Diagnostic Exercise
From The Davis-Thompson Foundation*
Answer Sheet
Case #: **237**; Month: **May**; Year: **2024**

Title: Paratuberculosis in cattle

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A chronic disease in cattle occurred on a dairy farm in southern Brazil (28° 47' 49'' S 53° 13' 22'' W). The herd consisted of 600 black and white Holstein dairy cattle, of which 280 were lactating cows. The attendant veterinarian reported an illness that affected lactating cows. During our visit to the farm, three cows were affected and four had already died. Clinical signs included weight loss over a few weeks, chronic intermittent diarrhea (Fig.1), abrupt drop in milk production, and subcutaneous submandibular edema. The owner used to spray bovine fecal slurry produced at the farm to fertilize the pastures on which where he kept the dairy cows. One cow was euthanized and necropsied.

Figure 1. Dairy farm in southern Brazil. An affected cow (left) flanked by a clinically normal cow from the same herd of 280 lactating cows that had weight loss, abrupt drop in milk production, and chronic and intermittent diarrhea (*inset*).

Necropsy Findings: Necropsy revealed poor nutritional status with serous atrophy of the epicardial and perirenal fat, edema of the mesentery, especially the mesocolon, and mesenteric and hepatic lymphadenomegaly. The lymphatic vessels of the small intestinal serosa had a tortuous, beaded trajectory, and the intestinal serosa was slightly opaque and edematous (Fig.2).

Figure 2 Jejunum of a cow with Johne's disease. Serosal edema and lymphangitis. The inflamed lymphatic vessels appear as thickened cords (*arrow*) beneath the intestinal serosa.

The small intestinal wall was thickened, especially in the ileum. The mucosa in these areas was corrugated, thick, and moderately hyperemic, with transverse folds that could not be undone manually (Fig.3). The ileocecal valve was enlarged and corrugated. The cut surface of the swollen lymph nodes had white nodules throughout the cortex that contrasted with tan medullary areas. The bulging white regions correspond to granulomatous inflammation (Fig.4).

Due to the characteristic lesions, a PCR was performed on formalin-fixed, paraffin-embedded fragments of affected ileum. The PCR for IS900 and ISMap02 elements for *Mycobacterium avium* subspecies *paratuberculosis* was positive. Authenticity of the PCR was confirmed by nucleic acid sequencing.
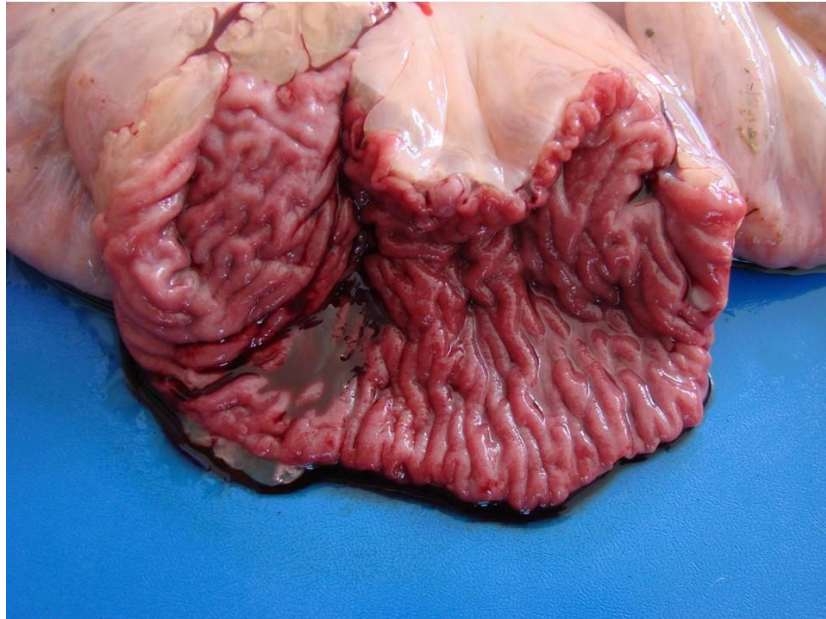
Figure 3. Ileum of an affected cow. The mucosa is corrugated, thick, and moderately hyperemic.
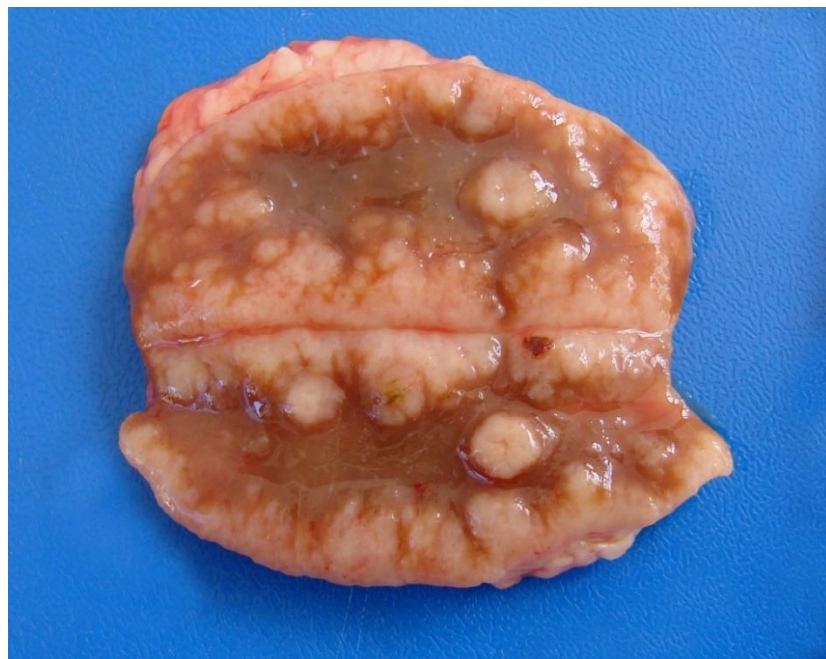

