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WHAT IS YOUR DIAGNOSIS?

What is your diagnosis? Cecal smear in a peafowl

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1 | CASE PRESENTATION

A 7-year-old intact female peahen was submitted to necropsy for weight loss at the Warrenton Animal Health Laboratory. No additional patient history was provided, and the specific genus and species are not known. Gross examination revealed a moderately prominent keel bone and decreased internal fat tissue. The cecum was dilated, with a thickened wall, and was filled with a white caseous material intermixed with feces. An impression smear of the cecum was performed and submitted to the Virginia-Maryland College of Veterinary Medicine for cytologic evaluation.

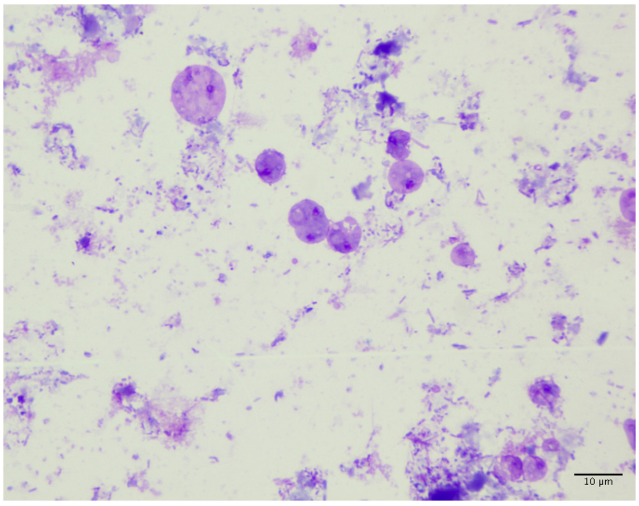

FIGURE 1 Wright Giemsa; ×100 objective, luminal cecal smear from a peafowl.

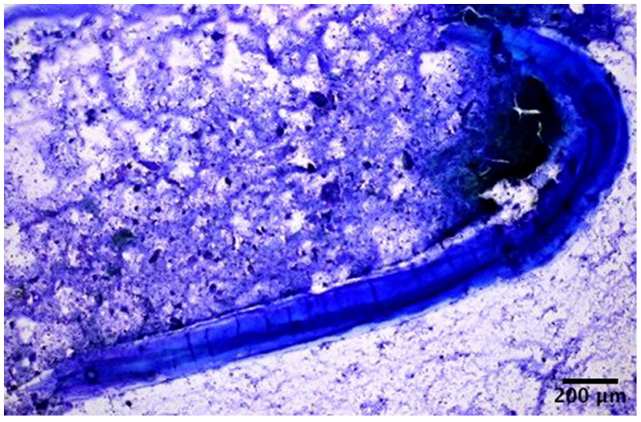

FIGURE 2 Wright Giemsa, ×4 objective, cecal smear from a peafowl.

Cytologic interpretation: Protozoal infection (presumed *Histomonas meleagridis*) with nematodosis.

The smear was of very low cellularity and good diagnostic quality and contained rare heterophils intermixed with frequent protozoal structures. The protozoal structures were ameboid (round to ovoid), measuring 8-12 µm in diameter with pale blue to eosinophilic cytoplasm, with an occasional area of central pallor. The nucleus was small (1-2 µm in diameter), eccentrically located, and irregularly rounded with an open chromatin pattern. The nucleolus is indistinct. No flagella were observed (Figure 1). Rare nematodes were also seen and measured 0.4-0.5 cm in length and 196 µm in width with a tapered end (Figure 2). Lastly, there was a plethora of bacteria amid a proteinaceous blue background with a large amount of debris. The bacteria represented a mixed population and were predominantly rod-shaped with fewer cocci.

2 | ADDITIONAL RESULTS

On histopathology, there was transmural ulcerative to granulomatous typhlitis. The mucosa was multifocally effaced and covered with a thick pseudomembrane composed of necrotic debris, degenerate heterophils, fibrin, numerous bacterial colonies composed of bacilli, and multiple nematodes with typical lateral alae, musculature wall, pseudocoelom, and intestine. Within the remaining mucosa and along the ulcerated areas, a dense granulomatous inflammation is dissected into the muscular layer of the cecum. In the cytoplasm of macrophages, low numbers of round (10 to 15 µm diameter) protozoal structures, with paracentral small nuclei, were noted. Inflammation reached the submucosa and was dissected between the bundles of smooth muscle. The diagnosis was typhlitis, ulcerative (pseudomembranous) to granulomatous, severe, chronic, with intralesional protozoal trophozoites (Figure 3; morphology suggestive of *Histomonas* spp.), nematodes (Figure 4), and bacteria.

