IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN: 2278-3008, p-ISSN:2319-7676. Volume 11, Issue 1 Ver. I (Jan. - Feb. 2016), PP 79-90
www.iosrjournals.org

Haemoproteus Columbae Infection and its Histopathological Effects on Pigeons in Qena Governorate, Egypt

Nermean M. Hussein, 1&2 and Eman A. Abdelrahim3
1 Zoology Department, Qena Faculty of Science, South Valley University, EGYPT.
2 Biology department, Faculty of applied science, Umm Al-Qura University, SAUDI ARABIA.
3 Histology department, Qena Faculty of Medicine, South Valley University, EGYPT.

Abstract: *Several health problems can affect pigeons but parasitic infections play a major role. The genus Haemoproteus includes a large number of intracellular protozoan parasites. A total of 103 pigeons were captured from different localities of Qena Governorate from March 2013 to April 2014, blood films were obtained, fixed, stained and examined microscopically. Lungs and liver were also examined histopathologically. The study demonstrated different stages of Haemoproteus columbae in the blood, liver and lungs of the pigeons captured with high prevalence (57.2%). In the liver and the lungs, the endothelium of the blood vessels was the main target of the parasite. In cases of high infection, round cell granuloma and massive destruction of the parenchyma of these organs occurred leading to sever illness or even death of the pigeons. There is a high prevalence of Haemoproteus columbae in Qena, Egypt. The parasite can severely affect vital organs like the lungs and the liver that may cause from severe illness up to death of the pigeons.*

Keywords: *Haemoproteus columbae, Pigeons parasite, histopathology, liver, lung.*

Corresponding author: *Eman A. Abdelrahim, e.mail: emaneweda@yahoo.com*.

I. Introduction:

Pigeons especially in Egypt have long been considered a sign of beauty, peace and love. In addition, they are the most favorite source of delicious bird meat in Upper Egypt. Several health problems can affect pigeons but parasitic infections play a major role. The genus *Haemoproteus* includes a large number of intracellular protozoan parasites of birds distributed all over the world1. It is the most common blood parasite of birds and has been reported from 67% of total bird species2. Asexual development of this parasite occurs in the peripheral blood of the birds and sexual development in the vector louse fly; *Pseudolynchia canarensis*3,4.

Most infections with parasites of the genus *Haemoproteus* produce subclinical infections. However, the parasite can affect some vital organs in the infected birds. Enlargement of the liver, spleen, kidneys and gizzards were previously reported5,6. *Haemoproteus* can also parasitize the lungs7. In some birds, anemia, anorexia and depression have also been reported8. The pathogenicity of this parasite can thus vary from altered physiology up to mortality9,10,11, depending upon the amount of infection.

Haemoproteus columbae and its vector *Pseudolynchia canarensis* are widely distributed in the world especially in tropical and subtropical regions12,13.

Studies on the prevalence and histopathology caused by *Haemoproteous* in pigeons in Qena governorate is very deficient. Qena governorate covers a stretch of the Nile valley in the southern part of Egypt (Upper Egypt) at 26.143ºN i.e. in the tropical zone14. This study was done to record the prevalence and histopathology of *Haemoproteous* parasites in the pigeon in Qena governorate. This study will give an idea about the magnitude of the problem caused by this parasite in our locality. It is a prerequisite to develop the strategies and the active measures for the prevention and control of this parasite that affects pigeons; which have a special economic significance especially in Upper Egypt.

Recent investigations reveal that some species of avian *Haemoproteids* cause pathology in birds15 and are sometimes lethal9,16,17. These parasites affect host fitness8,11,18, and thus warrant more fundamental research and attention in conservation projects. Studies on the diversity and distribution of *Haemoproteus* parasites are important to better understand wildlife diseases, particularly the virulence and mortality caused by these pathogens both in avian hosts16,17 and blood suckling insects19, which are poorly understood issues.

II. Materials and methods:

To study the prevalence and histopathology of *Haemoproteus Columbae*, pigeons were randomly collected from different localities of Qena Governorate from March 2013 to April 2014. At this period, the total number of pigeons examined were 103 (67 male and 36 female).

Collection and staining of the blood and identification of the protozoa:

After collection, sex of the pigeon was recorded and thin blood films were obtained from the wing vein. The smears were then air dried, fixed with absolute methyl alcohol and stained with Giemsa's stain as per standard method20. The slides were examined under high power light microscope (X400) and oil immersion lens (X1000) for the detection of the blood protozoa. Identification was based on the morphology as described by21,22,23.