Figure 4. Mesenteric lymph node of an affected cow. White nodules are distributed throughout the cortex, contrasting to tan medullary areas.

Histologic Description

The jejunal and ileal lamina propria and submucosa were expanded by a marked inflammatory infiltrate consisting predominantly of epithelioid macrophages and multinucleated giant cells, with fewer plasma cells and lymphocytes, and rare eosinophils (Fig.5). Perivascular lymphocytes, plasma cells, and macrophages were present in the muscular layers.
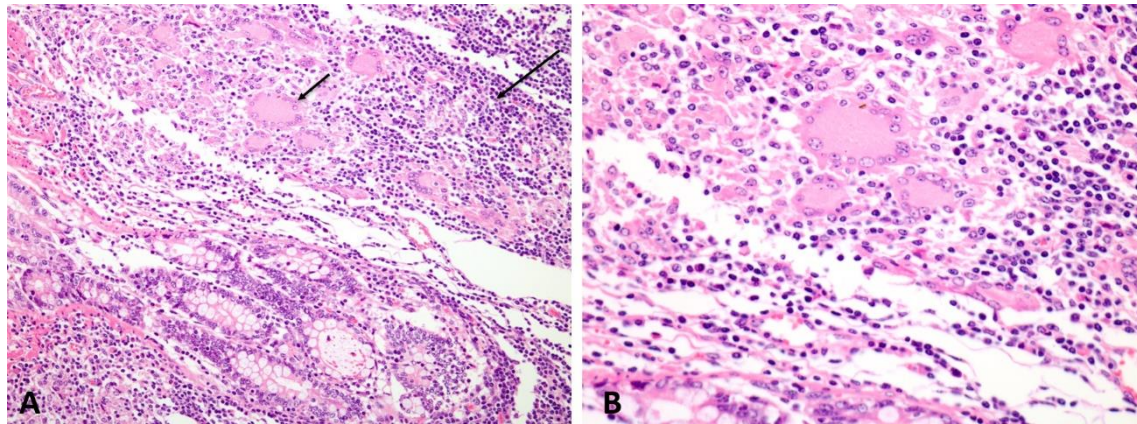
Figure 5. Jejunum of a cow with paratuberculosis. **A.** Inflammatory infiltrates in the mucosa consisting of multinucleated giant cells (*small arrow*) and lymphocytes (*long arrow*). **B**. Higher magnification of the section in A for details on the infiltrates.

