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Pathological Changes of Lung Tissues of Pigeons (*Columba livia domestica*) Infected with *Haemoproteus columbae* (Haemosporina: Haemoproteidae)

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Abstract: Large number of pigeons (n = 400) were surveyed for *Haemoproteus parasitemia*. Blood smearing revealed the presence of gametocytes-infected erythrocytes in 50 adult birds (12.5%). Infected erythrocytes as well as exo-erythrocytic schizonts were recognized in the examined lung imprints. Histopathology of the parasitized lung tissues also confirmed the existence of *Haemoproteus* schizonts in the pulmonary blood vessels. Granulomatous pulmonary tissue reaction was also detected at the site of the released merozoites. Electron microscopy revealed the presence of schizonts in endothelial lining cells of pulmonary blood capillaries. It was concluded that *Haemoproteus* infection can provoke significant pathological pulmonary changes in domestic pigeons.

Key words: *Haemoproteus columbae*, pigeons, lung, histopathology, ultrastructure

INTRODUCTION

Avian haemosporidian (haematozoan) parasites are a large number of species distributed throughout the families Leucocytozoidae, plasmodiidae and Haemoproteidae^{1,2}. Although these protozoa appear to be commensals, but under certain circumstances they become pathogenic^{3,4}. Some species of the families Leucocytozoidae and Plasmodiidae cause considerable mortalities in domestic flocks of ducks, geese, turkeys and fowls⁵⁻⁷. However, species of *Haemoproteus* have been considered of relatively low pathogenicity with little pathological effects and only provoke incidental mortalities in the avian hosts^{4,8-11}. *Haemoproteus*-infected tissues have been described by Earle *et al.*² and only lung congestion associating the presence of *Haemoproteus* schizonts in pulmonary vessels was reported. To the best of our knowledge, there is no published study focusing on the pathological changes of *Haemoproteus* infected lung tissues from avian hosts. Therefore, the present study was intended to identify the lung pathology of *Haemoproteus*-infected pigeons.

MATERIALS AND METHODS

Birds: Large number of suspected pigeons (n = 400) obtained from local breeding farms and showing respiratory distress, were surveyed during the summer

season (period extending from May to August, 2003) by blood smearing for *Haemoproteus parasitemia* (parasitemia of young gametocytes). Infected pigeons were necropsied and special attention was paid for the grossly affected lung tissues.

Blood smears and lung tissue imprints: The prepared thin blood smears from living birds, in addition to lung tissue imprints obtained from the necropsied ones, were air-dried, fixed by dipping in methanol, and stained with Giemsa's.

Histopathology

Preparation of paraffin sections: Tissue specimens from lungs, and other organs and tissues, of the infected pigeons were processed routinely for paraffin embedding technique, sectioned at 3-5 μ m and stained with haematoxylin and eosin (HE).

Preparation of semithin sections (1 μ m thickness):

Tissue specimens from the pigeon lungs were diced into the proper small pieces that were immediately fixed by immersion in 3% buffered glutaraldehyde (0.1 M sodium cacodylate buffer at pH 7.2) for 4 h at 4°C. The fixed pulmonary tissue specimens were thoroughly washed in the same buffer and then post-fixed in 1% osmium tetroxide (OsO₄) in 0.1 M sodium cacodylate buffer (pH 7.2) for 2 h. Subsequent dehydration of the fixed

tissues was done in ascending grades of ethanol and transferred to epoxy resin (Epon/Araldite mixture) via propylene oxide. Semithin sections (1 μm) were cut with glass knives on an ultramicrotome (Leica, UCT) and stained with toluidine blue.

Preparation of ultrathin sections (70-80 nm): Following tissue orientation and localization of the desired tissue sites, ultrathin sections were accordingly prepared using a diamond knife on the same ultramicrotome. Ultrathin sections were then double stained with uranyl acetate and lead citrate and examined under a Transmission Electron Microscope (TEM) (JEOL, 100 CX).

RESULTS

Necropsy findings: Necropsied birds showed edematus and congested lungs with accumulation of clear fluid in the peritoneal cavities. Other visceral organs including liver, kidney and spleen were also congested.

Blood smears and tissue imprints: Among the surveyed suspected pigeons, 50 adult birds (12.5%) (28 females and 22 males) manifested *Haemoproteus parasitemia*. Large number of erythrocytes in blood smears prepared from the infected birds revealed mature and immature gametocytes of *Haemoproteus* (Fig. 1). Infected erythrocytes as well as exo-erythrocytic schizonts were recognized in the examined lung tissue imprints (Fig. 2).