DNA was extracted from formalin-fixed paraffin-embedded cecal tissue (QIAamp DNA FFPE Tissue Kit, Qiagen, Germantown, MD USA). The internal transcribed spacer region was amplified using previously described primers and PCR conditions, modified using LongAmp Taq 2X Master Mix (New England BioLabs, Ipswich, MA, USA), and two rounds of PCR amplification.[2,3] The amplicon was Sanger-sequenced bidirectionally (Genomics Sequencing Center, Fralin Life Sciences Institute at Virginia Tech, Blacksburg, VA USA). The 353-bp fragment exhibited 97.5% sequence identity to *Histomonas meleagridis* isolate NCT1 (NCBI Accession HQ540394.1), confirming the identification of *Histomonas meleagridis* in this patient. The percent identity of the ITS fragment to other histomonads, such as *Histomonas* spp. GABB4 (Accession HQ334180.1), and related protozoa, such as *Dientamoeba fragilis* and *Tritrichomonas* spp., were all <90%.

The gross, cytologic, and histopathologic findings support a diagnosis of blackhead disease. Molecular testing confirmed the diagnosis of blackhead disease due to *Histomonas meleagridis* and the presumed intermediate host, cecal nematodes.[3]

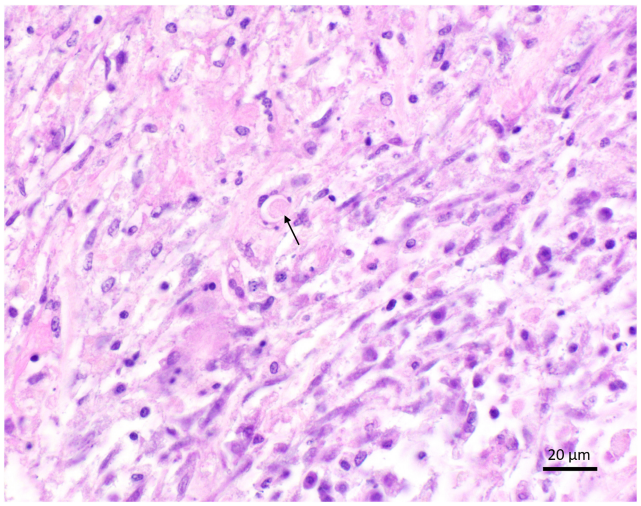

FIGURE 3 Hematoxylin and eosin (H&E), ×60 objective. Cecal tissue from a peafowl. Against a background of postmortem autolysis, there are numerous macrophages and elongated cells (interpreted as fibroblasts, as part of granulation tissue) effacing the cecal mucosa. Some of the macrophages contain round trophozoites (arrow).

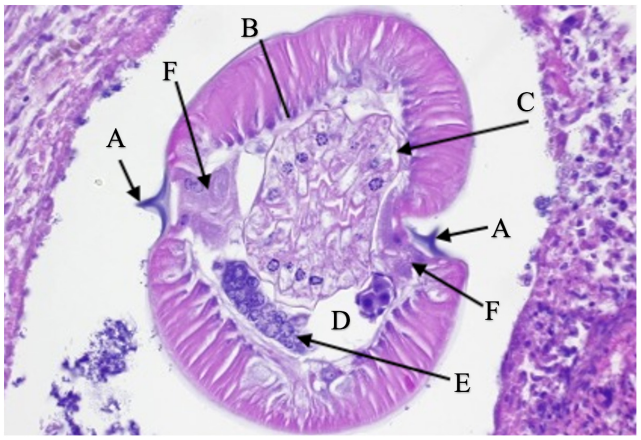

FIGURE 4 Hematoxylin and eosin (H&E), ×60 objective. Cecal tissue highlighting a cecal nematode in a peafowl. Nematode structure as labeled: (A) Lateral alae; (B) Coelomyarian musculature; (C) Intestine; (D) Pseudocoelom; (E) uterus filled with eggs; (F) Lateral cords.

