



## CASE REPORT

### ***Mycobacterium Avium* Subspecies *Paratuberculosis* Multibacillary Infection (Johne's disease) in a Teddy Goat**

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#### ABSTRACT

A 9-year old, debilitated, female teddy goat, with a body weight of about 23 kg, was subjected to smear staining, histopathological examination and ELISA for the diagnosis of Johne's disease (JD). During postmortem, the intestine showed thick corrugated mucosa and the mesenteric lymph nodes were found enlarged and edematous. Acid fast bacilli of multibacillary form were detected in Ziehl-Neelsen stained smears prepared from the tissues which showed gross pathology. Histopathologically, the ileal mucosa was intensely infiltrated by mononuclear cells and one of the ileum-associated lymph node revealed a large granuloma in its paracortical region. Special staining of tissue sections demonstrated the occurrence of acid fast bacilli. The JD case was confirmed by using indirect ELISA. The present case was differed from the previously reported studies in two aspects viz 1) A confirmed case of multibacillary form of JD in a teddy goat 2) Unusual presence of a large granuloma in the lymph node.

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#### INTRODUCTION

Johne's disease (JD) is a chronic infectious disease of domestic, zoo and wild ruminants and has been recognized all over the world (Maxie *et al.*, 2007). An acid-fast bacillus, *Mycobacterium avium* subsp. *paratuberculosis* (MAP) is the causative agent of JD, which is fairly resistant to harsh environment. Animals are greatly susceptible to infection in premature age (Timms *et al.*, 2011). JD exists in animals in two forms. The multibacillary form or lepromatous form in which the cytoplasm of macrophages is stuffed with bacilli and there is chronic diffused granulomatous enteritis (Biplab *et al.*, 2010), and the paucibacillary form in which lymphocytes with or without bacilli are present in intestinal mucosa (Clark *et al.*, 2010). It has been suspected that Crohn's disease in man is causally linked with MAP (Rita *et al.*, 2011; Timms *et al.*, 2011). Its proper control can be easily achieved by vaccination (Juste and Perez, 2011) but for this, an accurate diagnosis of subclinically infected animals is essential. On the basis of histopathological lesions, several classifications have been made in goats (AL-Dubaib and Mahmoud, 2008) and in sheep (Maxie *et al.*, 2007). Accurate prevalence of JD in small ruminants is not available and vaccination has not been performed in our country. The purpose of the study reported here is to

explain the gross/histopathological characteristics of Johne's disease, and its importance in teddy goats in developing countries like Pakistan.

**History and examinations:** A nine-year old, teddy goat of about 23 Kg weight, from local caprine herd was presented to slaughterhouse at Jhang municipal abattoir. The animal was debilitated and had normal appetite. Rumination was normal and feces were slightly pasty. Clinical examination revealed a poor body condition viz. body condition score-2 out of 5 (Villaquiran *et al.*, 2004) and had a rough hair coat. During postmortem, gross pathology was obvious in the some parts of small intestine, more specifically in the distal ileum. The tentative diagnosis was Johne's disease made on the basis of clinical signs, which was supported later by the postmortem examination. The findings were further strengthened by histopathology and serology. Intestines along with adjacent MLNs were collected carefully from the carcass soon after opening and were taken to the histology laboratory for further investigations. Lining of the intestinal mucosa was carefully wiped off with the edge of the slide and hard pressed impression smears were prepared. The smears were also made on centrally severed (cortico-medullary) portion of MLNs. Ziehl Neelsen (ZN) stain was used for staining of impression smears. Under

oil immersion lens the acid fast bacilli (AFB) were bright/rose-red rods with a blue background (Fig. 1). The ZN positive samples were processed further for histopathology. Small pieces of intestine and MLNs were fixed in 10% buffered formalin (neutral). Further processing, sectioning and staining of the tissues were routinely done following Bancroft and Gamble (2007). Blood sample was taken from jugular vein before slaughtering and serum was immediately obtained and stored in the freezer. Indirect ELISA test (Serelisa™ M.Para TB Ab Mono Indirect. Symbiotic Europe SAS, 2, rue Alexander Fleming, F-69367 Lyon, Cedex 07, France) was performed for the detection of antibodies in caprine serum as instructed by the manufacturers.

## RESULTS AND DISCUSSION

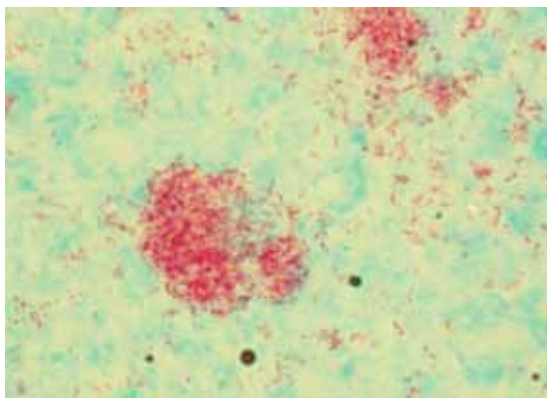
The thick edematous parts of the intestine, after opening, showed clear elevations of mucosa forming folds. The corrugations of different sizes towards lumen were observed. Some mucosal raised areas in small intestine did not disappeared even on stretching. Ileum-associated MLNs were found enlarged and edematous. The impression smears and tissue sections of intestines and MLNs, stained by ZN stain were found to be positive for acid fast bacilli (Fig. 1).