Histopathology: The common histological change in the examined infected lung tissues was the distention of the vasculature including blood capillaries and the large pulmonary vessels. Air capillaries were evidently emphysematous, parabronchial muscles were hypertrophied and diameter of the parabronchial lumina was reduced. Parabronchial epithelium was proliferated. At sites where the parasitic trophozoites were recognized (Fig. 3), endothelial cells were noticeably hyperplastic. Early schizonts were detected in the endothelial lining cells of the large pulmonary vessels (Fig. 4). Mature multinucleated schizonts, either intact or degenerated, were also discerned within the blood vessel walls associated with obvious vascular mural changes (Fig. 5). The viable schizonts contained masses of merozoites and the degenerated ones showed vacuolar structures. Schizonts were also encountered at the periphery of large blood vessels.

Released merozoites in the vicinity of the disintegrated schizonts, accompanied with deposition of pigment material, were a frequent histological finding (Fig. 6). These pulmonary foci disclosed remarkable

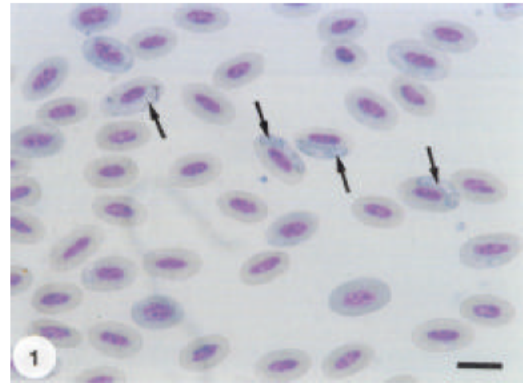


Fig. 1: Erythrocyte cells infected with *Haemoproteus* gametocytes (arrows). Blood smear stained with Giemsa's. Bar = 10 μm

endothelial cell hyperplasia and collapse of both air and blood capillaries. Infected erythrocytes harboring gametocytes were recognized within the parabronchial blood capillaries. Also, exo-erythrocytic schizonts were discerned within the pulmonary blood vessels (Fig. 7). An outstanding histological finding in the affected lungs was the formation of large nodular structures which occupied large proportions of the pulmonary tissues (Fig. 8). These granulomatous structures consisted of reacting macrophages, lymphoid cells, and plasmacytes and bordered by proliferated fibroblasts intermingled with released merozoites and pigment material (Fig. 9). The pigment material noticed within the granulomatous structures resembled that found at site of the merozoites released from the disintegrated schizonts. Occasional acidophilic intranuclear inclusions were detected in the degenerated parabronchial epithelial cells in the vicinity of the schizont-infected tissue structures (Fig. 10). Other tissues including heart muscles, liver and kidney showed the presence of varied-shaped schizonts.

Electron microscopy revealed the existence of schizonts within the endothelial lining cells of blood capillaries (Fig. 11). The infected endothelial cells were obviously distorted and many of them were protruded into the neighboring air capillaries. Nuclei of these endothelial cells were evidently pleomorphic and their organelles were degenerated. Most of the observed blood capillaries were distended as evidenced by the several lodged rows of erythrocytes. Basement membranes separating the endothelial lining cells from air capillaries were irregularly thickened. The lining cells of the air capillaries were frequently infiltrated with heterophils (Fig. 12 and 13). The heterophil cells were also seen lodged in the blood capillaries exhibiting infection of their lining endothelial cells.

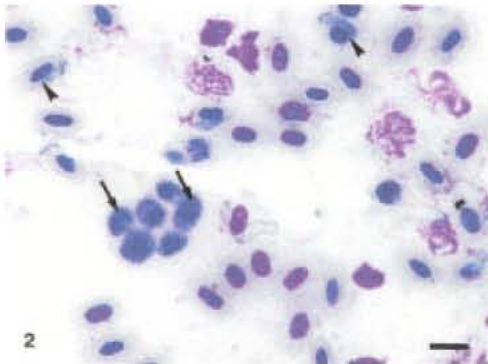


Fig. 2: *Haemoproteus* schizonts (arrows), filled with merozoites, in lung imprints, prepared from the infected pigeons. Gametocytes-infected erythrocytes (arrowheads) are also noticed. Giemsa stain. Bar = 10 μ m

