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Pathology of Caseous Lymphadenitis in Slaughtered Goats Associated Infection with *Corynebacterium Pseudotuberculosis*

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ABSTRACT

Present study recorded pathomorphology of 10 cases (2.16%) infected with *Corynebacterium pseudotuberculosis* in slaughtered goats that has caused suppurative bronchopneumonia accompanied by lymphadenitis. The lungs lesion was characterized by either formation of caseo-calcified nodules of multiple abscesses or fully lungs lobes replaced with cheesy greenish pus. The enlargement of mediastinal lymph node was recorded with greenish yellow inspissated pus giving the characteristic spherical onion-skin appearance. Histopathologically, the lung sections were characterized by a central caseo-necrotic core admixed bacterial colonies and infiltration of polymorphonuclear cells, lymphocytes, plasma cell and macrophages. The bacterial culture isolation was confirmed the bacteria as *Corynebacterium pseudotuberculosis*.

Keywords: Suppurative bronchopneumonia, small ruminants, *Corynebacterium pseudotuberculosis*

Amongst various livestock species of animals, sheep and goat contributes significantly to the economy of Asian countries. The small ruminants are rear by more than 80 % of rural families, involving women and children, in tropical countries. Various infectious diseases are responsible for the morbidity and mortality in goats subsequently leading to economic loss. Caseous lymphadenitis (CL) is a chronic bacterial infectious disease of goats and other small ruminants caused by *Corynebacterium pseudotuberculosis*. The genus *Corynebacterium* is a gram-positive, small or pleomorphic coccobacillus, spore-forming and the microaerophilic intracellular organism that multiplies inside the macrophage. It belongs to family *Corynebacteriaceae*, class *Actinobacteria* (Baird and Fontaine, 2007). The commonly occurring external form of CL is characterized by abscess formation in the superficial lymph nodes, while in the visceral CL abscessation occurs in various lymph nodes and other internal organs like liver, lungs, and intestine (Dorella *et al.,* 2006; Fontaine and Baird, 2008). The disease causes significant economic losses by downgrading and condemnation at slaughter and inspection, culling of affected animals, loss of fertility and reduced meat and milk yield. Moreover, it has great ability to persist in the environment and show poor response against various chemotherapeutic agents. Hence, once introduced into a flock, it is very difficult to control (Williamson, 2001). There are very few systemic studies regarding occurrence of CL in India. Owing to its economic importance and subclinical nature, present work was carried out to study pathomorphological changes in lungs and lymph nodes due C. *pseudotuberculosis* in slaughtered goats.

During the period from August 2015 to April 2016, 462 lungs and lymph nodes of adult goats were screened from different slaughterhouses (Delhi and U.P) and gross lesions were noted. These animals aged mostly between 2 to 5 years. The lungs and associated lymph nodes with gross

lesions were collected in 10% neutral buffered formalin (NBF) for histopathology and frozen tissues were stored at −20°C until further examination. The paraffin blocks of the tissues were then cut into 4 -5 µm thick paraffin sections by microtome and stained with hematoxylin and eosin technique (H&E) following standard procedure. For bacterial isolation, aseptically a loopful of the content was inoculated into nutrient agar, 5% sheep blood agar and MacConkey agar and incubated aerobically at 37°C for 24-48 hours. The isolates were identified according to the colony morphology, Gram's stain, as well as biochemical characters. For identification of *C. pseudotuberculosis*, the isolates were further inoculated on cystine tellurite blood agar and incubated at 37°C for 48 hours.

