

## **PASTEURELLA MULTOCIDA MASTITIS IN COW – CASE REPORT**

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### **Abstract**

*Pasteurella (P.) multocida* is a heterogeneous species of Gram-negative bacteria which are common commensals of the upper respiratory system of various mammal and bird species, but are also opportunistic contagious zoonotic pathogens which cause a wide spectre of infections in domestic animals and humans. *P. multocida* is a rare cause of mastitis in dairy cows. The source of infection mainly remains unknown, mastitis usually is acute, and the therapy by intramammary administration of antibiotics does not lead to satisfactory results. Lethality is possible due to presence of endotoxins in blood. Literature data on *P. multocida* mastitis in dairy cows is particularly scarce, which is why such a case is described in the current work, with past medical history, clinical findings, laboratory diagnostics and therapeutic approach.

**Key Words:** *Pasteurella multocida*, isolation, bovine mastitis, therapy

### **CASE PRESENTATION**

A case of *Pasteurella (P.) multocida* bovine mastitis occurred on a dairy farm with 14 cows, out of which 10 were milked. The annual average number of somatic cells in bulk milk samples was about 170,000/mL. The diseased cow was a red Holstein in its first lactation. On day eight post partum, when the first few squirts of milk were stripped from the udder into a strip cup before milking, flakes and clots in the milk from the left hind mammary quarter were noticed. No changes in the size or tissue consistency were observed in the affected quarter. The numbers of somatic cells in milk were detected

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automatically, using a Fossomatic™ FC counter (Foss, Denmark), and in milk from the affected quarter, was 2,200,000/mL. A registered veterinary surgeon treated the cow with intramammary application of a suspension of tetracycline, neomycin, bacitracin and prednisolone (Mastijet forte, Intervet International B.V.) at 12-h intervals for 2 days, but without any success. The somatic cell count following the treatment remained high – 1,800,000/mL, and the clots in the milk stayed visible.

Milk samples for bacteriological examination were collected from each quarter individually using an aseptic technique. From each milk sample 50 mL was taken and inoculated on two plates of Columbia blood agar base (CM0331, Oxoid, Basingstoke, UK) with 5% defibrinated ovine blood, MacConkey agar (CM0007, Oxoid, Basingstoke, UK) and Sabouraud dextrose agar (CM0041, Oxoid, Basingstoke, UK). The inoculated plates were incubated aerobically and in 5-10 per cent CO<sub>2</sub> conditions (blood agar) for 24-48 h (blood and MacConkey agar) or 5-7 days (Sabouraud dextrose agar).

After 24 h of incubation in aerobic and microaerophilic conditions, on the blood agar inoculated with milk from the diseased mammary quarter, the growth of relatively large, mucoid, greyish, non-haemolytic colonies, with a characteristic sweetish odour was observed. There was no bacterial growth on MacConkey agar. On Sabouraud dextrose agar, there was no visible growth of medically important yeast and mould. No clinically important species of bacteria or fungi were isolated from milk originating from the other udder quarters. Microscopic examination of Gram-stained slides revealed small, Gram-negative, bipolar-staining coccobacilli. The isolate was tested for catalase, oxidase, indole and urease production (Christensen urea agar), and fermentation of glucose, lactose, sucrose, xylose, maltose and mannitol (according Quin et al., 1998).

The isolated species was identified as *P. multocida* according to the following criteria: the colonies were greyish in colour, mucoid and with a sweetish odour, did not produce haemolysis, did not grow on MacConkey agar, and were Gram-negative bipolar-staining coccobacilli, oxidase and catalase positive, capable of producing indole, and fermenting glucose, sucrose and mannitol; they were urease-negative and did not produce acid from lactose, xylose or maltose.

The susceptibility of the isolate to antibiotics was tested with standard disk-diffusion tests on Müller Hinton agar, and preparations based on penicillin were recommended for the treatment of the infected quarter. The treatment was performed with intramammary infusion of 0.5 L of physiological saline solution in which 8,000,000 IU of penicillin was dissolved and 8 ml of Dexaforte (Intervet International B.V.), once a day, on 5 consecutive days. Seven days after the treatment, the California mastitis test was negative, and 14 days after the treatment, the number of somatic cells in the milk from the quarter was 126,000/mL.

The microbiological examination of milk sampled from the treated quarter was repeated 14 days after the end of the therapy but *P. multocida* was not isolated, which confirmed the infection was microbiologically resolved.

## DISCUSSION

*P. multocida* are often found as part of the normal microbiota of the oral, nasopharyngeal, and upper respiratory tracts. However, under predisposing circumstances, the organism is an opportunistic pathogen, the aetiological agent of infections of various tissues in immunosuppressed animals. The infections are usually endogenous, and develop following the dispersion of the pathogen via the blood or lymph system, whilst the exogenous transmission is possible via aerosols or by direct contact. In addition, *P. multocida* can also cause occasional, but severe mastitis. The incidence of *P. multocida*-mastitis is typically very low: 0.21% (Ribeiro *et al.*, 2010) or 1.25% (Langoni *et al.*, 1991). Mastitis can be subclinical (Wilson *et al.*, 1999), or cause only visible macroscopic changes in the appearance and consistency of milk: the milk consistency turns watery, with yellow clots (O'Sullivan & Bauer, 1971), thick, creamy-yellow, viscous secretion, sometimes with a foul odour (Swartz & Peterson-Wolfe, 2016). In more severe cases, udder oedema appears and systemic signs of infection such as fever, tachycardia and dyspnoea develop (Ribeiro *et al.*, 2010).