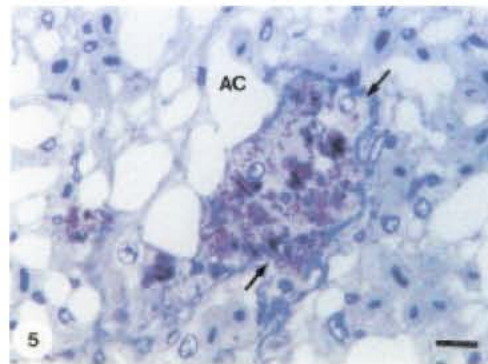


Fig. 5: Mature thin-walled multinucleated merozoites-filled schizont (arrows) in a markedly distorted endothelial lining cell in the lung of an infected pigeon. Air Capillaries (AC) are emphysematous. Toluidine blue stain. Bar = 25 μ m

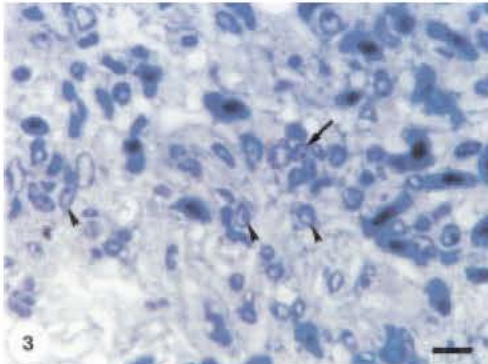


Fig. 3: *Haemoproteus* trophozoites (arrow) infecting an endothelial lining cell of pulmonary blood capillary. Capillary endothelial cells (arrowheads) are obviously hyperplastic and air capillaries are collapsed. Semithin section (1 μ m thickness) prepared from an infected lung and stained with toluidine blue. Bar = 50 μ m

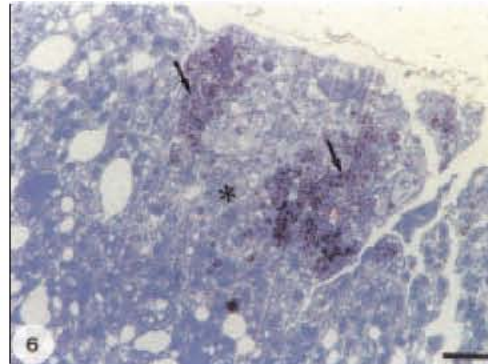


Fig. 6: Site of a ruptured schizonts showing released merozoites (arrows) in the lung tissue of an infected pigeon. Note the marked endothelial cell hyperplasia (*) at these pulmonary foci. Toluidine blue stain. Bar = 50 μ m

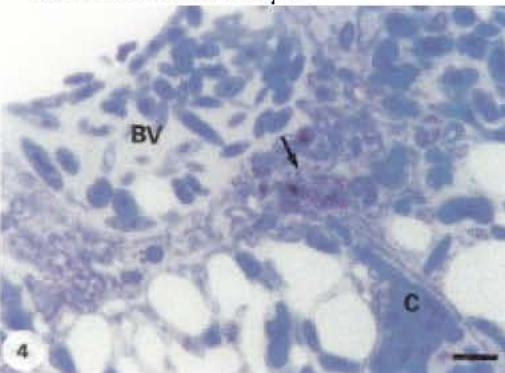


Fig. 4: Early schizont (arrows) in the endothelial lining cells of a large pulmonary blood vessel (BV) of an infected pigeon. Note the distended parabronchial blood capillaries (C). Toluidine blue stain. Bar = 25 μ m

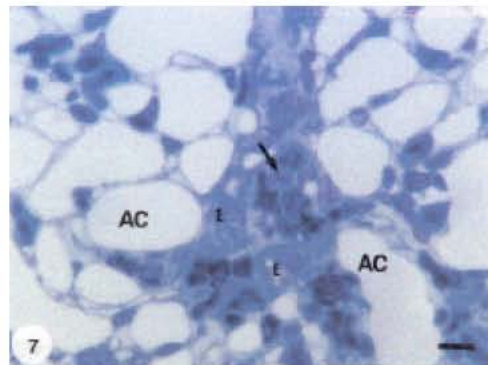


Fig. 7: Exo-erythrocytic schizont (arrow) in the lumen a pulmonary blood vessel of an infected pigeon. Note the swollen endothelial cells (E) and the obviously distended Air Capillaries (AC). Toluidine blue stain. Bar = 20 μ m