Submucosal lymphoid follicles were hyperplastic and sometimes coalesced to form an extensive cellular mantle. Central lymphoid necrosis was occasionally present. In sections of jejunum and ileum subjected to Ziehl-Neelsen staining, acid-fast bacilli were observed within macrophages and multinucleated giant cells (Fig. 6). Granulomatous lymphangitis occurred in the intestinal and mesenteric lymphatic vessels. In and around the lymphatic walls, many lymphocytes, plasma cells, macrophages, and multinucleated giant cells formed the inflammatory infiltrate (Fig.7). Many of these inflammatory cells formed clusters that adhered to the valves and lymphatic endothelium, causing partial or total obstruction of the vessel lumen.
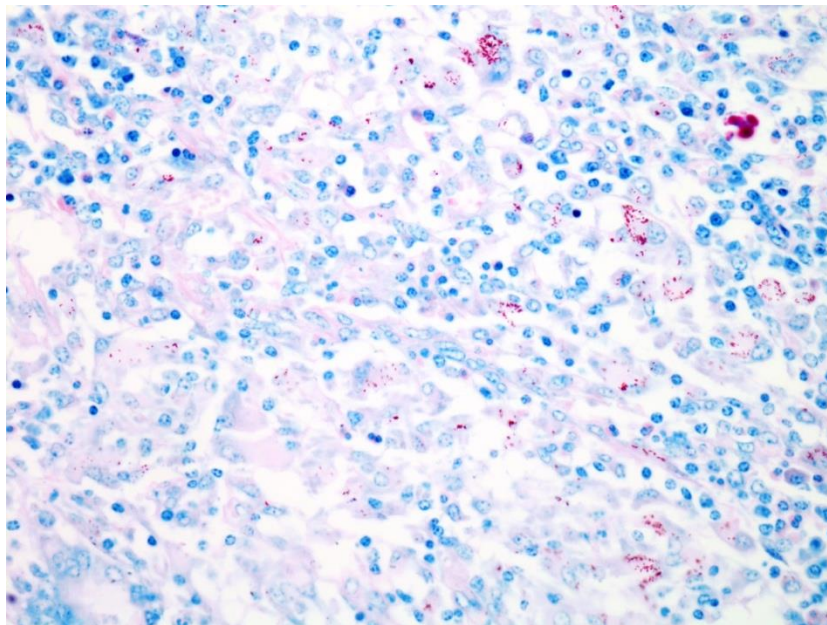

Figure 6. Paratuberculosis in a cow. Ileum. There are acid-fast bacteria in the cytoplasm of macrophages and multinucleated giant cells (Ziehl Neelsen stain).
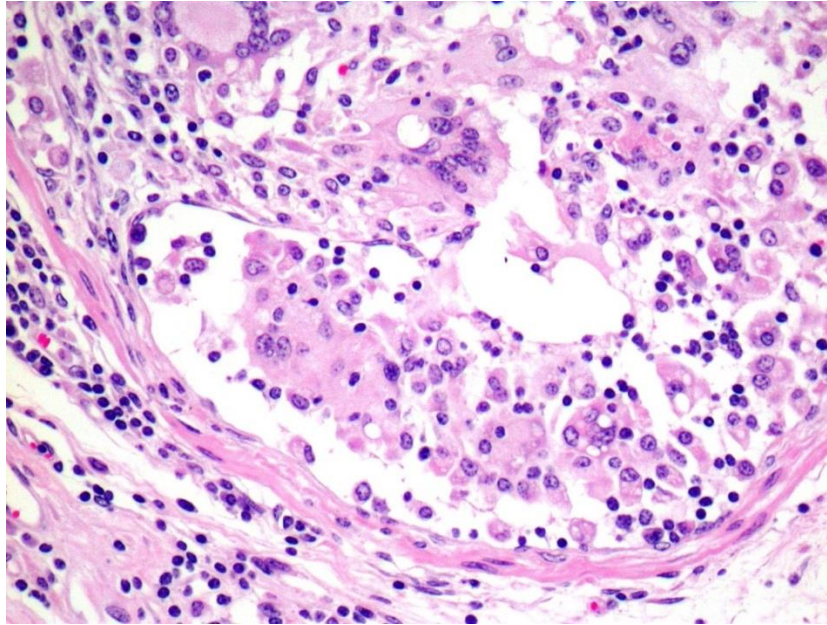
Figure 7. Subserosa of the ileum of a cow with paratuberculosis. Granulomatous lymphangitis with multinucleated giant cells.