3 | DISCUSSION

Histomonosis (blackhead disease) is caused by the protozoal organism *Histomonas meleagridis*. This protozoal disease is reported in gallinaceous birds with a mortality rate ranging from 80%-100% in turkeys and 10%-20% in chickens and can be higher in young birds.[4,5] Chickens can serve as asymptomatic carriers resulting in the recommendation that turkeys and chickens be reared separately.[5] Additionally, the lack of effective treatment options and the potential for high economic loss make environmental prevention highly

important.[4] Blackhead disease is reported uncommonly in peafowls but remains an important differential diagnosis in these species.

Transmission of histomonosis can be direct via cloacal drinking or an intermediate host. Commonly, the intermediate host is a poultry cecal worm, *Heterakis gallinarum*.[6] When the cecal worm is involved, the parasitic cycle begins in the cecum, where *H. gallinarum* incorporates the protozoan into its embryonated egg, protecting the organism. The infected egg can then be ingested by a novel host bird or enter a paratenic host (eg, earthworm), which may then be ingested by naïve birds.[5,6]

Infection with *H. meleagridis* relies on bacterial flora for the replication portion of its life cycle. Some intestinal bacteria (eg, *Escherichia coli*) have been implicated as being superior in their supportive role to this protozoal organism. The protozoa rely on bacteria for nutrients via phagocytosis of bacteria and the uptake of bacteria-produced proteins and metabolites.[7]

Most cases of histomonosis are diagnosed histologically, as clinical signs are non-specific and may include the following: lethargy, decreased appetite, depression, drooped wings, and watery, sulfur-yellow feces with blood or mucus. Gross pathology is often variable but can include an engorged cecum with sloughed mucosa, a thickened cecal mucosa with caseous cores, and ulceration and multifocal to coalescing areas of necrosis and granulomatous inflammation in the liver (often referred to as "bulls eye-like" lesions). In histologic samples, the protozoal organisms can be observed with routine stains and are identified most commonly in the liver or cecum.[6] However, Histomonads may stain poorly in some routine hematoxylin and eosin-stained sections. In these cases, periodic acid-Schiff (PAS) or Giemsa stain may be helpful in identifying the organisms.[1]

H. meleagridis must be differentiated from several other intestinal protozoa hosted in gallinaceous birds such as *Tetratrichomonas gallinarium*, *Trichomonas gallinae*, and *Histomonas wenrichi*. Cytologically, *H. meleagridis* can be observed in two forms, ameboid or flagellated, with a diameter of 8-12 μm. Uncommonly, larger diameters of up to 20 μm are reported in the literature. When flagellated, a single flagellum is expected, though, during cellular division, two flagella can be seen. In contrast, *H. wenrichi* is also observed in ameboid or flagellated forms with a larger diameter of 20-30 μm and 3-4 flagella. Differentiating *H. meleagridis* and *H. wenrichi* is important because the latter is considered non-pathogenic. Both species of *Histomonas* can be transmitted by the same cecal nematode, *H. gallinarum*.[3] *T. gallinae* are ovoid or pyriform with a diameter of 7-11 μm and contain four anterior and a single posterior flagellum (not free). *T. gallinarum* is pear-shaped with a diameter of 6-15 μm and contains four anterior and a single, free posterior flagella.[8]

Cytologically, *T. gallinarum* and *T. gallinae* can be differentiated from *Histomonas* spp. based on their size and number of flagella. However, given the overlap in the cytologic appearance of *Histomonas* spp., it is recommended to support a diagnosis of *H. meleagridis* using PCR. Histopathology is also useful in demonstrating the invasion of the organism into lesions, indicating pathogenicity.

To the authors' knowledge, this is the first report of histomonosis observed on cecal cytology with the presence of the cecal nematode intermediate host. The lack of cytological reports of cecal histomonosis is likely due to the fact that the diagnosis is more commonly made from necropsy tissue samples rather than cytologic antemortem samples.

CONFLICT OF INTEREST STATEMENT

We have no conflicts of interest to disclose.

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KEYWORDS

blackhead disease, cecal cytology, *Heterakis gallinarum*, Histomonosis, *Histomonas meleagridis*

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