Histopathological study:

Parts of the lungs and the liver were collected and fixed in 10% buffered neutral formalin for histopathological studies. Formalin fixed tissue samples were processed, dehydrated in ethyl alcohol, cleared in xylene and impregnated and embedded in paraplast. Tissue blocks were sectioned using manual microtome at 3-5 µm. Tissue sections were stained with Hematoxylin and Eosin for routine histopathological examination23 and with Masson trichrome for detection of collagen fibers. Tissue processing and staining methods were done according to Drury and Wallington24.

III. Results:

In this study, the total number of examined pigeons were 103 (67 male, 36 female), 59 (42 male, 17 female) pigeon from them were infected with *Haemoproteus columbae* by a prevalence of (57.2%).

Examination of the blood smears:

Haemoproteus columbae was detected in RBCs, where the intra-corpuscular gametocytes were elongated, crescent shaped and partially encircle the nucleus of the host cell. Microgametocytes were stained violet with Giemsa's stain, (Figure-1), macrogametocytes were stained dark violet with Giemsa's stain (Figure- 2).

The fully grown intracellular gametocytes of *Haemoproteus* were differentiated into microgametocytes (10.2-12.5 x 1.6-5 µm) and macrogametocytes (13.1-15 x 3.7-6.6 µm). Extra-corpuscular gametocytes (6.6-18.8 x 2.2-10 µm) were visible. Double gametocyte infection of *Haemoproteus* was occasionally present (Figure- 3).

Diagnosis of *Haemoproteus* infection is generally accomplished by microscopic examination of a Giemsa-stained peripheral blood smear. Gametocytes are mainly present within erythrocytes. Organisms may appear similar to *Plasmodium*, but the pigment within the intra-erythrocytic gametocytes is more dispersed and schizonts are not seen in the peripheral blood smears. These pigment granules (haemozoin) are derived from the digestion of haemoglobin found within the host's erythrocytes and appear as refractile, golden brown granules within the host's erythrocyte25. The gametocytes partially encircle the erythrocyte nucleus forming a halter-shaped appearance with little displacement of the host cell nucleus. *Haemoproteus* gametocytes often occupy over one half of the erythrocyte cytoplasm. The parasite may cause slight enlargement of the infected host cells and displacement of the red blood cell nucleus to one side. Based on the above generic characters, the genus is identified as *Haemoproteus*26. The concentration of the parasite was sparse (1–8 pars/100 RBC) but occasionally a high degree of erythrocytes parasitization was visible (30–40 pars/100 RBC). Occasionally, the parasite infected two adjacent cells (Figures-1a-g), also at times there was a close approximation of cells parasitized with micro and macrogametocyte (Figure- 3f).

Immature and mature gametocytes were visible in blood films. Young forms (Figures-1a-c, Figures-3a, e), immature gametocytes (Figures-1d-h,-3a, e, g, k). The measurements of the young and immature forms were (2.5 -8.7 µm in length x 2.5 -4.2 µm in width). They developed lateral to the host cell nucleus and have no contact with the host cell membrane or the host cell nucleus.

Mature forms could be differentiated into macrogametocytes (with randomly scattered granules and nucleus with clear margins) (Figure- 2) and microgametocytes (with polar granules and nucleus diffused with the cytoplasm) (Figure- 1).

Macrogametocytes (Figures-2a–p, 3f) are broadly sausage shaped, slightly halteridial and usually situated lateral to the erythrocytic nucleus. The fully grown parasite reached the poles of the infected erythrocyte and encircled its nucleus (Figures-2a, b, e, i, m, n, o, p). The margins were mostly smooth and rarely amoeboid. Variations in the shape of macrogametocytes were quite evident.

Sometimes, a large space between the gametocyte and the host cell membrane could be seen in the central zone and in such cases, the gametocyte was thin in the central zone and broad at the ends (Figures- 2g, h). On the other hand, gametocytes adhered to the host cell membrane (Figures- 2a,p) were also detected. Some gametocytes were broad at one end and narrow at the other (Figures- 2 b, c, d, g). Occasionally, the ends of the parasite curved around the erythrocytic nucleus (Figures- 2b,c,d).

Almost mature forms displaced the host nucleus towards one pole (Figure- 2c). Cytoplasm of these forms was moderately coarse and stained pale blue with Giemsa's stain. The granules were median or small sized and dispersed randomly in the whole cytoplasm averaging 20 per parasite. When the granules were small

and their number was higher. Granules were black to yellow- brown in color. The parasite nucleus was median and stained pink with Giemsa's stain, averaging 2.5 µm in length and 1.2 µm in width.

