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An Outbreak of Chicken Histomoniasis in the Absence of Normal Vectors

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Abstract : Acute and massive death was noted in 10-week-old chickens, broiler breeder, housed in the floor pens. The number of dead chickens exceeded 20 birds each day. Grossly, fibrinous peritonitis with adhesion of mesenteries and intestinal organs was noted. The ceca were enlarged, expanded, and thickened with congestion. Cecal lumen was distended with a caseous core composed of serous, fibrinopurulent, and hemorrhagic exudates with desquamated masses of epithelial cells. The liver had multifocal white irregular necrotic foci surrounded by a raised ring. Light and electron microscope revealed *Histomonas meleagridis* in the liver with its characteristic structures and not in the intestinal mucoca and submucosa. In this case, the examination of parasite, larvae and egg was conducted more carefully; however, we could not find eggs or worms of *Heterakis gallinarum* in the dead or live chickens and earthworms in the soils of floor pens. Therefore, we concluded that an outbreak of blackhead disease probably occurred by direct transmission of histomonads from chickens to chickens in this case.

Key words **:** Histomoniasis, *Histomonas meleagridis*, absence of *Heterakis gallinarum* and earthworm, chickens.

Introduction

Histomoniasis (blackhead disease) is a parasitic disease of many gallinaceous birds caused by the protozoan *Histomonas meleagridis* (2,12). The protozoan parasite is the only recognized species in the genus *Histomonas*. It is classified as a member of the family Monocercomonadidae in the class Trichomonada (5). The parasite is found in the cecum and liver, and is the cause of histomoniasis, infectious entero-hepatitis, or blackhead, in the turkey. Being world-wide in distribution, it is an important disease entity in turkeys, assuming great economic importance especially where turkeys are kept in large numbers. It may also occur in peafowl, guinea-fowl, pheasant, partridge and quail and is probably ubiquitous also in chickens, although the incidence of disease in these is low. Outbreaks are commonly seen in domestic chickens and turkeys when flocks are 3-7 weeks old (19).

The parasite is carried from flock to flock encapsulated in ova of cecal worm, *Heterakis gallinarum* (3,6). Blackhead infections result from ingestion of embryonated eggs of adult cecal worms, but not unembryonated worm eggs (7,20). Earthworm may eat eggs of *H. gallinarum* in soil and thus harbor the contained histomonads, transmitting the infection when the earthworm is eaten by a chicken and a turkey (14).

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Although histomoniasis in chucker partridges has been reported at the wild bird-raising farms in Korea (17), there are few reports so far in chickens. This report describes an outbreak of blackhead disease characterized by acute and high mortality in chicken in the absence of the cecal worm or earthworm vector.

Materials and Methods

The carcasses of chickens were transferred from a farm in Gyeonggi province following histomonad infections in June, 2009. The 10 week old broiler breeders were housed in floor pens. At that time, the number of dead chickens exceeded 20 birds/house/day. Liver, ceca, lung, heart, and bursa of Fabricius were fixed in 10% buffered formalin and processed with conventional histological techniques for paraffin wax sectioning. Paraffin sections were stained with routine H-E or PAS staining techniques for histopathological analysis and detection of histomonads in the tissues. The whole intestinal contents were examined by sugar floatation method and the contents were passed through the sieves for other parasites detection. The soils in floor pens were searched for earthworm.

For transverse electron microscopy, pieces of liver tissue were fixed with 2.5% glutaraldehyde solution in 0.1 M phosphate buffer (pH 7.4) for 4 hrs. After washing with buffer, the specimens were post-fixed with 1% osmium tetroxide at 4°C for 4 hrs. Afterward, the specimens were dehydrated in a graded ethyl alcohol series and two changes of propylene

oxide, and embedded in epon mixture. The ultrathin sections were stained with uranyl acetate and lead citrate.

Results

Grossly, a severe fibrinous peritonitis with adhesion of mesenteries and intestinal organs was observed in the abdomen. In addition, the ceca was enlarged, expanded, and thickened with serosal congestion. Serous, hemorrhagic and fibropurulent exudates with a hard caseous or cheesy core filled in the lumen of ceca, distending the walls (Figs 1-A,C). The liver had multifocal white irregular foci surrounded by a raised ring (Fig 1-B).

