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Cytopathological diagnosis of histomoniasis in two-week-old native chicks

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ABSTRACT

Histomonas meleagridis is the causative agent of histomoniasis (histomonosis), a significant protozoal illness of gallinaceous birds that mostly affects the liver and caeca and is typically linked to *Heterakis gallinarum*. In turkeys, the illness is particularly severe. Although they are comparatively less impacted, chickens may have clinical illness. In this case study, two-week-old native chicks from a private chicken farm in the Chittoor area of Andhra Pradesh, India, were found to have histomoniasis. The afflicted flock displayed anorexia, sadness, dullness, and sulfur-colored droppings before dying. Twelve chicks had swollen, thickened, and inflamed caeca with greenish material, elevated ulcerative lesions, and *Heterakis gallinarum* worms upon postmortem examination. Numerous clearly defined circular to ring-shaped necrotic lesions were present in the enlarged and congested liver. Numerous amoeboid to round or ovoid protozoal organisms, measuring around 8–12 µm, with pale blue to eosinophilic cytoplasm and small, eccentrically positioned nuclei with open chromatin, were seen in Field's stain of impression smears of hepatic and caecal lesions. There were no visible flagella. The presence of *H. gallinarum*, typical gross lesions, and cytomorphological characteristics were used to diagnose histomoniasis. Drinking water containing 25 mg/kg body weight of metronidazole for five days in a row gradually decreased mortality starting on the third day and produced clinical recovery on the fifth. The existence of histomoniasis in very young native chicks and the diagnostic value of the association between gross pathological and cytological results are highlighted in this case study.

Keywords: Histomoniasis, *Histomonas meleagridis*, Native chicks, Cytopathology, Cytological diagnosis, Caecal lesions, Hepatic necrosis, *Heterakis gallinarum*, Field's stain, Metronidazole, Poultry disease

Introduction

The protozoan *Histomonas meleagridis* is the cause of histomoniasis, a serious parasite disease of gallinaceous birds that is also known as histomonosis, blackhead disease, infectious enterohepatitis, or typhlohepatitis (McDougald, 2005; Mitra *et al.*, 2018; Beer *et al.*, 2022) [1, 2, 3]. Due to its severe clinical presentation and economic impact in vulnerable birds, the disease has been identified for over a century and continues to be a significant health issue in chicken industry (McDougald, 2005; Mitra *et al.*, 2018) [1]. Although "blackhead disease" is commonly used, the word "histomonosis" is seen to be more acceptable for scientific use because dark or cyanotic staining of the head is not a consistent or pathognomonic sign of infection (Beer *et al.*, 2022) [3].

Causative agent

A flagellated protozoan parasite in the order Trichomonadida, *Histomonas meleagridis* is the causative agent of histomonosis in chicken (Mitra *et al.*, 2018; Beer *et al.*, 2022) [2, 3]. Although pheasants and peafowl are among the gallinaceous birds that can contract the parasite, turkeys and chickens are the main hosts (Mitra *et al.*, 2018; Beer *et al.*, 2022) [3]. While chickens are typically more resistant and can experience less severe or subclinical infections, turkeys are particularly vulnerable to the disease and can develop severe clinical disease with substantial mortality (McDougald, 2005; Mitra *et al.*, 2018; Beer *et al.*, 2022) [1, 2, 3]. However, because they may aid in the parasite's maintenance and spread throughout the poultry community, infected chicks may be of epidemiological significance. The most significant feature of histomoniasis is its intimate epidemiological association with *Heterakis gallinarum*. *H. meleagridis* may be present in the nematode eggs, allowing the protozoan to survive in the environment for a far longer amount of time than it could on its own. The main way that birds contract the infection is by eating embryonated *Heterakis* eggs that carry the protozoan. In order to spread the infection, earthworms may also act as paratenic hosts, consuming *Heterakis* eggs before being eaten by vulnerable birds.

