Mesenteric caseous lymphadenitis in a cow calf caused by *Corynebacterium pseudotuberculosis*: a case report

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ABSTRACT: Caseous lymphadenitis caused by *Corynebacterium pseudotuberculosis* is mainly a disease of sheep and goats and is of zoonotic importance. The disease has rarely been recorded in cattle and mostly in its superficial form. The present communication deals with an extremely rare case of corynebacterium-induced mesenteric pseudotuberculosis in a cow calf. The gross, cytologic, histopathologic and microbial isolation as well as cultural characteristics of the organisms have been described, as well as the mode of spread of the disease to the mesenteric lymph nodes. To the authors' knowledge, this is the first report of mesenteric caseous lymphadenitis in a cow calf.

Keywords: caseous lymphadenitis; cow calf; *Corynebacterium pseudotuberculosis*; mesenteric; tape worms, CAMP

Caseous lymphadenitis is a chronic contagious disease of sheep, goat and occasionally cattle, deer, horse, camelids, water buffalo, wild ruminants, primates, pigs, and fowl. The disease is also of zoonotic importance as it may on rare occasions cause regional lymphadenitis in humans, particularly in farm workers and meat inspectors (Peel et al. 1997). Caseous lymphadenitis is characterized by the formation of caseous abscesses in superficial lymph nodes and/or internal lymph nodes and organs. The disease is caused by *Corynebacterium pseudotuberculosis,* which is a gram positive, pleomorphic, intracellular, non-motile, facultative anaerobe that grows well on blood agar, forming small, whitish, opaque colonies (Coyle and Lipsky 1990). Three major factors have been implicated in the pathogenicity and virulence of *C. pseudotuberculosis*: the high lipid content in its cell wall; production of phospholipase-D exotoxin and its intracellular ability to persist in macrophages (Yozwiak and Songer 1993).

The disease causes significant economic losses due to reduced weight gain, reproductive efficiency as well as condemnation of carcasses and devaluation of hides. Infection is rarely seen in cattle, although it was first described in 1888 as a cause of bovine lymphangitis. Later, it was documented that *C. pseudotuberculosis* caused mastitis either naturally (Shpigel et al. 1993), or experimentally (Aroch et al. 2003). Yeruham et al (2003) reported *C. pseudotuberculosis* infection in Israeli dairy cattle, where the condition was manifested in cutaneous, mastitis and visceral forms. The isolation of *C. pseudotuberculosis* from an ectopic site like the spinal canal in a goat has also been reported by Karimi et al. (2003). However, there is a paucity of literature on caseous lymphadenitis in cattle worldwide and, moreover, there is no report of the disease in cattle in India.

The present communication describes the pathology of a rare case of caseous lymphadenitis (visceral form) in a cow calf with the exclusive involvement of mesenteric lymph nodes, and places the present findings in context by reviewing previous such cases.

MATERIAL AND METHODS

Case history

A carcass of a six-month old male cross-bred calf was received for necropsy at the Department of Veterinary Pathology, GADVASU, Ludhiana, with a history of sudden death.

Diagnostic cytology. Impression smears were made from the affected lymph nodes, and were quickly dried and stained by Wright-Giemsa stain for cytological examination. The smears were also subjected to Ziehl-Neelsen and Good Pasteur staining to investigate the presence of bacteria (Bancroft and Gamble 2008).

Histopathology. Representative 0.5 cm thick tissue slices were collected from liver, spleen, intestines and lymph nodes for histopathological examination in 10% neutral buffered formalin and processed for paraffin sectioning and H&E staining (Bancroft and Gamble 2008).

Isolation, identification and characterisation of *Corynebacterium pseudotuberculosis*. The caseous material from the mesenteric lymph node was collected in sterile petri-dishes for microbiological analyses. The isolation, identification and characterization of *C. pseudotuberculosis* was done as per the standard protocols (Quinn et al. 1994). Briefly, the sample was inoculated onto 5% sheep blood agar aerobically at 37 °C for 48 hours. The morphological features were recorded by Gram staining and meatchromatic granules were stained with Albert's staining. Biochemical characterisation involved testing for catalase, oxidase, CAMP, nitrate reduction, urease production and fermentation of glucose, lactose, sucrose and maltose. An *Enterobacteriaceae* Identification Kit (Hi-Media, India) was used for other biochemical tests viz. methyl red (MR), Voges Prauskauer (VP), ONPG, indole, aesculin hydrolysis, lysine utilization, ornithine utilization, phenylalanine deamination, hydrogen sulphide production, citrate utilisation and fermentation of arabinose, xylose, adonitol, rhamnose, cellobiose, maltobiose, saccharose, raffinose, trehalose, and malonate.

