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RESEARCH ARTICLE

CORYNEBACTERIUM PSEUDOTUBERCULOSIS INFECTION IN HOLSTEIN DAIRY CATTLE IN VAN PROVINCE

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ABSTRACT

C. pseudotuberculosis is a Gram-positive, pleomorphic, intracellular, immobile, and facultative anaerobic bacterium which may cause various infections mainly in sheeps and goats, and at a lesser degree in humans, horses, camels, pigs, and buffalos. In this study, 15 sick cattle, raised in a farm within the Van province, were included. Clinical assessments of the cattle showed various pyogranulomatous skin lesions along with inflammation in the udders and joints. *C. pseudotuberculosis* was isolated and identified from the samples obtained from the milk and abscess content. Antibiogram test showed that the isolated pathogens were sensitive to penicillin G (100%, n=15), erythromycin (100%, n=15), ampicillin (100%, n=15), tetracycline (66.7%, n=10) , and clindamycin (60%, n=9); while they were resistant to cefazolin (100%, n=15) and cefoxitin (100%, n=15). Thus, this study was the first which determined C. pseudotuberculosis infection, a rare entity in cattle, in dairy cattle raised in the Van province.

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INTRODUCTION

C. pseudotuberculosis is a Gram-positive, pleomorphic, intracellular, immobile, and facultative anaerobic bacterium (Yeruham *et al*., 2003; Ipek *et al*., 2012). This bacterium is categorized in two groups relative to its ability in nitrate reduction. *C. pseudotuberculosis* infecting sheeps and goats is negative in biotype nitrate reduction test, while *C. pseudotuberculosis* infecting horses gives positive result with the same test. On the other hand, although *C. pseudotuberculosis* biotype infecting cattle often reduces nitrate, there are studies reporting the isolation of biotypes that do not reduce nitrates (Barakat *et al*., 1984; Yeruham *et al*., 1996). An exotoxin named as phospholipase-D (PLD) is reported to be present in the cell wall of *C. pseudotuberculosis* which is thought to underpin the pathogenicity of this bacterium (Biberstein *et al*., 1971; Hodgson *et al*., 1999; Dorella *et al*., 2006). While *C. pseudotuberculosis* infection is mainly seen in sheeps and goats, it may also lead to infections in humans, horses, cattle, camels, pigs, and buffalos (Peel *et al*., 1997; Silva *et al*., 2011; Sood *et al*., 2012).

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The *C. pseudotuberculosis* infection manifests itself in various forms in cattles such as pyogranulomatous, abscess formation, ulcerative lymphangitis, mastitis, and visceral forms (Yeruham *et al*., 2003; Aroch *et al*., 2003). Although the pathogen spreads in all seasons, it is more commonly encountered in the summer. The mosquitos occuring particularly during hot months have been reported to play an important role in the transportation and spreading of the disease (Yeruham *et al*., 2003; Addo, 1983). Other significant factors involved in the infection are media infected with the pathogen and environmental conditions (Augustine and Renshaw, 1982). Moisture and temperature of the environment are of utmost importance for the survival of *C. pseudotuberculosis*. The pathogen may be commonly found in the stables due to high moisture levels of wet grounds. The pathogens adhere to the wet skin of the animals and multiply after invading the epidermis. Subsequently, it spreads to the environment again by the purulen fluid draining from the ulcerative granulomatous lesions on the skin (Dorella *et al*., 2006). In this study, we aimed to investigate the presence and pathogenicity of *C. pseudotuberculosis* in cattle raised in Turkey, while also targeting to inform field veterinarians, various institutions figting against the disease, and scientists performing researches on this pathogen.

MATERIAL AND METHODS

A total of 15 Holstein cows were determined to be sick after their internal and external examinations in a farm housing 160 Holstein dairy cattle in the Tusba district of Van province. In cases with a moderate or severe course, microbiological aspirates of abscesses were taken from the granulomatous and lymphangitis lesions. In addition, subsequent to cleaning the udders of 3 cows suspected of having mastitis with 70% alcohol, the milk samples were put into sterile containers. The aspirates of abscesses and milk samples were transported to the Faculty of Veterinary Medicine Microbiology Laboratory under cold chain. Smears were prepared directly from the aspirates of abscesses and stained with the Gram method which revealed the presence of Gram-positive, rod-shaped, widely dispersed, and pleomorphic bacteria. The isolation of the causative agent was performed using 5% sheep blood agar (Salubris), MacConkey agar (Salubris), and Bile esculin agar (Salubris). The petri dishes containing the samples were incubated in an aerobic environment for 48-72 hours. The preparation made from the growing colonies were treated with Gram staining again. The identification and antibiogram test were carried out using BD Phoenix automated microbiological system (Becton Dickinson, US), biochemical tests (nitrate test, maltose test, glucose test, sucrose test), and CAMP test. Reptopen® (benzyl penicillin procain, dihydrostreptomycin sulfate), selected based on the resistancy/sensitivity test results of the cattle diagnosed with caseous lymphangitis, was applied via intramuscular route at a dose of 5 ml/100 kg for 7 days. The cattle diagnosed with acute mastitis received Tetra-Delta® (procain penicillin G, novobiocin, neomycin, dihydro streptomycin sulfate, prednisolone) at a dose of 10 ml applied to each udder once a day via intramammary route for 5 days.