In the parasitological examination, parasites and earthworms were neither detected in the intestinal contents nor soils in floor pens. Histopathologically, the most histomonad-associated lesions were found in the liver where they showed extensive hepatic necrosis with inflammatory exudates. In the necrotic zones, histomonads were seen surrounded by abundant mixed inflammatory cells such as macrophages, mononuclear cells, heterophils. Despite the absence of detectable parasites or earthworms, *H. meleagridis* had its characteristic (double-eyed) structure (Figs 2-A,B). In the ceca, there were severe epithelial necrosis and infiltration of inflammatory cells, predominantly consisting of mononuclear cells and heterophils, while the mucosa was covered with a layer of sloughed necrotic epithelium and inflammatory exudates (Figs 2-C,D). However, *H. meleagridis* was not detected. Minimal to

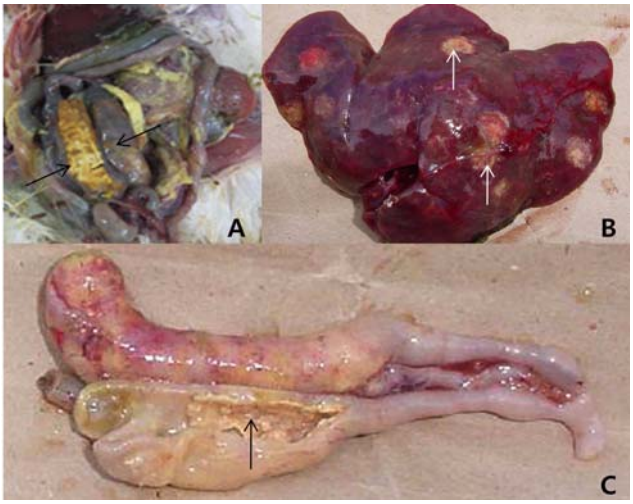

Fig 1. Gross view of Histomonad-infected chicken. (A) Note the engorgement of ceca (arrows). (B) Discrete pathognomonic lesions of liver (arrows) showing multifocal necrotic areas circumscribed by a raised ring. (C) Note the caseous core of ceca (arrow) and serosal congestion.