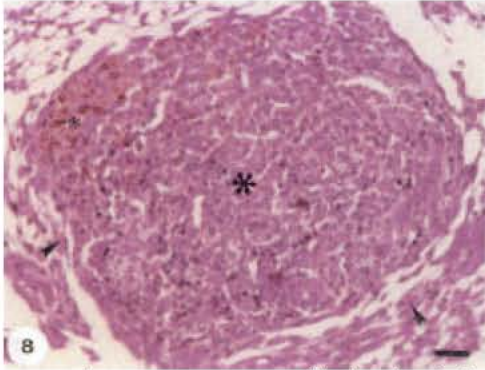


Fig. 8: Granulomatous structure (*) developed in the parasitized pulmonary tissue of an infected pigeon. This structure is delimited by the proliferated fibroblasts (arrowheads). HE. Bar = 40 μ m

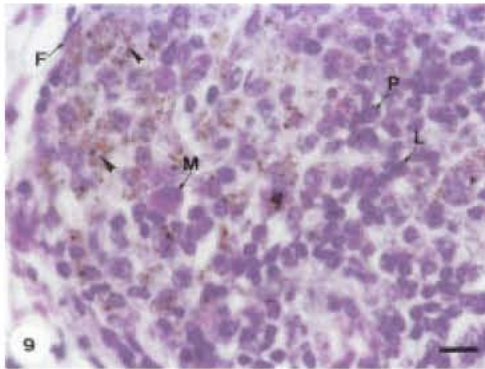


Fig. 9: Higher magnification for the pulmonary granulomatous structure showing the constituting cells which include; reacting macrophages (M), lymphoid cell (L) and plasmacytes (P) and bordered by proliferated fibroblasts (F). Note the pigmented granules within the granuloma (arrowheads). HE. Bar = 25 μ m

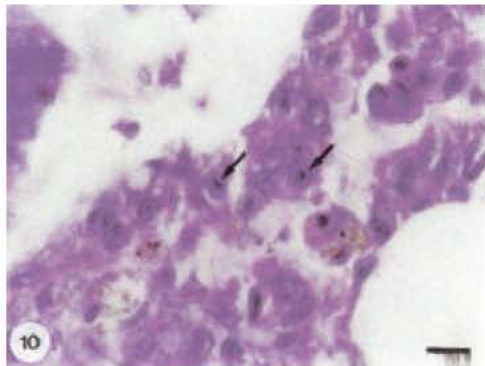


Fig. 10: Acidophilic intranuclear inclusions (arrows) in the degenerated parabronchial epithelial cells in the vicinity of ruptured schizonts. HE. Bar = 25 μ m

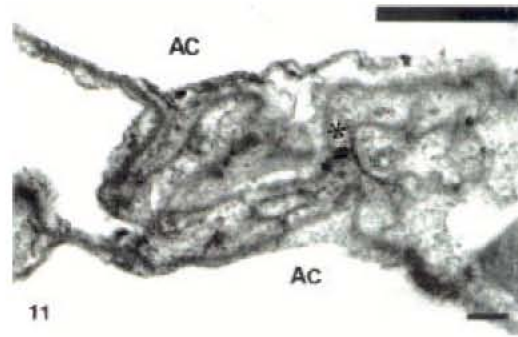


Fig. 11: Transmission electron micrograph showing a schizont (*) within an endothelial lining cell of a blood capillary. Note the irregular thickening of the basement membrane separating the endothelial lining cells from Air Capillaries (AC). Bar = 1 μ m

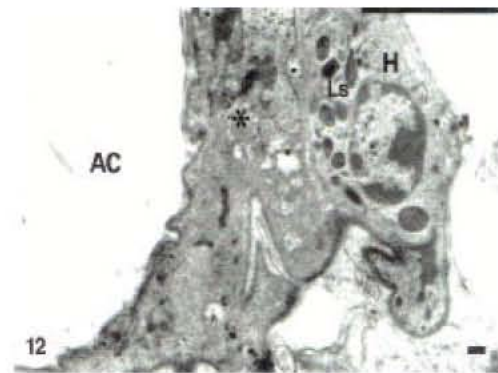


Fig. 12: Heterophil (H) infiltrating on an air capillary lining cell (*). Note the dense lysosomal structures (Ls) in the infiltrating heterophil. Bar = 1 μ m

