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SHORT COMMUNICATION

Concurrent infection of histomoniasis and colibacillosis in ducklings

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ABSTRACT

Ducklings (aged 45-50 days) from a nomadic flock had mortalities of 4-5 birds per day since one week was reported. Systematic postmortem was conducted. Samples were collected for histopathological, microbiological and parasitological studies. Gross examination revealed thin whitish membranous deposits over the pericardium and liver. Pericardium was tightly adhered with the heart. Caecum was slightly distended and showed multifocal, firm creamy yellowish-white adherent foci over the mucosa. Microscopically, chronic fibrino-purulent pericarditis, epicarditis, fibrinous perihepatitis, diptheritic typhlitis with trophozoites of *Histomonas meleagridis* was observed. *Histomonas meleagridis* was confirmed by parasitological examination. *Escherichia coli* was isolated from heart blood and liver swabs. Histomonads exacerbate the illness when concurrent infection with pathogenic bacteria prevails. The present study described the co-infection of histomoniasis and colibacillosis in ducklings under natural condition.

Keywords: Chronic granulomatous pericarditis, diphtheritic typhlitis, duckling, *Escherichia coli*, *Histomonas meleagridis*

Histomoniasis is a protozoan disease of gallinaceous birds caused by unicellular parasite *Histomonas meleagridis* belonging to the phylum Parabasalia, class Tritrichomonadea, order Tritrichomonadida and family Dientamoebidae[1]. Turkeys are highly susceptible with acute mortality reaching up to 100%[2], whereas Ring-necked pheasants are found to be relatively resistant[3]. However, histomoniasis found to occur in domestic and wild gallinaceous birds[2,4,5], it is rare in water fowls especially in ducks[6,7]. Histomoniasis is also reported to cause mortality in established infection of *H. meleagridis* along as a co-infection with internal pathogenic bacteria and intestinal nematodes *(Ascardia galli* and *Heterakis gallinarum)*[8]. Concurrent infection with grave malady of histomoniasis is reported on pair with *Enterococcus fecalis* in ducks[9] and with *Eimeria tenella*[10], colibacillosis[4] and ascariasis[11] in chickens. Histomoniasis was reported to occur in the absence of possible vectors by cloacal route[12]. The present report documented the co-infection of histomoniasis and chronic colibacillosis as a natural infection in an Indian runner duck.

A nomadic duck farmer reported a mortality of four to five ducklings per day since a week in 42 to 50 days old age groups in a flock holding 4000 birds. History showed that ducklings had yellowish watery diarrhea, twisting of head and limping for two to three days before death. Three dead ducklings were brought to the Department of Veterinary Pathology, Veterinary College and Research Institute, Orathanadu, Thanajvur, Tamil Nadu for postmortem examination. Complete and systematic postmortem examination was carried out. Representative tissue samples from coelomic organs and brain were collected in 10% formalin for histopathology. Heart blood and liver swabs were collected in sterile tubes for microbiological study. Caecal luminal contents were collected in sterile container for parasitological study.

Tissue samples were processed as per standard paraffin embedding technique. Tissue sections of 4-5 µm thickness were prepared and stained with haematoxylin and eosin staining protocol[13]. Duplicate sections were stained with MSB technique to demonstrate fibrin[14]. Heart blood and liver swabs were processed as per standard bacteriological procedure. Heart blood swab was nourished in nutrient broth and further streaked in MacConkey agar and Eosin Methylene Blue (EMB) agar. Caecal contents were subjected to direct wet film examination following centrifugation under light microscope.

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Grossly, carcasses were thin and feathers around the vent were matted with creamy whitish pasty contents. Birds had sunken eyes (Figs. 1 & 2). On pressing the lower abdomen near cloaca, whey coloured (yellowish green tint) watery discharge was found (Fig. 3). Internally, the liver was covered with thin, whitish pseudo-membranous layer (Fig. 4). The heart revealed thick, chalky-white pericardium. Pericardium was tightly adhered