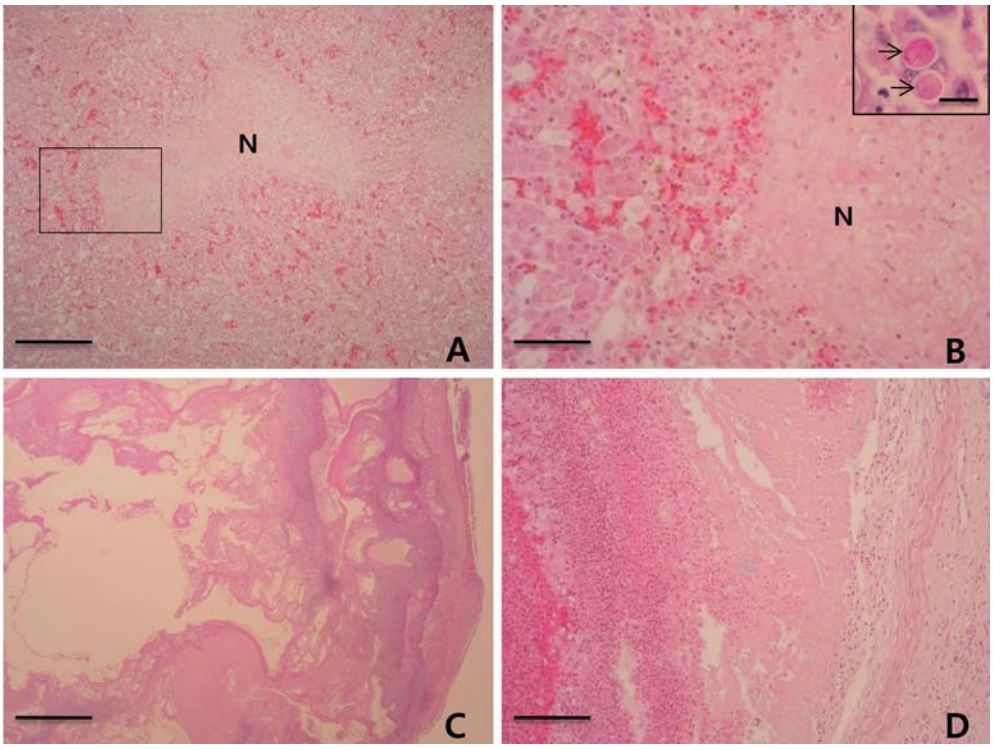

Fig 2. Histopathologic section of chicken infected with histomonas. (A) Note extensive hepatic necrosis (N) with mild congestion. Bar = 200 μm, H-E. (B) Higher magnification of A. Note severe hepatic necrosis (N) with inflammatory cell infiltration. Bar = 50 μm, H-E. Square: PAS positive histomonads (arrows). Note the characteristic (double-eyed) structure. Bar = 5 μm. (C) Section of chicken ceca. Note severe necrosis of intestinal wall and the lumen is filled with sloughed epithelium and inflammatory exudates. Bar = 500 μm, H-E. (D) Higher magnification of C. Showing severe inflammatory cells infiltration, predominantly consisting of mononuclear and heterophils. Bar = 100 μm, H-E.

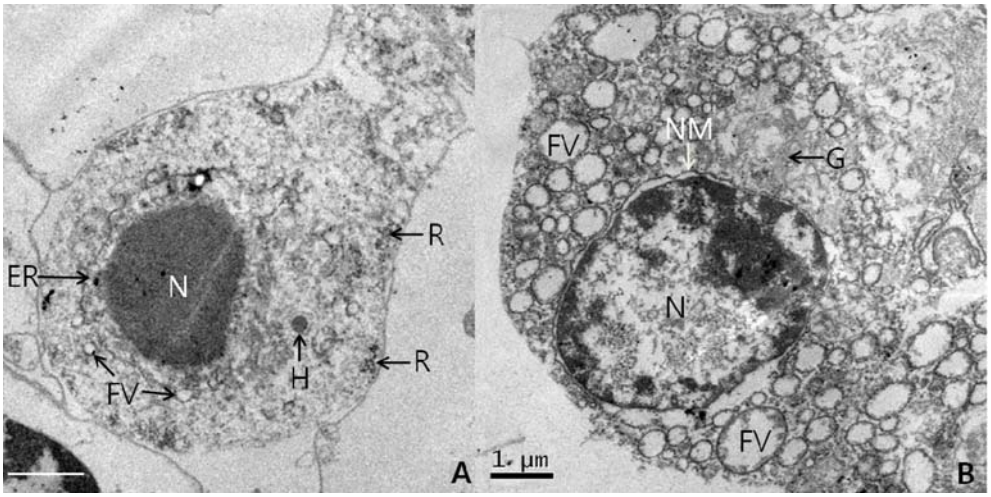

Fig 3. Electron micrographs of *Histomonas meleagridis*. (A) Smooth endoplasmic reticulum is situated periphery by some food vacuoles. The dark stained hydrogenosome is presented in single number and ribosomes are visible. (B) The nucleus is clearly visible inside the parasite, surround by a nuclear membrane. The Golgi apparatus is closely situated to the nucleus. The several food vacuoles is scattered in cytoplasm. ER: endoplasmic reticulum, FV: food vacuoles, G: Golgi apparatus, H: hydrogenosome, N: nucleus, NM: nuclear membrane, R: ribosomes. Bars = 1 μm.

mild pathological changes were observed in other organs.

In the ultrastructural examination, *H. meleagridis* from liver showed a spherical shape and a varying size of 14-20 μm. The nucleus showed an ovoid structure and was sized 2-4.5 μm in diameter. The nuclear envelope consisted of two membranes. A Golgi complex was found close to the nucleus. The several food vacuoles are scattered in cytoplasm (Fig 3-B). A few hydrogenosomes have an average size of 0.1-0.3 μm in diameter. These organelles were electron-dense (Fig 3-A).