RESULTS

We were informed about an outbreak among the Holstein dairy cattle raised in the Tusba district of Van province. The outbreak had first occurred about 25 days ago and continued spreading to a new cattle each day, and had already killed two cattle. Internal and external examinations revealed that 15 animals were sick. Six of the sick cattle were not able to stand on their own and were lying on the ground, however they were able to eat and drink. The remaining 9 sick animals were able to walk, albeit in a limited fashion. General examination among the sick cattle demonstrated acute and chronic swellings in various parts of the body, particularly in the subcutaneous area and joints; the animals with acute swelling had elevated body temperature along with increased respiratory and heart rates. Moreover, 3 animals exhibited redness, edema, and elevated temperature in the mammary tissue. In addition, the maintenance and feeding conditions in the livestock barn were observed to be remarkably poor. Physical examination of the sick animals showed a moderate-to-severe disease course. They had emaciation; swelling in various joints of the body, particularly in the joints of the hind limbs; and abscess formation in the subcutaneous areas of the caudal parts of the body (Fig 1). These abscesses were observed to be perforated in some sites, with a granulomatous purulent material leaking out. In more severe cases, the subcutaneous abscesses were found to have spread into the deeper tissues, even into the muscle tissue and through the lymph vessels. Thus, some cases had lymphangitis in the ventral abdominal lymph vessels.

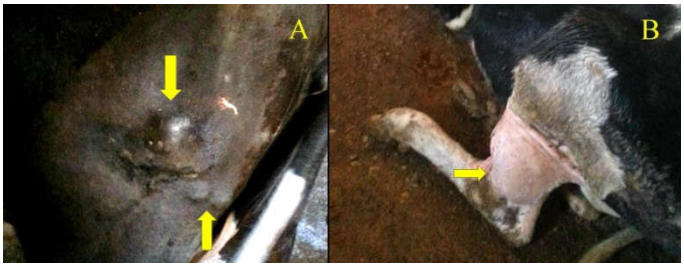

Fig. 1. (A) Subcutaneous abscess foci, (B) Acute swelling in the joint (arthritis)

Fig. 2. Macroscopic view of *C. pseudotuberculosis* in blood agar.

The microbiological cultures showed small, white and dry colonies of *C. pseudotuberculosis* encircled by a narrow margin of hemolysis in the 5% sheep blood agar (Fig 2). Moreover, the colonies were observed to become dry and pulverizable in the long-term incubations. Gram-stained samples prepared from these colonies exhibited Gram-positive, rod-shaped, and pleomorphic pathogens. No growth was observed in the MacConkey agar. The pathogen was found to be esculin-negative in the bile esculin agar. The pathogen isolated by biochemical tests demonstrated positive results in nitrate, glucose, and maltose tests, while giving negative result with the sucrose test. In the CAMP test performed for the isolation of the pathogen, the causative agent was observed to inhibit the ß hemolysis of *S. aureus* and increase the ß hemolysis by showing synergistic activity with *Rhodococcus equi*. In light of these analyses, the pathogen was identified as *C. pseudotuberculosis* by the BD Phoenix automatized microbiological system. The antibiogram test showed that *C. pseudotuberculosis* pathogens were sensitive to penicillin G (100%, n=15), erythromycin (100%, n=15), ampicillin (100%, n=15), tetracycline (66.7%, n=10), and clindamycin (60%, n=9), while they were resistant to cefazolin (100%, n=15) and cefoxitin (100%, n=15).

DISCUSSION

C. pseudotuberculosis causes infection worldwide and leads to productivity loss and mortality particularly in sheeps and goats by causing caseous lymphadenitis (Ipek *et al*., 2012). Nonetheless, it has been shown to induce a disease in humans

and many animal species as well (Sood *et al*., 2012; Baird and Fontaine, 2007). Although the local edematous swellings around the lesions induced by the causative agent are associated with the factors of virulence, they are believed to be essentially related to the effect of phospholipase-D exotoxin in the blood vessels and lymphatics around the lesions (Biberstein *et al.,* 1971; Hodgson *et al.,* 1999). In addition to its local pathologic effects, phospholipase-D exotoxin also leads to toxemic impacts on the internal organs. Similarly, the lipids of the *C. pseudotuberculosis* cell wall have a strong necrolytic effect over the tissues which is believed to be a major factor in the pathogenesis. Pathogens enter the epidermis layer by taking advantage of elevated cutaneus moisture level, poor maintenance and hygiene in the barn, and a wet skin surface, and start the infection (Dorella *et al.,* 2006).

