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Johne's disease in a pygmy doe: A diagnostic dilemma

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DESCRIPTION

A 2-year-old pygmy doe presented for suspected neurologic signs, lethargy and a 3-day history of diarrhoea with normal appetite. Suspected neurologic signs reported by the owner included lying in dorsal recumbency for extended periods, isolating itself from the herd and a tremor in the hindlimbs. The doe was housed on a farm with approximately 20 goats. The doe was in thin body condition (2/5), and was determined to be neurologically appropriate by an examination in which no proprioceptive deficits were observed. Complete blood count showed marked leukocytosis characterised by neutrophilia with toxic change and a progressively decreasing haematocrit. Biochemistry panel revealed a progressive hypoproteinemia, characterised by moderately low total protein and albumin. Despite therapy, the doe's condition declined and was euthanased. An enzyme-linked immunosorbent assay (ELISA, MAP ab kit, IDEXX; performed by the Washington Animal Disease Diagnostic Lab)

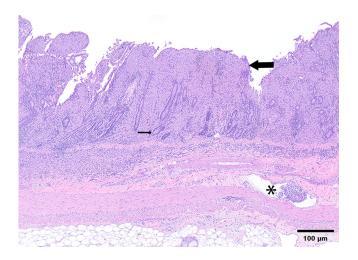


FIGURE 1 Histopathologic examination of the jejunum using H&E stain under low magnification (4×). The mucosal villi are thickened and blunted (large arrow), due to expansion of the lamina propria by sheets of epithelioid macrophages, separating intestinal crypts (small arrow), and extending into the submucosa. A dilated submucosal lymphatic vessel contains macrophages (asterisk).

LEARNING POINTS/TAKE-HOME MESSAGES

- Histopathologic examination of granulomatous inflammation with positive acid-fast staining of the intracytoplasmic organism and a positive test result by another ancillary test (i.e., culture, polymerase chain reaction) is the gold standard for definitive diagnosis of Johne's disease.
- Direct faecal polymerase chain reaction is a superior ancillary test compared to the enzyme-linked immunosorbent assay for detecting Johne's disease, particularly in subclinical patients. However, polymerase chain reaction is only superior if the animal is actively shedding the *Mycobacterium avium* subspecies *paratuberculosis* organism, and herd status should be taken into consideration.
- The lengthy and variable timeline of Johne's disease makes antemortem diagnosis challenging, especially in subclinical animals.

for *Mycobacterium avium* subspecies *paratuberculosis* (MAP), the causative agent of Johne's disease, was negative.

On postmortem examination, the ileal and jejunal mucosa was coarse and thickened. Mesenteric lymph nodes lacked corticomedullary distinction and had 5–10 mm diameter white, soft foci, corresponding to areas of necrosis and granulomatous inflammation histologically.

On histopathologic examination, the jejunum had blunt, thickened mucosal villi, expanded by sheets of epithelioid macrophages and dilated submucosal lymphatic vessels (Figure 1). Acid-fast staining confirmed the presence of intracytoplasmic bacilli in the jejunum, ileum, caecum and the surrounding mesenteric lymph nodes (Figure 2).

Clinical signs in this case were attributed to granulomatous enteritis and typhlitis caused by MAP organisms. A direct faecal PCR (internal test, Iowa State University Veterinary Diagnostic Laboratory) submitted after postmortem examination confirmed MAP organisms. Johne's disease is common

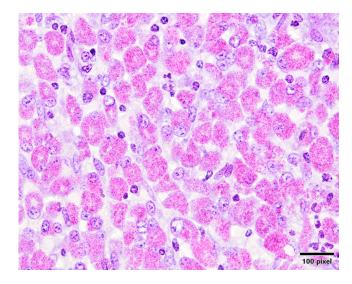


FIGURE 2 Histopathologic examination of the jejunum using acid-fast stain under high magnification (60×). The macrophages are filled with intracytoplasmic bacilli that stain bright pink-red. Similar intracytoplasmic bacilli were in the ileum and caecum.

in goats, and clinical signs manifest around 2–10 years of age.¹ ELISA is commonly marketed as a screening tool to identify subclinical infections.² However, antibody production should likely occur later in the disease process when clinical signs are present. As such, a false-negative ELISA result in a patient with clinical signs is unexpected versus a subclinical patient. ELISA has a lower sensitivity when compared to faecal PCR, especially in subclinical patients.^{2,3} Clinicians should consider all clinical and diagnostic information (taking into account the entire clinical picture including: history, clinical signs, ancillary testing, diagnostic test results, etc.) available when making diagnostic interpretations of Johne's disease status in goats.

AUTHOR CONTRIBUTIONS

Madison C. Callicott and Kaitlyn E. Upton initially drafted the manuscript. Joe S. Smith and Camille Cordero edited later drafts. Madison C. Callicott, Kaitlyn E. Upton and Joe S. Smith managed this case clinically and acquired antemortem data. Camille Cordero acquired postmortem data. All authors reviewed, edited and approved final manuscript.

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CONFLICT OF INTEREST STATEMENT The authors declare they have no conflicts of interest.

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ETHICS STATEMENT

No approval required due to the descriptive and retrospective nature of this manuscript.

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MULTIPLE-CHOICE QUESTION

What stain is best used to identify the causative agent of Johne's disease on histopathology?

POSSIBLE ANSWERS TO MULTIPLE-CHOICE QUESTION

- A: Gram stain
- B: Acid-fast stain
- C: Prussian blue stain
- D: Congo red stain
- E: Romanowsky stain

CORRECT ANSWER

B: Acid-fast stain.

This is the ideal staining method for any acid-fast bacteria, including the genus *Mycobacterium*. Samples are initially stained red using carbol fuchsin, and then decolorised with an acid. However, acid-fast bacteria have a large amount of lipoidal material in their cell walls that do not allow decolorisation, leaving them stained red as in the image above.