Inflammatory infiltrates were less intense in the cecum. Random areas of hepatocellular necrosis and hemorrhage were observed in the liver and were associated with a discrete inflammatory infiltrate composed of macrophages, lymphocytes, and plasma cells. The cytoplasm of centrilobular hepatocytes had microvacuolar degeneration. In the hepatic lymph node, clusters of inflammatory cells consisted mainly of epithelioid macrophages and Langhans-type multinucleated giant cells. These clusters were observed in the marginal sinus, the sinus trabeculae, and the paracortical zone (Fig. 8). In some areas, the paracortical zone was obliterated by macrophages. There was dilation of the medullary cords. In the capsule, there were foci of lymphohistiocytic inflammatory infiltrates. In lymph node sections subjected to Ziehl-Neelsen staining, acid-fast bacilli were highlighted within macrophages and multinucleated giant cells. In the spleen, there were macrophages with granular, golden-brown pigment (hemosiderin) in the cytoplasm, which were located within the splenic cords.
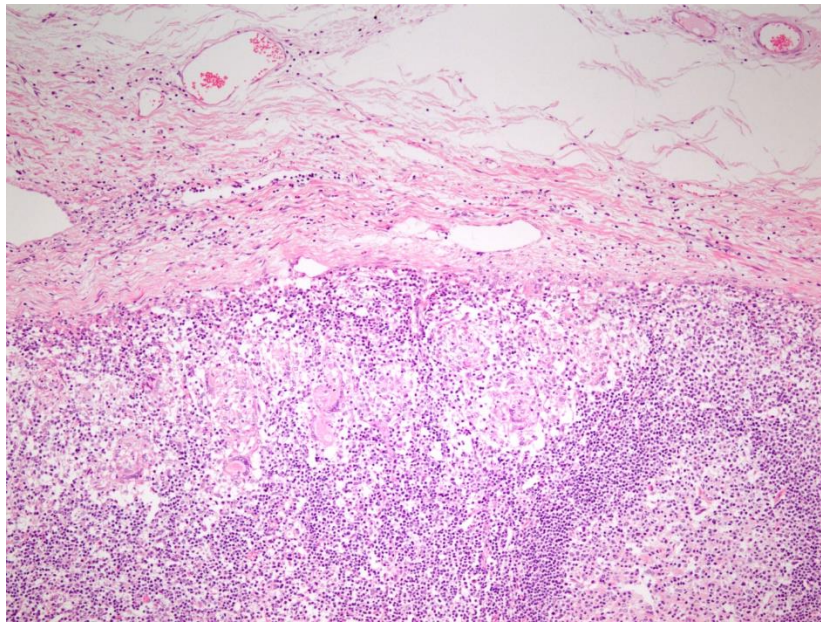
Figure 8 Mesenteric lymph node of a cow with paratuberculosis. The subcapsular sinus is obliterated by granulomatous inflammation with multinucleated giant cells.

Morphologic diagnoses:
● *Marked, diffuse granulomatous enteritis (jejunum and ileum) and lymphangitis with numerous intrahistiocytic acid-fast bacilli.*

● *Diffuse granulomatous mesenteric lymphadenitis with numerous intrahistiocytic acid-fast bacilli.*

Etiologic diagnosis:
● Mycobacterial enteritis

Etiology:
● *Mycobacterium avium* subsp. *paratuberculosis*

Name of the condition
● *Paratuberculosis*

Comments
Paratuberculosis (Johne's disease) is a chronic granulomatous enteritis of domestic ruminants (cattle, sheep, goats, camelids, and buffalo) and wild ruminants (cervids) that has a global distribution (2). The disease has also been reported in horses, donkeys, pigs, rabbits, stoats, foxes, and weasels (4). Paratuberculosis is caused by the intracellular bacterium *Mycobacterium avium* subsp. *paratuberculosis* (Table 1). The disease was initially reported from Germany as an atypical case of tuberculosis in a cow (5) and later named after the first author of that report.

The disease consists of chronic granulomatous enteritis caused by a slow-growing, difficult-to-cultivate bacteria. It results in enteric disease that leads to malabsorption and loss of essential proteins and nutrients by the damaged intestinal wall. Clinical signs include decreased milk production, progressive weight loss and wasting, diarrhea, and death (1).

The discovery of an insertion element (mobile genetic elements containing only genes related to insertion functions), IS900, specific for *M. avium* subsp. *paratuberculosis*, facilitates the detection and discrimination of this bacterium (1).

Table 1. **Characteristics of *Mycobacterium avium* subsp. *paratuberculosis*, the causative agent of paratuberculosis (**1,2**)**

Characteristic	Comment
Motility	No
Tinctorial features	Gram-positive, strongly acid fast
Shape	rod
Size of bacterium	2 μm x 0,5 μm
Presentation in feces and tissues	As clumps
Growth pattern	Slow
Special stains	Gram-positive, strongly acid-fast resistant
Special needs	Requires elevated levels of iron for multiplication; dependent on mycobactin (produce by other bacteria) used by the bacterium as an iron chelator
Resistance	270 days or more in water; 264 days in cattle feces; inactivated by most disinfectants

The slow development of the disease and the invisible and prolonged transition between stages of infection make it difficult to detect all infected animals in a herd. The concept "tip of the iceberg" adjusts to the epidemiology of paratuberculosis, as estimates point to the fact that when 5% of cattle in a herd have clinical disease, the frequency of infected subclinical cattle in the herd is 50% (6).