RESULTS

Gross lesions

Grossly, the animal was very weak and debilitated with pale mucous membranes. Necropsy revealed straw-coloured fluid (about 500 ml) in the peritoneal cavity. The liver and spleen were enlarged and congested. The intestines had fibrinous enteritis, besides the presence of tapeworms (*Moniezia* species). Mesenteric lymph nodes were enlarged and on cutting they revealed the presence of central caseous material (Figure 1). All other lymph nodes and visceral organs were apparently normal.

Figure 1. Enlarged mesenteric lymph node with cut surface showing the presence of caseous material in the centre

Cytology

Cytological examination of the caseous material revealed the presence of neutrophils (intact and degenerated) and a few lymphocytes along with bacteria, which appeared to be bacilli, at times in a Chinese Letter-like arrangement (Figure 2). Many neutrophils in place of a few neutrophils also harboured engulfed bacilli indicating phagocytosis and active infection.

Microscopic lesions

Liver. The liver showed a mild to moderate degree of degenerative change in hepatocytes as well as sinusoidal congestion.

Intestine. The intestine revealed diphtheritic enteritis. Cross sections of tapeworm were also seen in the small intestine.

Lymph node. The lesion in the lymph node revealed a central area of caseous necrosis. The laminated central caseous mass was surrounded by a thin layer of macrophages, epithelioid cells mixed with neutrophils and lymphocytes, surrounded by fibrous connective tissue (Figure 3). Dystrophic calcification was also noted in places.

Isolation, identification and characterisation of *Corynebacterium pseudotuberculosis*. The caseous material from the mesenteric lymph nodes inoculated onto 5% sheep blood agar revealed a pure growth of small, white and dry colonies which were

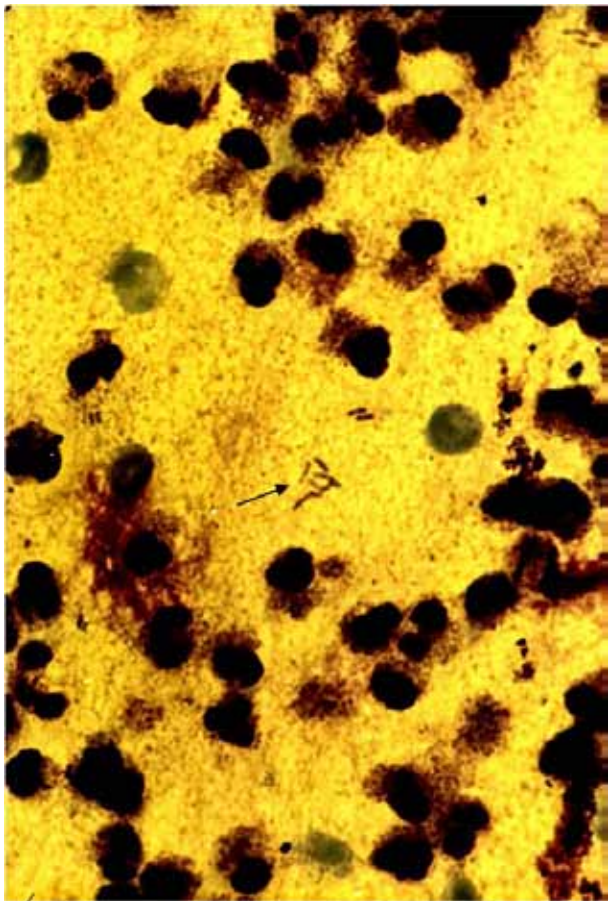

Figure 2. Lymph node impression showing the presence of several degenerated neutrophils, with a few lymphocytes along with rod-shaped bacteria creating a somewhat Chinese Letter-like appearance (arrow). Phagocytosed bacteria are also visible in most neutrophils. Wright-Giemsa, original magnification ×1000

Figure 3. Photomicrograph of a mesenteric lymph node showing central laminated caseous mass, dystrophic calcification (arrow) surrounded by an inflammatory zone and fibrous tissue encapsulation. H & E, original magnification ×100

β-haemolytic after 48 hours of incubation at 37 °C. Microscopically, gram-positive, pleomorphic rods arranged in a typical "Chinese Letter" arrangement of *Corynebacterium* spp. were observed (Figure 4). In addition, metachromatic granules were seen in Albert's staining. The bacteria were catalase- and urease-positive but negative for nitrate reduction and oxidase. Inhibition of the effect of staphylococcal haemolysins in a CAMP test confirmed these to be *C. pseudotuberculosis.* Biochemically, the bacteria were negative for aesculin hydrolysis, ONPG, ornithine utilisation, phenylalanine deamination, hydrogen sulphide production, citrate utilization, MR, VP and indole. In addition, glucose, lactose, maltose, arabinose, xylose adonitol, rhamnose, cellobiose, maltobiose, saccharose, raffinose, trehalose were fermented, whereas, sucrose and malonate were not fermented.