Through histopathological study of intestinal tissue sections, the enterocytes lining the intestinal villi had

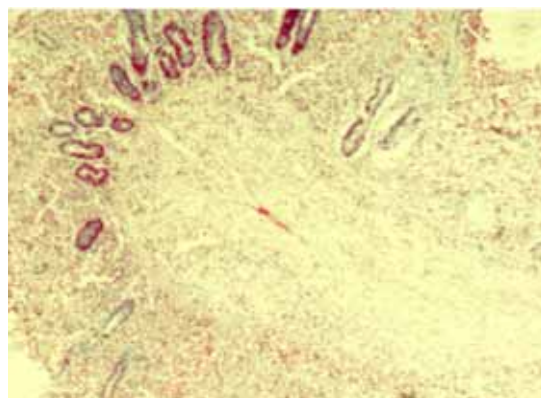
mostly sloughed off, and the lamina propria of mucosa was distended with epithelioid macrophages, lymphocytes and plasma cells. A little infiltration by the AFB-laden macrophages also took place along with lining epithelium of crypts of Lieberkuhn (Fig. 2). The intestinal crypts were small in size, reduced in numbers and were wide apart from each other owing to cellular infiltration. Lining epithelium of most of the crypts was detached and their openings were filled from exfoliated cells. The infiltrations by mononuclear cells (MNCs) in the muscularis and serosa were also observed in the small intestine.

Cytoplasm of epithelioid macrophages was overstuffed with red to pink AFB as reported by Clark *et al.* (2010) in red deer (*Cervus elaphus*). Large number of bacilli-laden epithelioid macrophages was observed in the mucosa and in the upper tela submucosa (Fig. 3). Macrophages in serosa contained only few microorganisms.

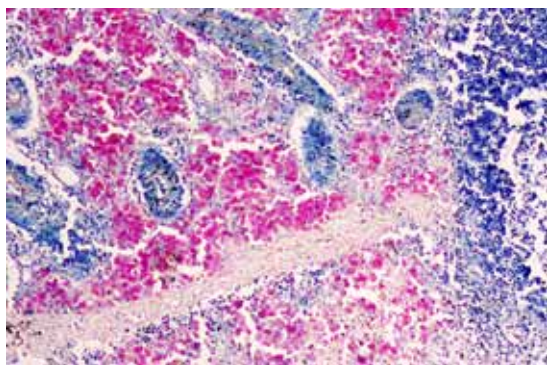
The thick capsules surrounding the mesenteric lymph nodes consisted of fibrous connective tissue and some smooth muscles. There were large variable sized, irregular areas containing brown, caseous, acellular homogenous substance with small quantity of calcified masses. The cortical and paracortical regions of the lymph nodes contained those areas. Same epithelioid macrophages which were observed in the intestinal sections were also present in the paracortical areas of the lymph nodes. Most of the cortical areas were replaced by the necrotic tissue



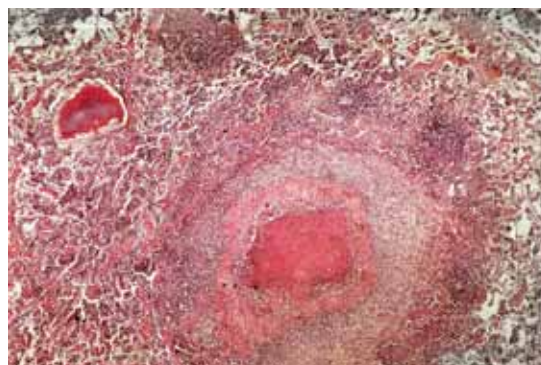
**Fig. 1:** Impression smear prepared from the mucosa of ileum. The bacilli appear as red rods in blue background. The ruptured cells were stuffed with bacilli of multibacillary form. ZN (1000X).



**Fig. 2:** Circular folds (plicae circulares) of duodenum. AFB Laden macrophages are infiltrated in the opening wall of the crypts of Lieberkuhn. ZN (40X).



**Fig. 3:** Macrophages laden with rose red AFB in the mucosa and submucosa of ileum. Crypts in mucosa are atrophied and present apart from each other. ZN (100X).



**Fig. 4:** Ileum associated mesenteric lymph node. A granuloma is present in the paracortical region. HE (40X).

mass. One large granuloma was observed in ileum-associated MLN (Fig.4). Center of that granuloma contained homogenous caseous red material, surrounded by several layers of inflammatory reaction predominantly MNCs. The JD case was confirmed by using indirect ELISA. According to the statement of Rita *et al.* (2011), the serology is very effective tool for the establishment of JD in a herd. The gross thickness and corrugations in the wall of intestine are due to MNCs infiltration in mucosa and in the tela submucosa, same as reported by Khan *et al.* (2010). Calcification and caseation in the mesenteric lymph nodes were observed in the current study, similar to the findings reported by (Sikandar *et al.*, 2012). Giant cells in caprine MLNs were also seen in the present study. This study has highlighted the importance of a careful serological and histopathological assessment of the intestine and mesenteric lymph nodes for diagnosis of JD. Because the information of JD in domestic animals is limited, therefore, it is not a priority disease to control in Pakistan and ongoing spread of Johne's disease among and within goat herds must be expected. It is predictable that this will be the topic for future examination.

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