure 2.** Goat, mammary gland. Extensive infiltration of neutrophils in alveolar lumen (arrows), intralobular and interlobular connective tissue of the affected half (asterisks). Basophilic bacterial colonies and thrombosis were evident within the secretory alveoli (arrowheads) and the blood vessels of the interlobular connective tissue, respectively. Hematoxylin and Eosin, 100 $\times$ .



**Figure 3.** Goat, mammary gland. Severe cell swelling, infiltration of neutrophils and cell debris of necrotic alveolar epithelium (arrows), and basophilic bacterial colonies (arrowheads). Hematoxylin and Eosin, 400 $\times$ .



**Figure 4.** Goat, mammary gland. Moderate to severe presence of bacterial colonies in red color in the alveolar lumen (arrows). Brown and Brenn, 400 $\times$ .

The second part of the udder tissue sample was transported to the laboratory and immediately cultivated in 5% sheep blood and MacConkey agar medium in sterile conditions. These streaked plates were incubated under aerobic conditions at 37 $^{\circ}$  C for 24-48 h. After initial bacterial colonies growth on these plates, wet microscopic

smears of suspected colonies were prepared and then stained with a Gram technique for tissues in order to identify morphological properties of the bacteria. On bacteriological examination, round, smooth, non-motile and non-hemolytic colonies were observed on the blood agar plate. Growth test on MacConkey agar medium was negative. Finally, according to macro- and microbiological morphology as well as biochemical characteristics by Quinn et al. (17), *P. multocida* was identified.

### Discussion

Mastitis, as the multiple etiological and the most economically important disease of the mammary gland tissue, is prevalent in dairy ruminants especially cattle (11, 21). The severity of goat mastitis can be affected by numerous factors including farm conditions, breed, old age, multiparity and stage of lactation (2, 15). Ali et al. (1) stated that high milk production for long periods, multiple numbers of parturitions and reduced immune responses can induce stress and favor conditions for infectious agents growth. Poor milking management and sanitary conditions, lack of therapeutics and control strategic plans, traumatic injuries and cutaneous ecthyma can lead to the development of mastitis (1, 2). Thus, mastitis can cause colossal and significant economic damages to the dairy goat's production by increasing the culling of morbid animals and their milk.

Mastitis is most commonly caused by infection with pathogenic bacteria (intramammary infection) that affects milk quantity and quality, but may also be due to fungi, teat injuries and less commonly allergy and neoplasia (2, 4, 9, 12). Several bacterial pathogens have been associated with mastitis in goats. *Staphylococcus* spp. (*S. aureus*) is recognized as the most common etiological agent of caprine mastitis, followed by minor occurrence of Enterobacteriaceae, coagulase-negative staphylococci (CNS), *Mannheimia* (formerly *Pasteurella*) *haemolytica*, *E. coli*, *Clostridium perfringens*, *Streptococcus* spp., *Pseudomonas* spp., *Mycoplasma* spp., *Nocardia* spp. and *Corynebacteria* (2, 9, 18). The mammary gland tissue is usually sterile, however, the flow of milk can be halted by acute inflammatory exudates and injuries caused by bacteria (11).

In the present case, the clinicopathological and microbiological features of acute mastitis due to *P. multocida* are described in a goat. The tissue Gram (Brown and Brenn) staining of the affected udder tissue sections confirmed red bacterial colonies demonstrating the presence of Gram-negative bacteria. The histological lesions of the present *Pasteurella* spp. mastitis including diffuse and severe suppuration characterized by marked exudation of neutrophils were more severe at the alveolar lumen than in the ducts. These histological changes along with the vacuolation and exfoliation of the alveolar epithelial cells without chronicity features such as infiltration of mononuclear inflammatory cells in the interstitial and fibrous connective tissue replacing the

glandular parenchyma revealed the acute process of this disease. To the best of our knowledge, *P. multocida* infection of the mammary gland tissue was not been reported in goats.

The Gram-negative bacteria *P. multocida* is a commensally common organism of the tonsils and nasopharynx of healthy sheep and goats and it is considered as a primary pathogen, such as in hemorrhagic septicemia (HS) in cattle and fowl cholera in birds, and a secondary invader, for example in cases with pneumonic lesions (7, 10). Most infectious agents such as small (bacteria), large (leeches) pathogens and foreign material can enter the mammary gland in an ascending fashion via the papillary duct. Some instances including the retroviruses of caprine arthritis and encephalitis, ovine maedi-visna, and *Mycoplasma* spp., home to the mammary gland tissue from systemic infection, but their number is small (11). There is the possibility of transmission of the pathogenic organisms such as *S. aureus* and *Mannheimia* species from the contaminated skin of the teat or oral cavity of the lambs to the ewe's udder following lamb sucking (5, 13). It is possible that the causative organism hide in the mammary gland tissue in a latent form and eventually in the case of traumatic teat injuries, the bacteria become infectious and produces the functional and structural abnormality in the affected udder and clinical mastitis (18). It appears that the present isolated bacteria persist either in the environment or within the oral cavity of kids and directly enter the orifice of the teat canal following sucking or after the entry to the body and the establishment in the target organs being introduced hematogenously to the udder.

The present case revealed that *P. multocida* bacteria should be considered in the differential diagnosis of causative agents of acute mastitis in goats. On the other hand, the main reasons for the occurrence of clinical mastitis are not known but it seems that environmental contaminations such as poor hygiene practices were involved in the development of the disease in this particular dairy goat. Bergonier et al. (2) stated that environmental factors like humidity, wet bedding, forage, housing, feedstuffs, clusters, equipments, human and other animals are the major determinants of mammary gland pathology in dairy goats. Caprine and ovine mastitis should be considered as an important and critical economic problem likewise in dairy cows because of the limitations of milk and meat production, mainly in the geographic areas where goats and sheep are the dominant species of livestock.

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