

## Morphological diagnosis of Johne's disease: a case report in cattle

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Received: 02.06.2018 • Accepted/Published Online: 18.10.2018 • Final Version: 10.12.2018

**Abstract:** The present case report deals with the morphological (gross and histopathological) and histochemical characterization of Johne's disease in a cow. The carcass was presented in a hidebound condition with sunken eyes and with a history of persistent and nonresponsive diarrhea. Necropsy revealed generalized gelatinization of the visceral and peripheral fat. Thorough gross examination unveiled major pathological changes in the digestive tract. The gallbladder was distended, with severely thickened walls and blood mixed with bile. The small intestine was severely congested and the mesenteric lymph nodes were enlarged. The opening of the small intestine showed marked thickening of the intestinal walls, with a corrugated appearance. Diffuse granulomatous lesions were observed in the mesenteric lymph nodes. Histopathological examination revealed severe infiltration of intestinal layers and mesenteric lymph nodes by macrophages, lymphocytes, and epithelioid cells with multiple Langhans giant cells. Ziehl-Neelsen staining demonstrated multiple acid-fast bacilli in the cytoplasm of macrophages from impression smears.

**Key words:** Paratuberculosis, Crohn's disease, giant cells, cattle, epithelioid cell

### 1. Introduction

Johne's disease (paratuberculosis; JD) is caused by the acid-fast bacterium *Mycobacterium avium* subsp. *paratuberculosis* (MAP) and is characterized by a chronic wasting disease of ruminants, accompanied by nonresponsive diarrhea and ultimately death. The disease has been reported across the globe in various wild and domestic ruminants including cattle, sheep, goats, deer, camel, antelope, and bison (1,2). MAP is shed into the feces of the infected animals and is mainly transmitted through the oral-fecal route. Contaminated feed and water, bedding material, and soiled udders act as major sources of MAP dissemination. Animals younger than 6 months of age are considered to be most susceptible, acquiring the infection through contaminated milk (3). Intrauterine infection has also been reported (4). The disease is of huge economic significance due to its direct impact on delayed/reduced breeding, infertility, culling, mortality, and milk reduction. Additionally, JD has gained much attention due to its close association with Crohn's disease in humans (5).

Several methods for diagnosis of subclinical/clinical cases of JD have been adopted, their use varying among institutions. Although bacterial culture is considered

the gold standard for JD's diagnosis, it is a time-consuming process and requires special media. Molecular techniques for diagnosis of JD such as PCR and ELISA are quite sensitive and rapid; however, they require special instrumentation. Under such circumstances, histopathological and histochemical characterization of lesions from infected animals may serve as a cost-effective and specific technique for JD's diagnosis. Additionally, histopathology and histochemical analysis are also more practical under field conditions. Subclinical cases of JD may exhibit low-grade lesions with lower numbers of bacteria and may be difficult to diagnose with histopathology. For sensitive detection of subclinical cases, other tests may be employed to complement histological diagnosis of JD.

### 2. Case history

A dead cow was brought to the Division of Pathology, Indian Veterinary Research Institute, Izatnagar, for postmortem study. The breed of the animal was nondescriptive and the animal was aged 4 years. The animal had a history of persistent diarrhea over the previous several months and was nonresponsive to antimicrobial and anthelmintic treatment. During the entire course of the disease, the

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appetite of the animal was normal; however, there was mild inappetence during the last few days before death. There was a progressive loss of body condition; the animal was 7 months pregnant. The animal was brought for a postmortem examination on the day of death, and the postmortem was conducted approximately 5 h after the death of the animal. Before the carcass was opened, it was examined for the presence of any external injury or the presence of ectoparasites. A systemic postmortem was conducted, and different organ systems were examined for the presence of any lesions. The tissue samples from organs showing gross lesions such as the gallbladder, small intestine, and mesenteric lymph nodes were collected in 10% neutral buffered formalin. Mesenteric attachments were removed and the intestinal contents were flushed out to examine the lesions in the intestinal mucosa. Impression smears from intestinal and mesenteric lymph node lesions were prepared, heat-fixed, and stained with Ziehl-Neelsen (ZN) stain to test for the presence of acid-fast bacilli (AFB). Formalin-fixed tissues were embedded in paraffin wax, sectioned at 4  $\mu$ m thickness, and stained with hematoxylin and eosin (H&E).