Discussion

H. meleagridis was first described under the name *Amoeba meleagridis*, but discovery of flagellate characteristics led Tyzzer to rename the protozoan *H. meleagridis* (21). Chickens become infected with *H. meleagridis* and suffer from severe cecal lesions and morbidity. Experimentally, mortality has reached 100% in turkeys. Although mortality from histomoniasis in chickens is generally low, mortality has exceeded 30% in some naturally occurring infections. Occasionally, a strain of *Histomonas* with high virulence for chickens is found (15). In our case, over 20 chickens died everyday.

It is well known that the primary lesions of histomoniasis develop in ceca and liver. Caseous core of ceca and enlargement, necrosis and parasites in liver have been reported (12). In our study, the typical pathological signs of histomoniasis were seen in both ceca and liver and the characteristic structure of histomonads was only seen in the liver by the light and electron microscope.

In general, the transmission of *H. meleagridis* is known to occur through the embryonated cecal worm eggs or adult worm of *H. gallinarum* (6) or earthworms (7,13,20). But this mechanism however, did not explain the phenomenon of rapid spread of histomonasis through a flock of birds, and led to some question whether other intermediate hosts might be involved or not. In turkeys, the transmission of histomonads was successful in initiating the disease (22), but other researchers concluded that birds could not become infected by pecking at fresh, infected droppings (8,14). This question was addressed in experiment where uninfected turkeys were placed in pens alongside other directly inoculated poults, in the absence of any other possible host or carrier (16). This led to discovery that turkeys could become infected from direct contact with birds or contaminated feces (4,16). Now the life cycle of *H. meleagridis* has been reconsidered.

Direct infection of turkeys in the absence of cecal worm ova or other vector appeared to involve the phenomenon of cloacal drinking during exposure to liquid droppings from sick turkeys (10,11). The phenomenon of cloacal drinking is also well known in chickens (18). Recently, the rapid spread of histomoniasis in duck flock and the high incidence of mortality denoted a direct transmission of infection (1). Hu *et al.* (9) suggested that the dynamics of blackhead transmission in chicks differs significantly from that of turkeys, where transmission from bird to bird is rapid and effective in the absence of vectors. These differences are not easily explained. They supposed that the behavior or moisture content of droppings may influence the survival of histomonads. Also, they observed that turkeys tend to huddle more than chickens, particularly if sick.

We have not detected the eggs or worms of *H. gallinarum* in any of postmortem and surviving chickens or earthworms in any of soils from pens. Although the direct transmission in turkeys was demonstrated in experiment, the rapid bird to bird spread of blackhead disease in chicken flocks will be more studied in the absence of vectors.

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매개체 부재 하에 발생한 닭의 흑두병 증례

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요 약 : 10 주령의 육계 종계가 급성으로 폐사 하였다. 치사율은 20 수/계사/일 이상 이었고 사육환경은 평사였다. 분변검사에서 맹장 및 장으로부터 기생충은 검출되지 않았으며, 부검 소견에서 장간막과 장조직의 유착을 동반한 섬유소성 염증이 관찰되었다. 또한, 맹장의 충 · 출혈을 동반한 종대, 확장 및 비후가 관찰되었으며, 맹장의 내강에서 염증성 삼출물에 의한 단단한 치즈양 core가 관찰되었다. 간의 육안소견에서는 원형의 융기된 괴사 반점이 관찰되었다. 광학 및 전자현미경을 통하여 전형적인 칠면조편모충이 간조직에서 관찰되었으나 장점막에서는 관찰되지 않았다. 한편, 본 예에서 흑두병을 매개하는 닭맹장충을 부검 및 생존한 닭에서 관찰할 수 없었으며 계사의 토양에서 지렁이를 전혀 관찰할 수 없었다. 따라서 본 증례는 닭에서 매개체 없이 칠면조편모충이 직접전파에 의해 감염된 사례로 판단된다.

주요어 : 칠면조편모충, 닭맹장충 및 지렁이 부재, 흑두병, 육계.