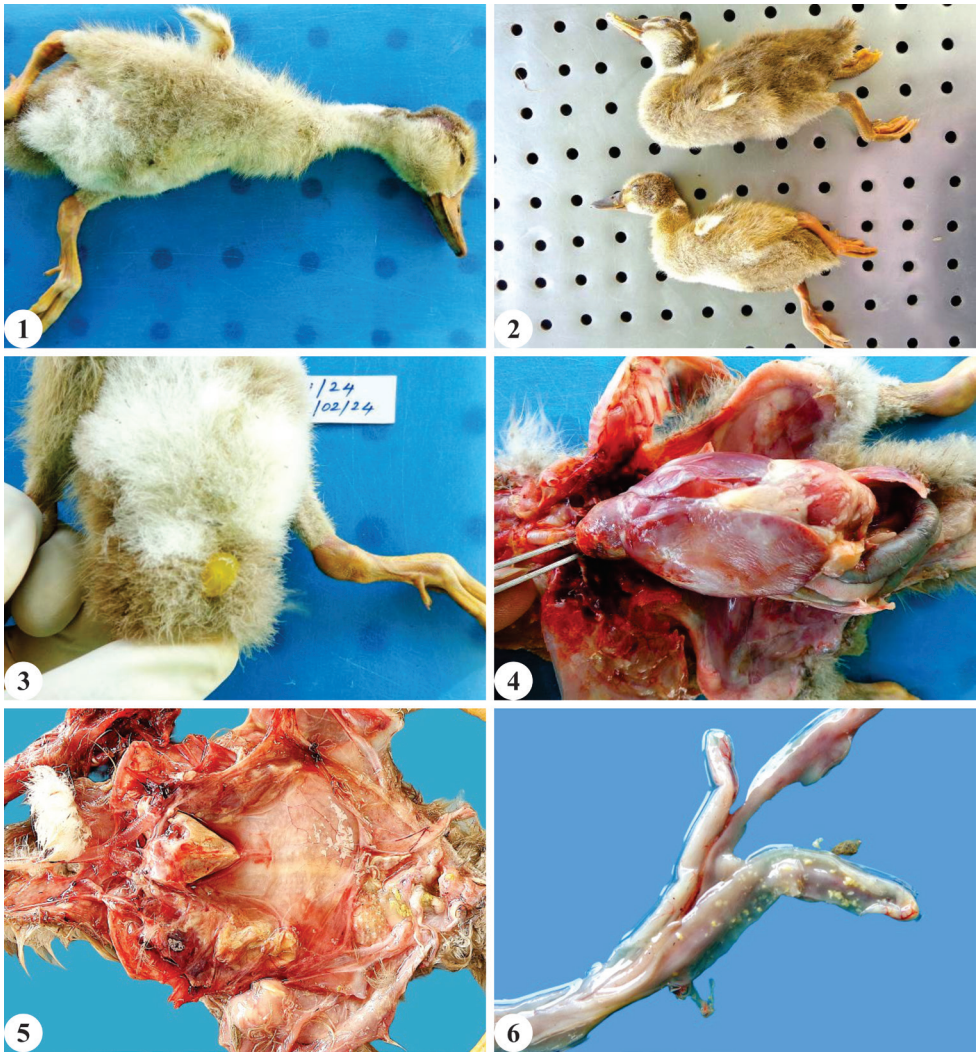

Fig. 1. Duckling with ruffled down feathers; **Fig. 2.** Ducklings with torticollis; **Fig. 3.** Yellowish-green (Whey-like) cloacal discharge; **Fig. 4.** Thin, whitish membranous deposits over liver; **Fig. 5.** Yellowish white, thick adherent pericardium; **Fig. 6.** Multifocal, creamy yellowish-white firm diphtheritic areas in the mucosa of caecum.

with the epicardium (Fig. 5). Both the caecum was slightly distended and contained yellowish watery contents. Mucosa of caecum revealed multifocal, creamy yellowish white, raised, firm diphtheritic foci which were found adhered to the underlying tissue (Fig. 6). Other visceral organs showed mild to moderate congestion. Brain revealed mild congestion of meninges.