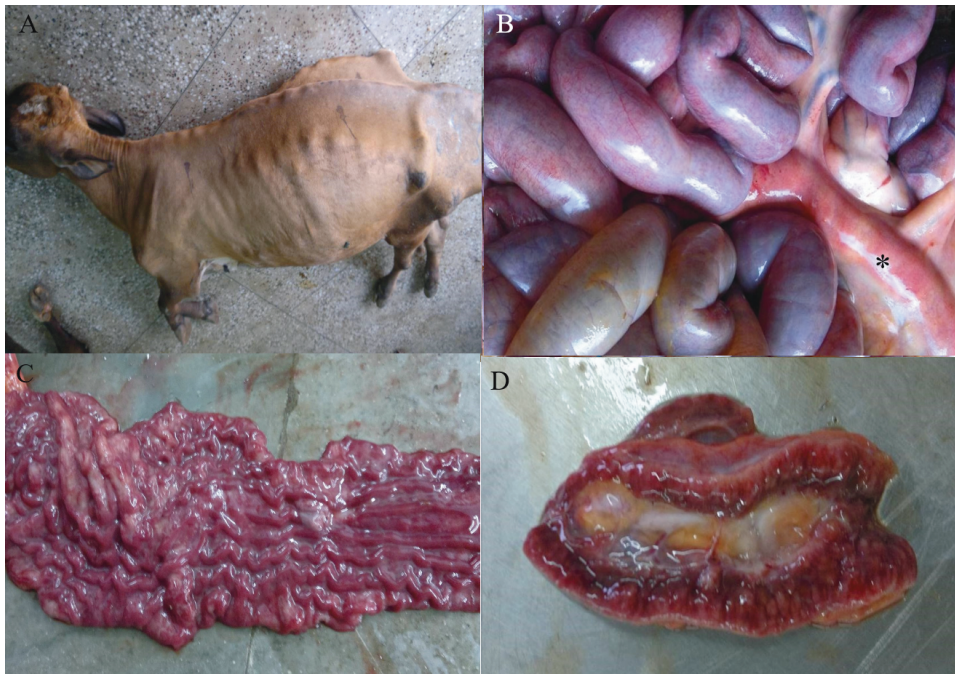
### 3. Results and discussion

JD in animals is classified into 4 stages based on the detection of MAP and the manifestation of clinical signs (6). Latent and subclinical stages are characterized by the absence of any clinical signs, with no to occasional detection of MAP in feces and MAP-specific antibodies in the blood, respectively. The clinical stage is characterized by emaciation, normal appetite, and periodic diarrhea. Advanced clinical stage, on the other hand, is characterized by extreme emaciation and permanent diarrhea. Based on the history of nonresponsive and persistent diarrhea, the animal in the present study could be classified into the advanced clinical stage of JD and would be expected to be a heavy shedder of MAP into the environment.

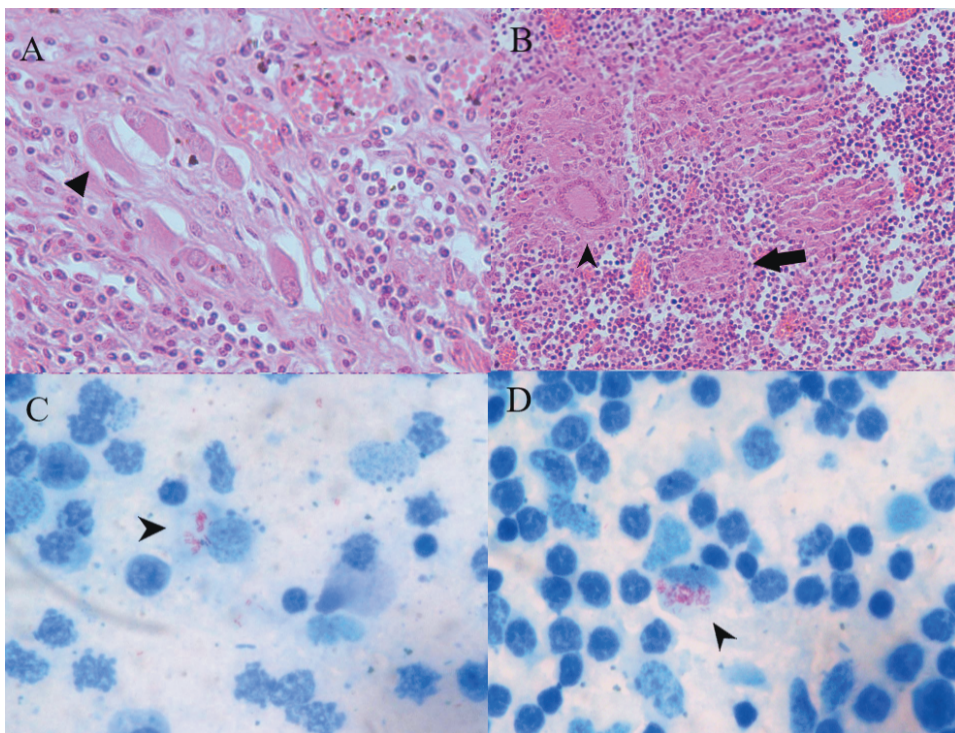
A thorough examination of the animal revealed the presence of full rigor mortis and stiffening of muscles in both forelimbs and hindlimbs. This early onset of rigor mortis may be attributed to the emaciated condition and low glycogen storage in the animal's body as a result of prolonged diarrhea. Eyes were sunken, revealing the dehydrated state of the animal. Body condition was hidebound, as ribs and spine were prominently visible (Figure 1A). No sign of ectoparasitism or external injury could be seen. The opening of the carcass showed generalized gelatinization of the visceral and peripheral fat. A small volume of straw-colored ascitic fluid was present in the peritoneal cavity. Mild emphysematous changes could be observed in the lungs. Marked yellowish staining of the perirenal fat was observed, and a dead fetus with no characteristic lesions was present in the uterus.