In the present study, 462 lungs and lymph nodes were screened from slaughtered goats and gross lesions were recorded. Suppurative bronchopneumonia pneumonia with lymphadenitis was observed in 10 cases (2.16%) which is characteristic lesion for *Corynebacterium pseudotuberculosis* infection. Grossly, small pea to the walnut size of multiple abscesses (discrete nodules) were also recorded in the lung parenchyma (Fig. 1A). The affected lungs were red to gray, solid and pale. Sectioning of affected lobes oozed suppurative exudates (greenish pus) (Fig.1B). Moreover, variable sized caseo-calcified nodules were found in different lobes and even over the chest wall. Mediastinal and trachea-brocnhial lymph nodes were diffusely enlarged and replaced by abscesses having calcified granules. The cut surfaces of the mediastinal lymph node were showing thick semi- solid-fluid with greenish yellow inspissated pus in lymph node and concentrically lamellated layers of fibrous connective

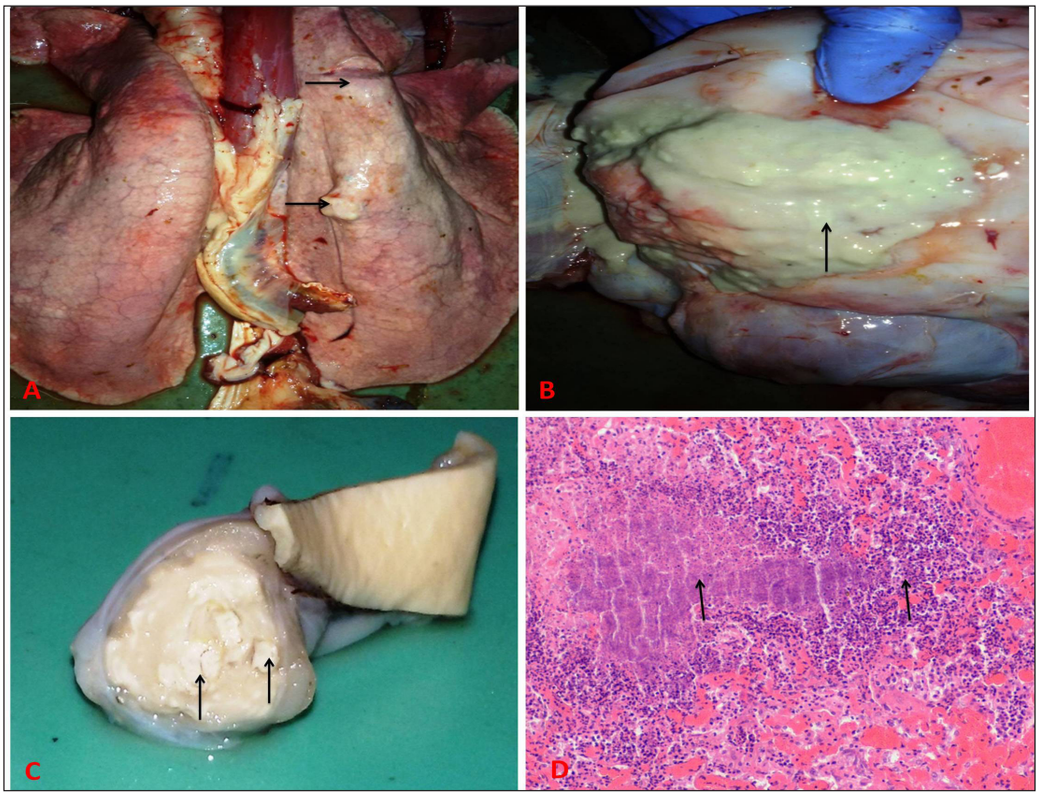

Fig. 1: Gross and histopathological lesions of pseudotuberculosis affected goats. **(A)** *Corynebacterium pseudotuberculosis* affected lung showed caseo-calcified nodules of variable sizes in pneumonic lung (arrows). **(B)** Gross lesion of *Corynebacterium pseudotubercosis* affected lung showed suppurative exudates (greenish pus) oozed out after cutting surface of lungs lobe (arrow). C) The mediastinal lymphnode showed characteristic lamellate (onion skin or onion ring), pathognomonic signs of CLA (arrow). **(D)** *Corynebacterium pseudotuberculosis* **abscess** in lung section of affected goat with caseative necrotic mass and bacterial colonies within the lung parenchyma (arrows).

tissue with alternating zones of caseous and friable material which gives it spherical onion-skin appearance (pathognomonic signs of CL) (Fig. 1C).