Microscopically, pericardium was thick and revealed fibrinopurulent inflammation characterized by deposition of fibrin with necrosis and degenerative heterophils, bacterial clumps surrounded by mononuclear cells and row of multinucleated giant cells (MGCs) (Fig. 7). MGCs' were foreign body type. MGCs' showed mixed population of Langhan's type with peripheral row of nuclear arrangement (Fig. 8) and as foreign body type with central nuclear cluster as well. Underlying epicardium revealed superficial infiltration with mononuclear cells into the myocardial fibers. In another carcass, pericardial cavity was filled with fibrino-purulent necrotic and highly cellular contents with bacterial colonies. Pericardium was found to be strongly adhered with the underlying epicardium which was anchored by columnar type giant cells. These giant cells were taller and had two rows of multiple nucleus (4-5) arranged one above the other pointing on either side; one from the visceral pericardium and another from the epicardium (Fig. 9). Underlying epicardium showed inflammatory cell infiltrations. Liver revealed thickening of Glisson's capsule with inflammatory cell infiltrations (Fig. 10). Parenchyma showed moderate to severe congestion, atrophy of hepatic cords and vacuolar degeneration of hepatocytes. Caecum revealed multifocal diphtheritic typhlitis characterized by mucosal necrotic plug adhered with the underlying mucosal cells (Fig. 11). Mucosal epithelium and glands showed atrophy with intraluminal trophozoites of *Histomonas meleagridis* (Fig.

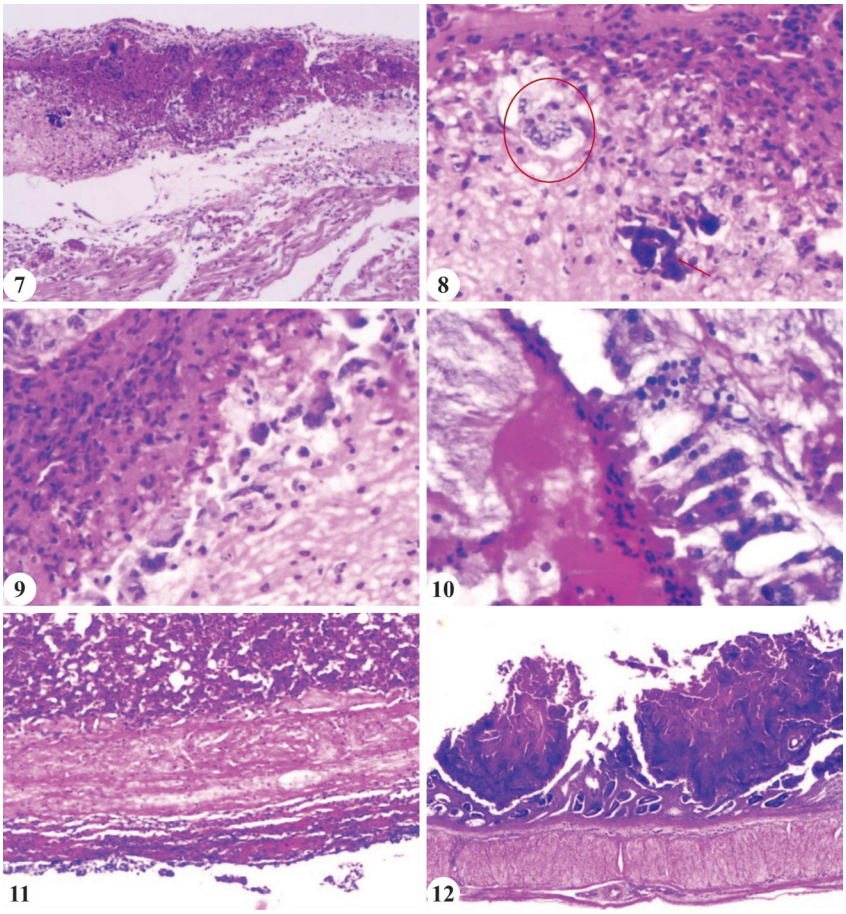

Fig. 7. Fibrino-purulent exudate with chronic pericarditis, MNC infiltrations into the epi and myocardium (H&E stain x100); **Fig. 8.** Chronic pericarditis, Giant cells (circle) and bacterial colonies (arrow) (H&E stain x400); **Fig. 9.** Degenerated heterophils and row of Langhans type giant cells (H&E stain x400); **Fig. 10.** Columnar type giant cell strands showing row of multinucleation of anchoring the epicardium and visceral pericardium (H&E stain x400); **Fig. 11.** Perihepatitis showing thickening of Glisson's capsule and peripheral MNC infiltration and inflammatory exudate (H&E x100); **Fig. 12.** Diphtheritic necrotic plaques adhered with mucosa (H&E stain x100).