Transmission and epidemiology

The close correlation between the epidemiology of histomonosis and the cecal nematode *Heterakis gallinarum* is one of its most prominent characteristics. An significant pathway for environmental persistence and bird-to-bird transmission of *H. meleagridis* is through embryonated *H. gallinarum* eggs that include the protozoan (McDougald, 2005; Cupo *et al.*, 2019) [1, 4]. Controlling *Heterakis gallinarum* is crucial to preventing histomonosis because it has a straightforward life cycle and is widely distributed in gallinaceous birds (Cupo *et al.*, 2019) [4]. Ingesting feed, water, litter, soil, or nematode eggs tainted with *H. meleagridis* can infect susceptible birds. Particularly in outdoor and free-range chicken production systems, earthworms and other invertebrates can act as paratenic hosts, aiding the parasite's survival and spread (Cupo *et al.*, 2019) [4]. The epidemiology of histomonosis is more complex than a straightforward parasite–nematode interaction, as evidenced by experimental evidence for direct transfer of *H. meleagridis* between turkeys in addition to transmission via *H. gallinarum* eggs (McDougald *et al.*, 2005) [5].

Pathogenesis

H. meleagridis travels to the ceca and infiltrates the cecal mucosa after infection. The parasite causes tissue damage and an inflammatory reaction as it grows inside the cecal mucosa. Caseous material buildup in the cecal lumen and widespread necrosis are possible outcomes of progressive lesions of the cecum. From the ceca, the organism can enter the portal circulation and proceed to the liver, where it produces distinctive regions of hepatic necrosis (McDougald, 2005; Beer *et al.*, 2022) [1, 3]. The outcome of an infection is also largely determined by the host immune response, with variations in the cellular and intestinal immune responses between turkeys and chickens contributing to variations in the severity of the disease (Mitra *et al.*).

Clinical significance

Depression, anorexia, ruffled feathers, drooping wings, diminished growth, frailty, dehydration, and unusual yellowish or sulfur-colored droppings are all possible clinical manifestations in affected birds. Severe infections can cause increasing weakening and mortality, especially in turkeys, which can lead to significant flock losses (McDougald, 2005; Beer *et al.*, 2022) [1, 3].

Characteristic pathological lesions

The liver and ceca have the most distinctive gross lesions. There may be an accumulation of caseous yellowish-white or greenish material, ulceration, thickening, inflammation, and enlargement of the ceca. In extreme circumstances, the cecum's contents could turn into cores. Numerous distinct, round or irregular necrotic foci are typically seen in the liver (McDougald, 2005; Beer *et al.*, 2022) [1, 3]. Their appearance is a key diagnostic characteristic of histomoniasis, and they are frequently referred to be target-like or ring-shaped. The gross pathological and cytological findings of histomoniasis in two-week-old chicks are described in this case, with particular attention to the detection of protozoal entities in impression smears from hepatic and cecal lesions.

Methods

a flock of two-week-old chicks during a two-week mortality period. Before they died, the afflicted birds displayed clinical symptoms of dullness, anorexia, sadness, and the passage of sulfur-colored droppings. Twelve dead birds in all were sent for post-mortem analysis to determine the most likely cause of death. Every bird that was presented underwent a thorough post-mortem inspection. On sterile glass slides, representative impression smears were created from the liver and caecal lesions. After air drying, smears were fixed in methanol for one minute. Field's stain was applied to the fixed smears. Field's stain A was applied for 30 seconds and Field's stain B for 60 seconds. Using a 100× oil-immersion objective lens, the dyed slides were examined under a microscope to determine the identity and morphological traits of possible protozoal organisms.

Results

Case history

For almost two weeks, a flock of native chicks from a private chicken farm in the Chittoor area of Andhra Pradesh, India, shown mortality. The chicks were about two weeks old. Affected birds perished after exhibiting clinical symptoms of depression, dullness, and anorexia. A thorough post-mortem and cytological examination were performed to ascertain the cause of death because the clinical history indicated an infectious illness of the liver and caeca.