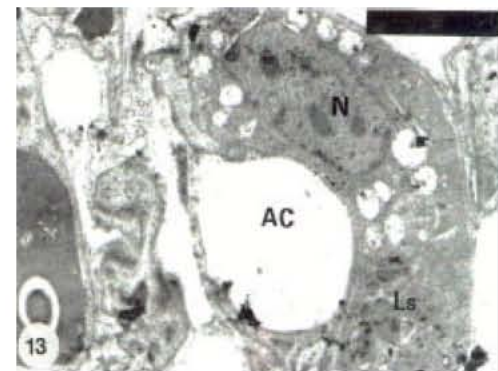


Fig. 13: Lysosomal structures (Ls) seen at site of a lining cell of an air capillary (AC). Organelles of this cell are degenerated. N, nucleus of the air capillary lining cell. Bar = 1 μ m

DISCUSSION

The currently demonstrated numerous gametocytes in the peripheral blood of infected pigeons were that of *Haemoproteus columbae* and this confirms the identity of the encountered tissue parasitic forms. Thus, blood smearing approved to be a reliable tool to exclude any confusion concerning the identification of tissue schizonts. In this respect, schizonts of the genera *Leucocytozoon* and *Haemoproteus* are confused and probably impossible to distinguish between them^[2,12].

The observed *Haemoproteus* merozoites-filled schizonts in the pulmonary vessels were thin-walled and irregular in shape resembling the schizont morphology of *Haemoproteus* species described by other researchers^[13-15]. In the present cases schizonts were located in the vascular walls within the endothelial lining cells. This schizont-endothelial cell association indicates parasitic development in the endothelial cell cytoplasm. The variability of schizonts shapes in the lung tissue was possibly related to the site of schizont development as a trial to conform with the local parasitized histological structures.

The noticed free extravascular schizonts were most likely a sequence to damage of the vascular walls caused by the effect exerted by the large-sized parasitic stages. The noticed large-sized schizonts possibly develop as a result of two or more generations of schizogony. The currently observed free merozoites presented an evidence for the vascular damage and also for the rupture of the degenerated schizonts. Rupture of megaloschizonts and release of merozoites are conditioned by the remarkable schizont swelling^[12].

The frequent finding of the various parasitic stages of *Haemoproteus*, including exo-erythrocytic stages (schizonts and merozoites) and erythrocytic stages (gametocytes) in the examined lungs approves that pulmonary tissue is a main target for *Haemoproteus* sporozoites and a major site for *Haemoproteus* schizogony.

Presently a marked pulmonary tissue response was noticed and represented by the granulomatous reaction in the lung tissue (granulomatous pneumonia). This inflammatory reaction was most probably a response to the existence of free merozoites released after vascular damage and schizont rupture. *Haemoproteus* is known to infect lung capillaries and the growing schizonts greatly distort the endothelial lining cells with a consequent vascular damage^[14]. On contrast, another histopathological study on tissue stages of *Haemoproteus columbae* reported rare or no inflammatory reaction in the parasitized tissues including lung^[2].

Appearance of *Haemoproteus* gametes in the peripheral blood of the presently investigated pigeons indicates that these birds survived the infection despite the significant lung lesions. Undoubtedly, such lung lesions compromise the total respiratory efficiency and may lead to disease conditions such as the pulmonary hypertension syndrome and the subsequent cardiac complications^[17]. These possible sequelae of *Haemoproteus*-induced lung lesions may reflect the significance of the present study.

The demonstrated intranuclear inclusions in the degenerated parabronchial epithelial cells were probably an evidence of unidentified viral infection. This finding may support the presumption that the immune status of the infected birds was lowered which opened the door for other pathogens to accentuate the existing tissue damage.

The present pathological study emphasizes the damaging effect provoked by a *Haemoproteus* species in the lung tissues of domestic pigeons. Some of the relevant previous studies^[11,15] also reported tissue damage caused by *Haemoproteus* species, however, these studies have not focused on the affection of lung tissue. The currently reported *Haemoproteus* infection is considered as a heavy one which was possibly arisen by re-infection of the birds which were in continuous contact with the source of *Haemoproteus* infection (insect vectors).

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