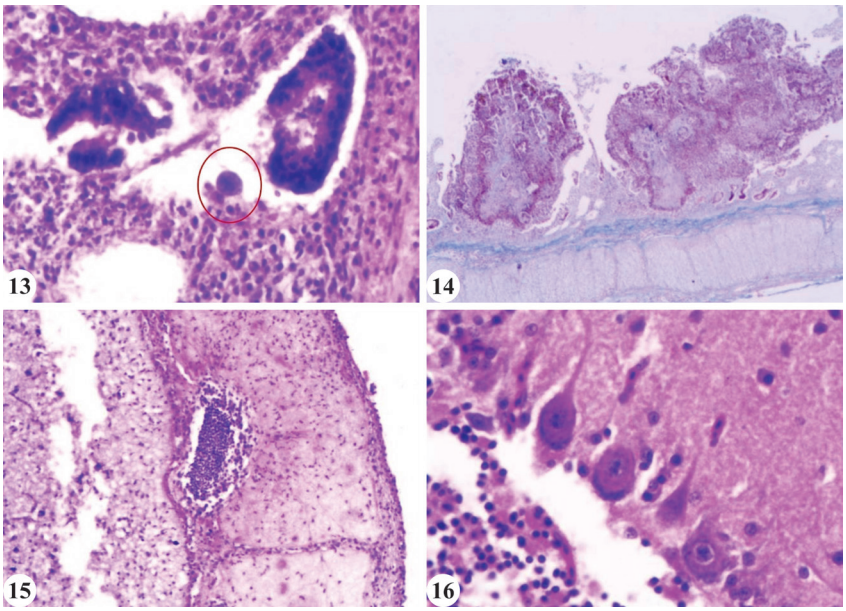

Fig. 13. Trophozoites of *Histomonas Meleagridis* (circle) with in the mucosa of caecum (H&E stain x400); **Fig. 14.** Red coloured fibrin in the diphtheritic plaques (MSB technique x100); **Fig. 15.** Sub-acute eosinophilic leptomeningitis (H&E x100); **Fig. 16.** Peripehral condensation of Nissl substance in Purkinje cells of cerebellum (H&E stain x400).

12). Necrotic plug showed high density of fibrin with MSB staining technique (Fig. 13). Meninges revealed subacute eosinophilic leptomeningitis (Fig. 14). Cerebellum showed mild to moderate peripheral condensation of Nissl substances in Purkinje cells (Fig. 15).

Caecal contents on wet film examination revealed numerous motile single flagellate trophozoites suggestive to *Histomonas meleagridis*. Dried smear stained with H&E stain revealed oval to round bodies surrounded by clear space (Fig. 16). *Escherichia coli* was isolated from heart blood and liver swabs. Colonies from heart blood swab produced green metallic sheen in EMB agar and produced typically bright pink, smooth, circular, moist colonies in MacConkey agar.

Histomoniasis was known to cause entero-hepatitis characterized by necrotic hepatitis and diphtheritic typhlitis. Turkeys were long known to be the susceptible host for *H. meleagridis*[15]. It was documented that other gallinaceous birds such as chicken, Pheasants, Partridges, guinea fowl, geese, ducks and peafowl were found to suffer with *H. meleagridis* infection both naturally and experimentally[3-5,7,11,16-18]. Histomoniasis was found to be rare in water fowls[8]. Ducks were reported to be an unsatisfactory host for establishment of *H. meleagridis* infection[6]. Experimental studies on histomoniasis in mule and Muscovy ducks do not exhibit any overt clinical signs other than diarrhea when compared to turkeys[7]. In parallel, Abd El-Wahab *et al.*[16] reported that fattening turkeys affected with histomoniasis had typical lesions in liver but did not show any apparent clinical signs of the disease before slaughter. The above documentary evidence, although puzzling, natural infection with *H. meleagridis* resulted in yellowish (whey-like) watery diarrhea in ducks as observed in the present study.