The measurements of the mature forms varied from 13.1 to 15.0 µm in length and 3.1–6.2 µm in width (average length 14.05 µm and width 4.65 µm). The parasite occupied approximately three quarters of the host cell. Microgametocytes (Figure-1) were slightly smaller than macrogametocytes (Figures-1h-p), slightly halteridial and usually lateral to the host cell nucleus as macrogametocytes. The ends of the parasites are usually rounded and the margin entire. The gametocytes almost adhere to the host cell membrane at the polar zones but sometimes, in the central zones as well.

A fully-grown microgametocyte fills the poles of the affected erythrocytes (Figures- 1i-p). Cytoplasm of the mature forms was fairly granular and stained only lightly with Giemsa's stain, occasionally it was colorless. The granules were localized only at the poles of the parasite averaging 11 per parasite and are yellow–brown or black in color. Parasite nucleus was diffused with the cytoplasm and was not easily distinguishable from the cytoplasm of the parasite. Microgametocytes varied in size from 10.2 to 12.5 µm in length and 1.6 to 5 µm in width (average 11.35 µm in length and 3.3 µm in width). Mature forms occupied the major part of the infected erythrocytes. Intra-corpuscular *Haemoproteus* displaced the host cell nucleus.

Extra-corpuscular forms (Figures-4, 5-a–i) macrogametocytes could be seen lying free in the plasma. The extra-corpuscular forms lying in the plasma were elongated (Figures-4d,i), rounded (Figures- 4b, c), S-shaped (Figure-4h) or spindle-shaped (Figures-4a, e, f). Cytoplasm was granular, granules being dispersed throughout the parasite (Figures-4a- i). The extra-corpuscular forms varied in size from 6.6 to 18.8 µm in length and 2.2 to 10 µm in width while the average measurement was 12.7 µm in length and 6.1 µm in width. The nucleus stained pink with Giemsa's stain and is situated at the center of the parasite averaging 2-3.8 µm in length and 1.8-3 µm in width. Double gametocyte infection (DGI) (Figures- 3a- n), and trigametocyte infection (TGI) (Figures- 3 o, p) were observed.

Histopathological examination:

In this study, two vital organs were examined histopathologically; the lungs and the liver.

1- Examination of the lung:

A. By hematoxylin and eosin:

Examination of the lung tissue of the infected pigeons revealed the presence of schizonts of the parasite. Schizonts could be detected within the endothelial lining of the pulmonary blood vessels which were usually dilated and congested (Figure- 5a). Schizonts were also seen eroding the pulmonary blood vessels and invading the lung parenchyma (Figure- 5b). Within the lung parenchyma schizonts could invade the alveoli destroying their wall (Figure- 5c). They could also be seen within the pulmonary interstitium with a concomitant hypercellularity of the interstitium (Figure- 5d). Most of the pulmonary alveoli were collapsed (Figure- 5a), while few were dilated with destruction of their wall and lysis of their endothelial lining (Figure- 5c&d). Round cell granuloma could also be detected around some pulmonary blood vessels (Figure- 6a). Areas of complete destruction of the lung parenchyma or marked dilatation of the pulmonary alveoli with lacking of their endothelium were observed (Figure- 6b). Beside the lysis of type I pneumocytes (the alveolar endothelium), prominant type II pneumocytes, that were located at the alveolar angles, were observed. Type II pneumocytes appeared protruded with foamy cytoplasm. Sometimes, an extracted nucleus of a type II pneumocyte leaving an empty space could be detected (Figure- 6b). Within the intrapulmonary bronchi schizonts also could be detected. They were seen replacing most of the nuclei of the lining epithelium of the bronchi. They were also detected within the subepithelial blood capillaries (Figure- 6c). They also replaced most of the nuclei of the smooth muscles of the bronchial wall (Figure- 6d).

B. By masson trichrome:

Examination of trichrome stained slides revealed the presence of the green stained collagen fibers in most areas of the pulmonary parenchyma. They could be detected around blood vessels (Figure- 7a), within the pulmonary interstitium, and around alveoli which appeared destructed and dilated. Collagen fibers could also be detected around alveolar ducts forming a honeycomb fibrosis (Figure- 7b).