Postmortem findings

Upon postmortem examination, the lumen had a lot of greenish material and the caeca were swollen, inflammatory, and enlarged. On the mucosal surface of each caecum, two to three circular, elevated ulcerative lesions were seen. The caecal lumen also contained morphologically roundworms that resembled *Heterakis gallinarum*. Numerous clearly defined circular to ring-shaped necrotic depression lesions with a diameter of around 1-2 cm covered the surface of the enlarged and congested liver. The neighboring, seemingly normal liver parenchyma was clearly distinguished from the hepatic tumors. A provisional diagnosis of histomoniasis (histomonosis) in the afflicted chicks was strongly supported by characteristic multifocal hepatic necrotic lesions, caecal ulceration, and caecal worms (*Heterakis gallinarum*).

Cytological examination

The damaged liver and caecal lesions were used to create impression smears, which were then microscopically inspected using a 100× oil-immersion objective lens. Numerous amoeboid-shaped protozoal organisms were visible in the smears. The majority of the creatures had a diameter of 8–12 µm and were spherical to ovoid. With sporadic central pallor, the cytoplasm was pale blue to eosinophilic. The nucleus was tiny, eccentrically positioned, irregularly spherical, and had an indistinct nucleolus and an open chromatin pattern. There were no flagella visible in the examined cytological preparations. Based on the observed morphological traits and their location within the hepatic and caecal lesions, which were linked to the presence of *Heterakis gallinarum* worms and distinctive gross pathological findings, the protozoal organisms were morphologically consistent with *Histomonas meleagridis*.



Figure 1. Gross pictures: A, B, C depicts circular to ring shaped necrotic depressed lesions in the liver (arrows); D and E depicts circular elevated ulcerative lesion in the caecum; F indicates *Heterakis gallinarum* worms (arrows).

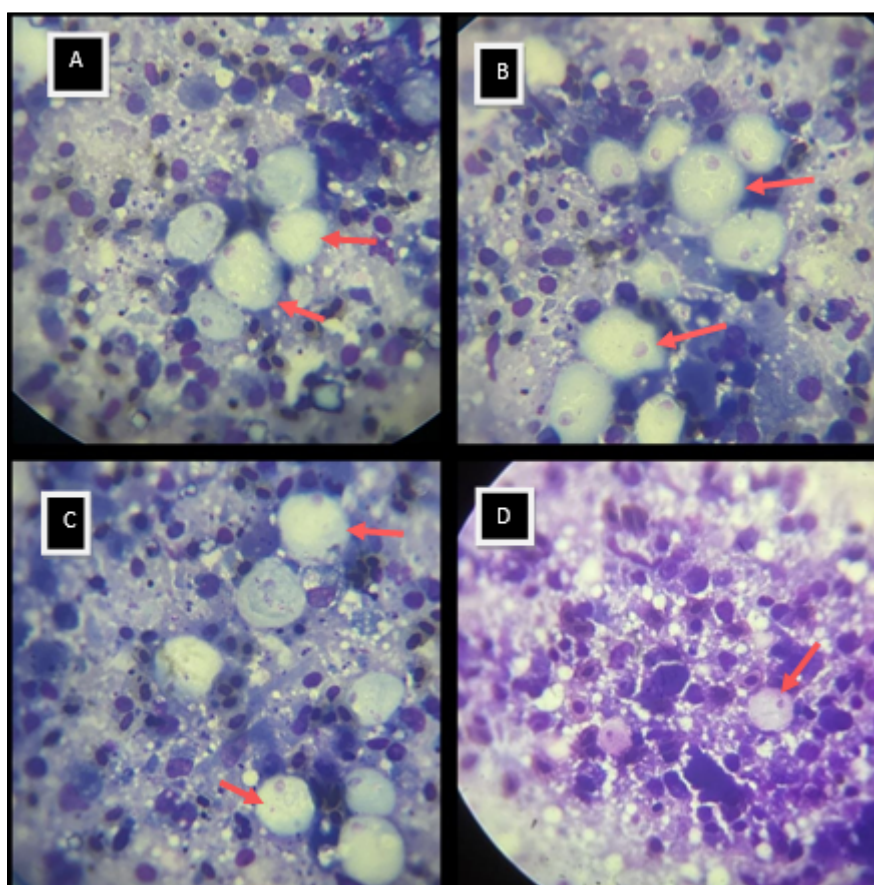


Figure 2. Cytological pictures: A, B, C, D impression smears of liver and caecum, revealed numerous amoeboid shaped organisms which are spherical to ovoid with sporadic central pallor, the cytoplasm was pale blue to eosinophilic, the nucleus was tiny and eccentrically positioned with indistinct nucleoli.