Striking lesions were present in the digestive system. The gallbladder was severely distended with blood mixed with bile. The opening of the gallbladder showed severely thickened walls with extensive hemorrhagic mucosa. Severe congestion of intestinal serosa, particularly in the terminal region of the small intestine, was observed (Figure 1B). When opened, the terminal region of the small intestine exhibited widespread congested mucosa accompanied by a corrugated appearance, which is characteristic of JD. The intestinal wall was severely thickened, with longitudinal folds that could not be relieved by stretching (Figure 1C). The mesenteric lymph nodes were congested and enlarged (Figure 1B). A cut section of the mesenteric lymph nodes revealed granulomatous/nodular lesions throughout the lymph node parenchyma (Figure 1D). The lesions were suggestive of JD, which is grossly characterized by the thickening and corrugation of the intestinal mucosa due to granulomatous enteritis, enlargement of mesenteric lymph nodes, and hepatic granulomas (7).

Histopathological examination revealed marked thickening of the intestinal wall due to infiltration of the mucosa and submucosa by macrophages and lymphocytes. Abundant epithelioid cells were present in the lamina propria of the villi and in between the intestinal crypts as scattered cells, as well as in cell nests (Figure 2A). Intestinal mucosal vessels were congested, and the submucosa was distended with edematous fluid. The villi were distended due to the accumulation of mononuclear cells and occasionally fused with each other. Multiple Langhans giant cells were observed in the lamina propria of the intestinal villi. The mucosa was infiltrated by a mixed population of macrophages and lymphocytes, whereas the submucosa was predominantly infiltrated by lymphocytes. Depending on the gut pathology and type of cell infiltration, JD has been described as of 3 different types: type 1, type 2, and type 3 (8). Gut lesions may vary with the formation of well-defined granulomas in Peyer's patches only (type 1 disease), Peyer's patches and overlying submucosa and mucosa (type 2 disease), and extension to the other regions of the gut along with the regional lymph nodes (type 3 disease). Type 3 lesions are further subclassified on the basis of the degree of cellular infiltration, cell type, and the number of AFB in the lesions (8). On the basis of the histopathological changes in the gut, the disease in the present study could be classified as type 3a disease. The most characteristic changes in the mesenteric lymph nodes include the formation of multiple microgranulomas. Multifocal clusters of epithelioid cells along with Langhans giant cells were observed in the cortical and paracortical areas of the mesenteric lymph nodes (Figure 2B). Furthermore, the majority of blood vessels were engorged with red blood cells, which was accompanied with mild dilatation of the paracortical



**Figure 1.** Characteristic gross morphological changes in cattle affected by JD. Hidebound condition (A); severe congestion of serosal capillaries in small intestine along with cording (asterisk) of mesenteric lymph nodes (B); marked thickening of the intestinal wall with typical longitudinal corrugations (C); multinodular appearance of mesenteric lymph node (D).



**Figure 2.** Characteristic histopathological changes in the intestine and mesenteric lymph node of cattle infected by JD. Accumulation of epithelioid cells in nest (arrowhead) in lamina propria of small intestine, H&E, 400 $\times$  (A); Langhans giant cells (arrowhead) and formation of microgranuloma (arrow) in the parenchyma of mesenteric lymph node, H&E, 200 $\times$  (B); multiple AFB in the cytoplasm of macrophages (arrowhead) in impression smear prepared from the small intestine, ZN, 1000 $\times$  (C); mesenteric lymph nodes, ZN, 1000 $\times$  (D).

sinuses. Extensive hyperplasia of lymphocytes in the mesenteric lymph nodes, which is a peculiar finding in JD cases, could not be observed in the present case. Histopathological changes in the gallbladder were in agreement with the gross pathological lesions. Pronounced thickening of the submucosal layer with fibrotic changes was observed. Mucosal and submucosal vessels of the gallbladder were noticeably dilated and engorged. However, no inflammatory response could be seen in the gallbladder, suggesting a chronic and compensatory reaction in response to a lesion elsewhere. No lesions were found in the liver. Similar findings with a lack of epithelioid microgranulomas in the livers of ewes affected with type 3a lesions have been reported (9). Additionally, depending on the load of MAP in lesions, JD has been classified into 2 phenotypes: paucibacillary (no or a low number of AFB) and multibacillary (high number of AFB) (8,10). ZN

staining of impression smears prepared from the intestinal mucosa and mesenteric lymph nodes revealed a large number of AFB within the macrophages (multibacillary form of the disease) (Figures 2C and 2D).

JD is not only an economically costly disease of ruminants but is also a major public health concern around the globe due to its close association with Crohn's disease in humans. Since the disease is considered incurable (11), its prompt and accurate diagnosis is important under field conditions to determine pathogen dissemination. Although several techniques are available for detection of JD, characterization of gross and histological lesions in animals suffering from clinical disease forms the most practical approach under field conditions and in laboratories lacking specific molecular facilities. Conclusively, gross and histopathological changes in cattle gut remain consistent and can be used for JD's diagnosis.

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