Paratuberculosis has a varying prevalence from country to country and within countries, with many nations yet to determine its extent (1). The available data, derived from slaughterhouse registries, bacteriological surveys, and antibody detection in serum or bulk-tank milk samples, paint a concerning picture. In Denmark, for instance, 9.8% of adult cattle were culture positive. A sample tank-milk ELISA was taken from 900 herds, and 70% were classified as positive. Serological testing in the Basque Country (Spain) revealed that 67% of cattle herds were positive. In Belgium, the seroprevalence was 17.4% at the herd level and 1.2% at the animal level. The true prevalence in the Netherlands was estimated to be between 30% and 70% at the herd level and between 2.7% and 6.9% at the animal level. Despite the methodological differences that complicate result comparison, the widespread prevalence of paratuberculosis in domestic livestock is a global concern (1).

Data on the prevalence of paratuberculosis in livestock in Brazil are sparse, but there are several reports of outbreaks from virtually every region. Most cases are in dairy cows, but cases have also been reported in beef cattle, sheep, goats, and water buffaloes (6). A high frequency of antibodies (70%) occurred in two farms in Northeast Brazil, where there were clinical cases of paratuberculosis. In 21 of 36 farms without a history of the disease, an average frequency of 10% was detected (6).

In certain regions of the USA, up to 30% of herds are infected, while other surveys in the USA and Canada suggest lower prevalence rates of 1.6% to 5%. The economic toll of paratuberculosis is staggering, with data from 1989 estimating annual losses exceeding $15 billion in the USA alone. These losses stem from the deaths of clinical cases and subclinical effects such as reduced weight gain, milk yield, and fertility, underscoring the urgent need for effective control measures (3).

The main problems associated with the lesions in paratuberculosis include (7):

● Lesions to the lining epithelium in the small intestine and disturbances in the proteins of the extracellular matrix of barrier systems in the small intestine mucosa.

● Compromise in the drainage of afferent lymphatic vessels in the lamina propria of villi of the small intestine, which disrupts the intestinal normal functioning.

● Injury to the monocyte-macrophage system and other cell populations in the lamina propria of intestinal villi.

Resulting gross lesions include granulomatous enteritis, mesenteric granulomatous lymphadenitis, and lymphangitis. Granulomatous enteritis most commonly affects the ileum and ileal-cecal junction; mesenteric granulomatous lymphadenitis is characterized by enlarged mesenteric lymph nodes that on cut surface have discrete and coalescing areas of white or yellow granulomatous inflammation. Mineralization of the intima of the ascending aorta and the endocardium of the left atrium occurs in up to 25% of affected cattle. The mechanism is related to activated-macrophage synthesis of vitamin D3 metabolites (1).

Young stocks ingest the bacterium in manure-contaminated fomites in the environment. In our outbreak, the infection most likely occurred due to the use of fecal slurry on the pasture where the cows were held. *M. avium* subsp. *paratuberculosis*, when swallowed, gains access via peristalsis to the intestines, binds to receptors on the surface of intestinal M cells, and is internalized. The time from the initial encounter with the bacterium to the expression of clinical disease is usually 12 months or longer (7).

The zoonotic potential of paratuberculosis has been speculated since *Mycobacterium avium* subsp. *paratuberculosis* was isolated, some forty years ago, from Crohn disease, a chronic inflammatory bowel disorder of human beings (1). The European Commission Directorate-General Health and Consumer reported that the currently available evidence is insufficient to confirm or to disprove that *M. avium* subsp. *paratuberculosis* is a causative agent of at least some cases of Crohn disease.

The diagnosis for clinical cases like we observed in this outbreak is less challenging. Chronic diarrhea and loss of weight are red flags. Culture can be performed in the feces and body tissues, and necropsy findings are characteristic (6). PCR further confirmed the diagnosis in our case.

Differential diagnoses for paratuberculosis include gastrointestinal endoparasitism, specially paramphistomosis and schistosomiasis, bovine virus diarrhea/mucosal disease, enzootic bovine leukosis, salmonellosis, and renal amyloidosis (2).

Two groups of diagnostic methods for paratuberculosis are available (1). The detection of *M. avium* subsp. *paratuberculosis* can be achieved by microscopic examination of fecal smears, culture of the organism from fecal samples, and detection by PCR. The second group consists of immunological tests such as enzyme-linked immunosorbent assay (ELISA), complement fixation test (CFT), agargel-immuno diffusion test (AGID), delayed type hypersensitivity test or intradermal test, and gamma-interferon test. Conventional bacterial culture of from fecal samples in advanced stages of the disease is highly sensitive.

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