Diagnosis

The case was diagnosed as *Histomonas meleagridis*-caused Histomoniasis (Blackhead Disease) in Poultry, based on the discovery of suitable amoeboid protozoal organisms in liver and cecal impres-

sion smears, the presence of *Heterakis gallinarum*, the clinical history, and distinctive hepatic and cecal lesions.

Treatment and outcome

For five days in a row, the afflicted flock was given metronidazole via drinking water using Metro Pet Liquid at a dosing rate of 25 mg/kg body weight. From the third day of treatment, a progressive reduction in mortality was noted, and clinical symptoms were gradually going away. All afflicted birds had made a clinical recovery by day five of treatment, and no more mortality was seen.

Discussion

The protozoan that causes histomoniasis (also known as histomonos) A serious illness that mostly affects the liver and caeca in gallinaceous birds is *Histomonas meleagridis*. While hens are relatively more resistant, they can develop clinical infection, particularly in favorable epidemiological conditions (McDougald, 2005; Beer *et al.*, 2022) [1, 3]. In contrast, turkeys are highly vulnerable and can develop severe disease with substantial mortality. The mortality of two-week-old native chicks makes the current situation noteworthy. Anorexia, dullness, depression, and sulfur-colored stools were all clinical indicators of histomonos. A post-mortem examination showed an enlarged and congested liver with distinctive numerous, well-defined, circular to ring-shaped necrotic lesions, as well as thickened, inflamed caeca with greenish material and elevated ulcerative lesions. This kind of concurrent hepatic and caecal lesions is thought to be indicative of an *H. meleagridis* infection (McDougald, 2005; Beer *et al.*, 2022) [3].

Since *H. gallinarum* is crucial to the persistence and spread of *H. meleagridis*, the discovery of caecal worms suggestive of *Heterakis gallinarum* offered additional epidemiological support. By serving as paratenic hosts, earthworms may also aid in transmission (Cupo *et al.*, 2019; Beer *et al.*, 2022) [4, 3]. Cytological analysis of impression smears from the liver and caeca revealed a large number of amoeboid to round/ovoid protozoal organisms with small eccentric nuclei and pale blue to eosinophilic cytoplasm, measuring about 8–12 µm. These tissue preparations' lack of flagella was in line with the mostly amoeboid type linked to tissue invasion. A diagnosis of histomoniasis was highly supported by the cytomorphological results, the presence of *Heterakis*, and the distinctive physical lesions (Beer *et al.*, 2022) [3]. Metronidazole 25 mg/kg body weight administered in drinking water for five days in a row reduced mortality starting on the third day and appeared to improve clinically on the fifth. However, in the lack of parasitological or molecular confirmation after therapy, the therapeutic response should be regarded as a clinical observation rather than definitive proof of cure. In summary, the current case highlights the importance of including histomoniasis in the differential diagnosis of young chicks, despite the fact that they are comparatively less vulnerable than turkeys. It also shows the diagnostic value of correlating clinical signs, distinctive gross lesions, *Heterakis* infection, and cytological findings.

Conclusion

Histomoniasis should be considered as a differential diagnosis of mortality and hepatic–caecal lesions in young native chicks, however chickens are less susceptible than turkeys. In the present case, characteristic caecal ulceration, multifocal hepatic necrosis, presence of *Heterakis gallinarum* and presence of amoeboid protozoal organisms in impression smears were highly suggestive of the diagnosis. This case exemplifies the utility of cytological examination as a rapid and practical ancillary diagnostic approach when correlated with clinical and gross pathological findings. Early diagnosis and proper management may help reduce mortality and economic losses associated with affected poultry flocks.

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