1- Examination of the liver:

A. By hematoxylin and eosin:

The first noticeable finding on examination of the liver of the infected pigeons was the lymphocytic (round) cell infiltration of the portal areas at the corners of the classic liver lobules (Figure- 8a). Magnification of the infiltrated portal areas revealed a granuloma-like round cell infiltration formed mainly of small lymphocytes, many plasma cells, and schizonts of the parasite in and around the granuloma (Figures- 8c&d). Schizonts were also seen invading the bile ducts in the portal area extruding and replacing the nuclei of their

epithelial lining (Figures- 8c&d). Schizonts also invaded the endothelial lining of both the central vein and the hepatic sinusoids eroding their wall and invading the hepatic parenchyma (Figures- 8b&9c). Within the hepatic parenchyma, areas of hepatocyte degeneration with vacuolated cytoplasm and pyknotic nuclei were frequently detected (Figures- 8d& 9a, b&d). Small round cell granulomas could also be observed especially near areas of massive hepatocyte degeneration (Figure- 9b). Apoptotic bodies were occasionally observed beside the invading schizonts. In many areas, schizonts were seen extruding the usually pyknotic nuclei of the infected hepatocytes and replacing them (Figure- 9d).

B. By masson trichrome:

Examination of trichrome stained slides revealed an obvious deposition of the green stained collagen fibers around the central vein, at the borders and in between the hepatic lobules (Figure- 10a), and a marked deposition was observed in and around the portal area but lacking at its center (Figure- 10b).

IV. Discussion:

The parasites of genera *Haemoproteus*, *Plasmodium*, and *Leucocytozoon* are well-known avian *haematozoa* and can cause declined productivity and high mortality in wild birds27. Diagnosis of *Haemoproteus* infection is generally accomplished by microscopic examination of a Giemsa-stained peripheral blood smear. Organisms may appear similar to *Plasmodium*, but the pigment within the intra-erythrocytic gametocytes is more dispersed and schizonts are not seen in the peripheral blood smears. These pigment granules (haemozoin) are derived from the digestion of haemoglobin found within the host's erythrocytes and appear as refractile, yellow to brown granules within the host's erythrocyte25. The gametocytes partially encircle the erythrocyte nucleus forming a halter-shaped appearance with little displacement of the host cell nucleus. *Haemoproteus* gametocytes often occupy over one half of the erythrocyte cytoplasm. Parasite may cause slight enlargement of the infected host cells and displacement of the red blood cell nucleus to one side, the mature gametocytes of *Haemoproteus columbae* occupy the host cell completely. They distort it and push the host cell's nucleus to one side13. This criteria are in agreement with our study where almost mature forms displaced the host nucleus towards one pole as in (Figure- 2c), mature forms occupied the major part of the infected erythrocytes as in (Figure- 2a –p). The parasite may also have a comma shape as in (Figure- 2c) as previously recorded by Dey et al.6.

Based on the above generic characters, the genus of the current study was identified as *Haemoproteus*26.

The currently demonstrated numerous gametocytes in the peripheral blood of infected pigeons were those of *Haemoproteus columbae* which confirms the identity of the parasitic forms encountered in tissues.

Occasionally, DGI and TGI were observed in this study. It was previously mentioned that DGI and TGI are rarely reported in vertebrate erythrocytes. This phenomena has been suggested to enhance *apicomplexon* transmission28.

The prevalence of *Haemoproteus columbae* in Qena governorate at the studied period (from March 2013 to April 2014) was 57.2%. The prevalence of bird *Haemoproteus* in two wetland sites in Bangladesh was 13.2%27. The prevalence of *Haemoproteus* in different areas of Mymensingh district of Bangladesh during July to December, 2007 was 20%6. In India, the prevalence for *Haemoproteus* was 18% and in Myanmar, it was 40%29. The prevalence of *Haemoproteus columbae* was 21% in pigeon in Iran, with the highest infection rate was observed in autumn 44%, while the lowest was in spring 12%30. It was 46.55% (27/58) in urban areas of Lages, in the state of Santa Catarina, Brazil31. In Queensland, Colombia, Bulgaria and the United States the prevalence rate for *Haemoproteus* ranged from 20% to 32%. Collectively, studies, to date, have determined that the most common blood parasite found in pigeons is *Haemoproteus columbae* and the infection rate may be as high as 75% ranging from 6 to 86%13.

By comparison, the prevalence of *Haemoproteus columbae* in the current study is one of the highest of all previous studies in nearly similar tropical areas, regardless of the difference in the season and the year of the study. It means that this parasite is a big problem in Qena governorate and measures for its control should be done as rapid as possible to save our pigeons and our environment.

The parasitemia of *Haemoproteus* in this study (in Qena, Egypt) was 1-8% which is greatly higher than that detected in other tropical areas as in Mymensingh district of Bangladesh where it was 0.2-0.5%6.