In the present study, chronic gross lesions finding in lung and associated lymph node are in agreement with the lesions described previously by various workers in goat affected with *C. pseudotuberculosis*. (Radostits *et al.,* 2007; Sonawane *et al.,* 2016; Singh *et al.,* 2017). In our study, mediastinal lymph node cut section given pathogonomic lesion for pseudotuberculosis in goats. This finding is in agreement to previous reports (Fontaine and Baird, 2008; Radostits *et al.,* 2007, Ali *et al.,* 2016). Mahmood *et al.* (2015) described lung lesion in experimentally induced infection by *C. pseudotuberculosis* in goats, which showed similar finding to present study. In CL development of chronic lesions with multiple abscesses are attributed to the ability of bacteria to evade the host immune system and spread to various organs, because of virulence factor like phospholipase D and mycolic acid (Baird and Fontaine, 2007).

Histopathologically, the lung lesions were characterized by focal areas of suppuration with multiple necrotic areas. The suppurative areas were characterized by a central caseo-necrotic core surrounded by infiltration of polymorphonuclear cells few mononuclear cells, lymphocytes, plasma cell and macrophages. Most of the cases had caseo-purulent material admixed with bacterial colonies and surrounded by thick connective tissue capsule of dense fibrous connective tissue mixed with multiple viable and degenerated neutrophils cuff with rare macrophages, plasma cells and lymphocyte. (Fig. 1D). The results of this study were consistent with earliar reports (Radostits *et al.,* 2007; Sonawne *et al.,* 2016; Singh *et al.,* 2017) who stated the classical presentation of the histopathological feature of the pyogranulomatous inflammation in lungs. Osman *et al.* (2012) described similar histopathological lungs lesion in a mouse model, lungs showed congestion, hemorrhage, and development of micro- abscesses, caseous necrosis, and presence of tubercular like granuloma that surrounded with neutrophil and macrophage.

The bacterial culture examination showed the presence of bacterial growth forming cream white, dry, waxy colonies with a narrow zone of β-haemolysis. They were stained gram-positive cocco-bacilli arranged in Chinese pattern indistinguishable from *C. pseudotuberculosis*. (Fig. 2).

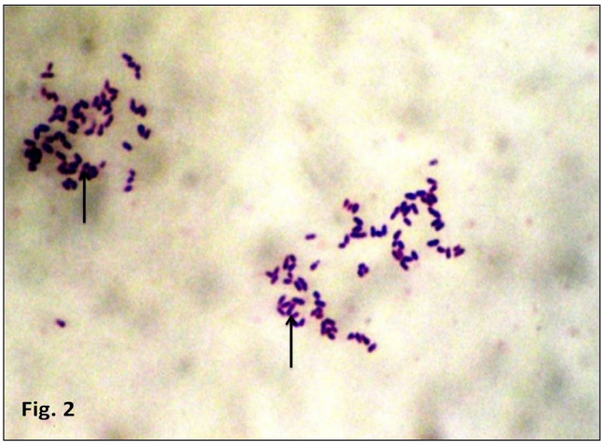

Fig. 2: Bacterial culture of *Corynebacterium pseudotuberculosis* gram' staining showed gram positive cocco-bacilli arranged in Chinese pattern (arrows)

These results are in agreement with previous studies (Fontaine and Baird, 2008; Radostits *et al.,* 2007). Chirino-Zarraga *et al.* (2006) have described the bacteriological characterization of *C. pseudotuberculosis* from 18 Venezuelan goat flocks.

CONCLUSION

The present study concludes that the pathomorphological and bacteriological could help in the diagnosis of *C. pseudotuberculosis* in small ruminants population. Further explorative surveillance studies are suggested to know the real magnitude and impact of this disease-affecting goat's population in India.

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