No apparent gross lesions of histomoniasis were observed in liver except mild caecal lesions in the present study and was in accordance with earlier reports[6,7]. Callait-Cardinal *et al.*[7] documented that histomoniasis affected ducks showed thickening of caecal wall with partial luminal occlusion with caecal core. The findings of multifocal, bran-like necrotic areas in the caecal mucosa was in parallel with the findings of Lund *et al.*, (1974)[6] and de Araujo *et al.*, (2015)[19], who reported that the infection with *H. meleagridis* revealed rudiment of adherent core in the caecal mucosa of experimentally infected geese and yellowish friable caseous contents over the mucosa in natural infection of free-range chicken respectively. On whole, *H. meleagridis* seldom associated with overt necrosis of liver and caecum put forth by Clarke *et al.*, (2017)[4] was found to be justifiable in the present report with lack of discrete lesions of histomoniasis.

Histologically, multifocal diphtheritic necrotic plaques with the presence of trophozoites of *H. meleagridis* recorded in the present study was in line with earlier report in duck[7] and in chicken[19]. Earlier, necro-fibrinous typhlitis was recorded in mule and Muscovy ducks experimentally infected with *H. meleagridis*[7]. It was suggested that proteases released by the protozoa likely to cause digestion and destruction of epithelial cells of the caecal mucosa which subsequently leads to necrotic typhlitis and formation of caecal core[1]. Subacute eosinophilic meningitis observed in the present study might possibly be responsible for nervous signs exhibited by ducks before death. Although histomonads were not found in the meningeal lesion of the present study, molecular localization of histomonads was reported in multisystemic infection of histomoniasis affecting visceral organs including brain[20].

It was postulated that *H. meleagridis* alone does not represent substantial danger to duck[7] rather, co-infection with *Eimeria tenella*, pathogenic bacterium such as *Escherichia coli*, *Bacillus subtilis* or *Clostridium perfringens* and round worm, *Ascaridia galli* could aggravate the clinical signs, exuberate the illness and increase the mortality rate[10,16,11,21]. It was documented that haemorrhagic enteritis virus occurred currently with histomoniasis in turkeys[18]. Further it was reported that colibacillosis was much frequent co-infection following histomonas infection representing that *E. coli* translocates from the gut to internal organs via blood stream probably due to loss of gut membrane integrity[2,22]. The above statement was acceptable in the present study where affected ducklings showed fibrous perihepatitis and granulomatous pericarditis and epicarditis characteristic of colibacillosis. Further the *E. coli* infection in the present malady was confirmed by microbiologial study. Histological changes in pericardium and epicardium suggests that the malady induced by bacteria was of chronic lesions.

Histomoniasis was once thought to be primarily transmitted by ingestion of embryonated eggs of *Heterakis gallinarum* from neighbouring chicken flock to turkeys. Incidence of histomoniasis in chicken and other gallinaceous birds in the absence of caecal worm and vector/reservoir host such as darkling beetles, earthworm and lesser mealworm suggested the possibility of direct spread via cloacal route of infection[12,23]. This was justified with the primary lesion of histomonaisis in bursa of Fabricius of desi chicken infected with *H. meleagridis* with mild caecal lesion[21,24]. Cloacal drinking/cloacal kissing was proposed to be one of the reasons of direct spread for *H. meleagridis*[1,24]. High moisture droppings, huddling of ducks in closed rearing in backyard farming pose risk for cloacal kissing in ducks[12]. The above statement was plausible in the present study where no vectors (caecal worm or eggs) were found by parasitological examination.

CONCLUSION

The present study concludes that mortality of ducks were due to co-infection with *H. meleagridis* and *E coli*. Direct spread of histomonas followed by secondary *E.coli* infection aggravated the disease. Further, the chronic colibacillosis with newer pericardial lesions thought to be rare findings in ducks. On par with other gallinaceous birds, ducks were found to be susceptible for natural infection by *H. meleagridis* followed by secondary colibacillosis. Additionally, *Histomonas* established in caecum with tone-down lesions in the present study suggested that ducks may act as reservoir and or carrier host for *H. meleagridis* to other poultry when reared together. Similar statement as already proposed by earlier workers[7] also need to be investigated.

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