The route of infection generally remains unknown. Udder infection can result from haematogenous or lymphogenic dissemination of *P. multocida* from the respiratory tract (Swartz & Peterson-Wolfe, 2016). In addition, it can occur via the teat canal or minor lesions on the mammary papilla, from the oropharynx of infected, suckling calves (Jubb & Kennedy, 2013). The infected cow can become a source of infection for other individuals in the herd, since the pathogen can be transmitted by contact, especially while milking, and through milking equipment (Ribeiro *et al.*, 2010). Unless an infected cow is excluded from the herd, which is of the utmost importance for epidemiological reasons, it should be milked with a separate unit, or separated and milked last (Swartz & Peterson-Wolfe, 2016). Cows with teat injuries are at a higher risk of developing mastitis. Due to the capability of *Pasteurella* species to readily multiply in injuries, a means of prevention is certainly avoiding teat injuries. New infections can occur at any time during lactation.

There are multiple factors thought to facilitate the survival of *P. multocida* in the host: iron acquisition mechanisms vital for *in vivo* growth, membrane lipopolysaccharide that confers resistance to complement, a capsule which renders phagocytosis impossible, surface components providing adherence properties, extracellular matrix-degrading enzymes (e.g. hyaluronidase, neuraminidase and proteases), which help in colonization and/or dissemination (Wilson & Ho, 2013).

In the present case, the infection of the left hind udder quarter caused by *P. multocida* was cured successfully by intramammary administration of penicillin and corticosteroid dissolved in a large volume of isotonic saline solution. Bovine mastitis caused by bacterial species of *Pasteurellaceae* family are generally considered to be refractory to treatment with several different antibiotics (O'Sullivan *et al.* 1971; Andersen & Jensen, 1997, Petersson-Wolfe *et al.*, 2013; Swartz & Peterson-Wolfe, 2016), but there are also reports of spontaneous cure (Wilson *et al.*, 1999). Typically, for the treatment of bovine

diseases caused by *P. multocida*, penicillin G, tetracycline antibiotics or sulphonamides in combination with trimethoprim, florfenicol, gentamycin, streptomycin or enrofloxacin are recommended (Ribeiro et al., 2010).

Multiple resistance to antibiotics has been noticed in *P. multocida* isolated from dairy cows and might be directly connected to the use of antibiotics in mastitis therapy. Although plasmids are not necessarily present in *P. multocida*, they have been detected in isolates originating from various sources. Their size usually varies from 1 kb to 6 kb, but they can measure as much as 100 kb. Most of them confer resistance to one or more antibiotics, most often to  $\beta$ -lactams, tetracycline, chloramphenicol, streptomycin or sulphonamides (Wilson & Ho, 2013). Given that many plasmids responsible for antibiotic resistance are exchangeable not only among the species of the *Pasteurellaceae* family, but can also be transferred to other Gram-negative bacteria (Wilson & Ho, 2013), it is not surprising that there is a growing problem of resistance.

The empirical approach to the treatment of this particular mastitis case in a cow, which included the administration of tetracycline, neomycin, bacitracin and prednisolone suspension, proved inadequate. Although *P. multocida* is a rare causative agent of mastitis in cows, it does occur sometimes as a pathogen leading to the inflammation of the udder. *Pasteurella* mastitis is considered difficult to be cured with usual antibiotic therapy. Thus, the treatment was modified and a large volume of penicillin and corticosteroid solution was used to enhance the contact surface of the antibiotic and the udder tissue and prevent endotoxic shock. Hereby, we would like to emphasize the necessity of aetiological diagnosis in each case of mastitis in dairy cows, and of the use of therapy based on the results of pathogen susceptibility testing to antimicrobials.

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### **Declaration of conflicting interests**

The authors declare the absence of financial or personal relationship with any people and organizations which could bias or inappropriately influence the content of the presented work.

### **Authors' contributions**

DM carried out the microbiological laboratory diagnostics and antimicrobial susceptibility testing of the isolated and identified bacteria, and wrote the manuscript.

DB and DT collected milk samples for analyses, treated the diseased cow and monitored the health of the animals on the farm where the disease occurred.

NA critically reviewed the literature, the manuscript and ensured that its content, language and form met the criteria for publishing.

DM read and approved of the final version of the manuscript.

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## **MASTITIS KOD KRAVE IZAZVAN BAKTERIJOM *PASTEURELLA MULTOCIDA***

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### **Kratak sadržaj**

*Pasteurella multocida* je heterogena vrsta gram negativnih bakterija koje su uobičajeni komensali prednjih partija respiratornog sistema različitih vrsta sisara i ptica, ali i oportunistički, kontagiozni i zoonotski patogeni, koji izazivaju širok spektar infekcija kod domaćih životinja i ljudi. *P. multocida* spada u retke uzročnike mastitisa mlečnih krava. Izvor infekcije za mlečnu žlezdu uglavnom ostaje nepoznat, mastitis obično ima akutni tok, a terapija intramamarnom aplikacijom antibiotika ne daje zadovoljavajuće rezultate. Letalni ishod je moguć usled razvoja endotoksemije. U literaturi ima veoma malo podataka o mastitisima mlečnih krava čiji je uzročnik *P. multocida*, zbog čega u ovom radu opisujemo jedan takav slučaj, uz prikaz osnovnih anamnestičkih podataka, kliničke slike, laboratorijske dijagnostike i terapijskog pristupa.

**Ključne reči:** *Pasteurella multocida*, izolacija, mastitis krave, terapija