DISCUSSION AND CONCLUSIONS

Caseous lymphadenitis is mainly a disease of sheep and goats (Benham et al. 1962; Vathsala et al. 2006) and the source of natural infection and the means of entry into cattle are not well documented. Several clinical forms of the disease caused by *C. pseudotuberculosis* have been described in cattle: pyogranulomatous reactions; abscess formation; ulcerative lymphangitis and mastitic and visceral forms (Yeruham et al. 1997; Steinman et al. 1999). Demonstration and isolation of a pure growth of *C. psudotuberculosis* from the caseous lymphadenitis confirmed it to be the causative agent, resulting in the death of the calf. However, no report is available in which only the mesenteric lymph nodes were infected by *C. psudotuberculosis* and all

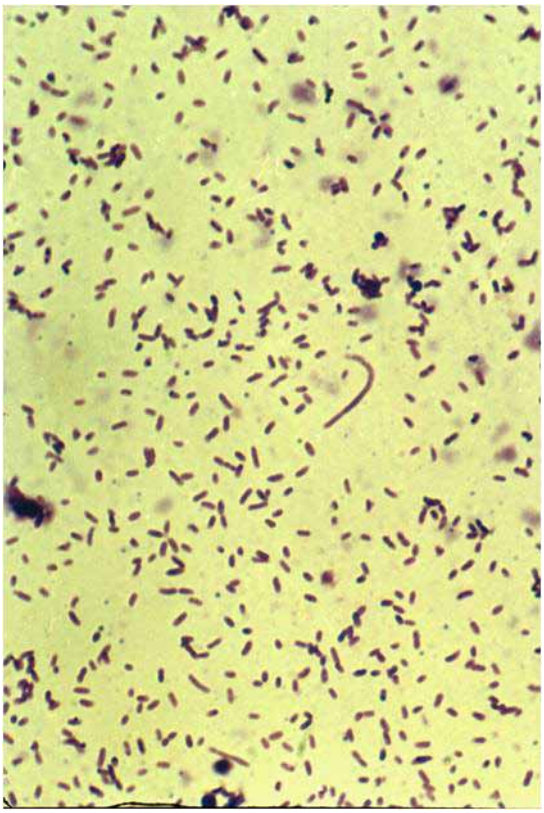

Figure 4. A smear from cultured corynebacterium showing bacilli in a typical Chinese Letter-like arrangement. Gram staining, original magnification ×1000

the other visceral lymph nodes spared. In India, *C. pseudotuberculosis* infection has been reported in sheep by Vathsala et al. (2006). However, no case of caseous lymphadenitis has been recorded in cattle in India, and the present study is the first such report.

Histological examination of the lesions in the lymph node was in accordance with earlier studies (Yeruham et al. 2003). It has been reported that young animals were more susceptible than older ones (Doherr et al. 1998) as was also the case in this study.

A possible role of arthropod vectors in the interspecies transmission of *C. pseudotuberculosis* has been suggested (Yeruham et al. 1996; Braverman et al. 1999). Also, as sheep, goat and cattle farms are usually located in close proximity, the disease might have spread to the cow calf from small ruminants (Yeruham et al. 2003). In addition, *C. pseudotuberculosis* has been shown to survive for prolonged periods in contaminated environments under favourable conditions (Benham et al. 1962). Possibly, the infection might have spread through mechanical transmission by house flies (Yeruham et al. 1996; Braverman et al. 1999). The concurrent presence of tape worms in the intestine might in turn have caused significant pathological damage facilitating entry of the organism to the mesenteric lymph nodes via infected macrophages (Yozwiak and Songer 1993).

Based on gross lesions, the case was initially suspected to be one of mesenteric tuberculosis; however, the impression smear cytology gave the first clues towards the right diagnosis. Negative staining of the impression smears by Ziehl-Neelsen and their gram-positivity further authenticated the diagnosis. Later, the isolation and identification of *C. pseudotuberculosis* confirmed the diagnosis of pseudotuberculosis. Therefore, diagnostic cytology seems to be of value in differential diagnosis of such complicated cases. To the authors' knowledge this is the first report of pseudotuberculosis from India involving the mesenteric lymph nodes of a cow calf.

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