While *C. pseudotuberculosis* leads to various infections in cattle, there is limited data concerning the presence of the causative agent in cattle. The infectionsoccurs sporadically or endemically (Yeruham *et al.,* 2003). Barakat *et al*. (1984) conducted a study in Egypt and reported that Bovine Skin Disease occuring sporadically in buffalos was caused by *C. pseudotuberculosis* which was a first in the literature. They observed elevated body temperature and large-sized swellings over the skin in the sick buffalos. Moreover, the causative agent isolated from the adult buffalos was injected intradermally to the buffalo calves and thus it was proved that the disease could be induced experimentally, as well. However, Sood *et al*. (2012) performed a study in India and reported that they isolated *C. pseudotuberculosis* in a calf with mesenteric caseous lymphadenitis. Similarly, Silva *et al*. (2011) conducted a study in Israel and reported the isolation of *C. pseudotuberculosis* bacteria in dairy cows with severe mastitis. Moreover, Yeruham *et al*. (1996) isolated *C. pseudotuberculosis* in dairy cows with mastitis and determined that it was transported via mosquitoes. Yeruham *et al*. (2003) also carried out a large-scale study comprised of 45 herds for 13 years, investigated *C. pseudotuberculosis* associated diseases, and observed epidemic form of granulomatous infection in 19 herds and sporadic form of the infection in 26 herds. The investigators noted that granulomatous lesions were of 3 clinical types: cutaneous, mastitis, and visceral; they reported the isolation of *C. pseudotuberculosis* in all three forms. Similarly, in the present study, *C. pseudotuberculosis* was isolated and identified in the abscess content and milk samples collected from the sick cattle. In this study which was conducted parallel to the studies investigating the presence of *C. pseudotuberculosis*, a sick cattle that had been added to the study herd was found to start the infection. The animal who had been added to the study herd two weeks previously had subcutaneous swellings and the infection was determined to have started after its arrival. Moreover, the maintenance and feeding conditions were poor. Similar studies underscore the rapid spread of the infection due to presence of active patients as well and poor barn conditions are reported to contribute to the occurrence of the disease (Ozturk *et al*., 2010; Makav and Gökce, 2013). In the present study, it is believed that a similar mode of infection has occurred in the herd.

In this study, the animals were found to have been infected with mild or moderate degree of the disease. The infected animals had lesions over various parts of the body and these lesions were determined to be of pyogranulomatous character (cutaneous, mastitis, and lympangitis forms). Nonetheless, the lesions were in the form of either acute or chronic abscess, occuring over various parts of the body such as head, neck, back, and legs. Acute abscesses were edematous and painful, while chronic abscesses were wavy, purulent, and often perforated. While three of the infected animals had the clinical profile of mastitis, 8 animals had the clinical profile of lymphadenitis in the regional lymph nodes and lymphatics. Four animals had necrosis and severe inflammation in the foot. Almost all of the infected animals were emaciating due to loss of appetite, and the volume of the subcutaneous abscesses and inflammed joints were increasing. Most of our findings were similar with those provided by Yeruham *et al* (2003).

Studies have reported the use of various antibiotics in infections with *C. pseudotuberculosis* (Senturk and Temizel, 2006; Yeruham *et al*., 1996; Yeruham *et al*., 2003). Yeruham *et al*. (1996) conducted a study on dairy cattle and reported successful treatment of mastitis caused by *C. pseudotuberculosis* with penicillin and amoxicillin. Nonetheless, the authors noted that 69% of the isolates of *C. pseudotuberculosis* were sensitive to penicillin, while 93.3% were sensitive to amoxicillin. Similarly, a study on sheeps with caseous lymphadenitis in whom *C. pseudotuberculosis* was isolated, reported the combined use of rifamycin and oxytetracycline in the treatment of the disease (Senturk and Temizel, 2006). In the present study, penicillin G and erythromycin were employed in the treatment of infected animals. The antibiogram test results of the *C. pseudotuberculosis* pathogens isolated from the infected animals were found to be sensitive to penicillin G (100%, n=15), erythromycin (100%, n=15), ampicillin (100%, n=15), tetracycline (66.7%, n=10), and clindamycin (60%, n=9). Nonetheless, they were resistant to cefazolin (100%, n=15) and cefoxitin (100%, n=15). The difference of our resistance and sensitivity rates from those of other studies in the literature may be associated with differences in geographic location and duration of antibiotic use. In the current study, as mentioned in the Material and Method section, the infected animals received local and parenteral treatment with various antibiotic groups. The treatment was observed to be effective in the acute period, while it was not effective in the chronic period during which the animals were very emaciated.

In conclusion, *C. pseudotuberculosis* was determined for the first time in cattle raised in the Van province. The Van province may be influential regarding the spreading potential of the disease because it is situated at a location transpassed by many stock routes The presence of *C. pseudotuberculosis* in cattle is important in terms of spreading and transmission of the disease which pose a risk for human health, as well. Future studies on *C. pseudotuberculosis* infection in cattle are required to develop effective control measures and treatment methods.

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