In the current study, schizonts of the parasite were massively detected in the studied organs. They were seen invading both the lungs and the liver of the infected pigeon. They were mainly directed towards the endothelial lining of the blood vessels and the epithelial lining of other luminal structures like the pulmonary bronchi and the bile ducts replacing their nuclei. They also replaced the nuclei of the smooth muscle fibers of the musculosa of the pulmonary bronchi.

The observed *Haemoproteus* schizonts in the studied tissues were thin walled and irregular in shape resembling the schizont morphology of *Haemoproteus* species described by other researchers7,32. In the present

study, 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 the schizont shape in tissues was possibly related to the site of the schizont development in a trial to conform to the local parasitized histological structures. The noticed free extravascular schizonts were most likely a sequence to the damage of the vascular walls caused by the effect exerted by the large- sized parasitic stages7.

On invasion by schizonts, many histopathological changes could be detected within the studied organs. Erosion of the blood vessels including the pulmonary blood vessels, the central hepatic vein and the hepatic sinusoids with lysis of their endothelial lining was the most prominent finding. At this point, it was previously reported that during the life cycle of *Haemoproteus* sporozoites found in the salivary glands of the vector fly *Pseudolynchia canariensis* infect a new host they are directed towards the endothelial cells of the blood vessels and lungs, liver and spleen with the production of schizonts33. It was also detected that the schizogenous phase of the *Haemoproteus* life cycle takes place in the endothelial cells34.

The vascular lesion due to invasion by schizonts consists of degeneration and dissolution of endothelial cells and it is impossible to detect the endothelial cell lining in most of the affected vessels6. Mubarak and Abed7 added that *Haemoproteus* in the lung is known to infect lung capillaries and the growing schizonts greatly distort the endothelial lining cells with a consequent vascular damage.

Schizonts in the lung were also detected invading, eroding and destructing the alveolar wall with lysis of the alveolar endothelium (type I pneumocytes). Many alveoli appeared small and collapsed and others were abnormally wide and emphysematous. At the same time, numerous hypertrophied type II pneumocytes were observed. In addition to, fibrosis of the pulmonary interstitium and fibrosis of the interalveolar septa forming a honeycomb fibrosis in trichrome stained infected lung tissue. Massive cellular infiltration of the pulmonary interstitium in addition to, the detection of small round cell granulomas particularly near the blood vessels were very clear.

Similar to the present findings a previous study approved that pulmonary tissue is a main target for *Haemoproteus* sporozites and a major site for schizogony. Histopathology of the parasitized lung, as previously reported, confirmed the existence of schizonts in the pulmonary blood vessels and granulomatous pulmonary tissue reaction was detected at the site of released merozites. In addition to, distension of the vasculature, emphysematous air capillaries and in some areas collapse of both air and blood capillaries7. They also observed cellular infiltration and marked pulmonary tissue response as presented by granulomatous reaction in the lung tissue (granulomatous pneumonia), which is most probably a response to the existence of free merozoites released after vascular damage and schizont rupture. They were concluded that *Haemopoteus* infection could provoke significant pathological pulmonary changes in the pigeon.

Histopathologically, the reactive signs observed in the infected lung tissue due to invasion by the parasitic schizonts are called interstitial pneumonia. Interstitial pneumonia is characterized by homogenous expansion of the pulmonary interstitium by inflammatory cells, hyperplasia of type II pneumocytes, honeycomb fibrosis and fibrosis of the pulmonary interstitium, fibrosis patchy areas of alveolar consolidation, and thickening of interlobular septa35. Interstitial pneumonia is usually a fatal condition36.

In the liver, in addition to erosion of its blood vessels by schizont invasion, infiltration by round inflammatory cells and formation of round cell granuloma especially around the portal areas were the most prominent findings. The granulomas were mainly formed of lymphocytes and plasma cells were frequently encountered, it also contained brown pigments and many schizonts of the parasite. Similar to the current finding, Mubarak and Abed7 described the cellular composition of the pulmonary granuloma of the *Haemopoteus* infected pigeons to be formed of macrophage, lymphoid cells, plasmacytes, bordered by proliferated fibroblasts and intermingled by released merozites and pigment materials.

The observed pigment granules whether inside the infected tissues or inside the intra-corpuscular stage of the parasite are haemozoin pigment granules. They are derived from the digestion of haemoglobin found within the host's erythrocytes and appear as refractile, yellow to brown granules25.

In the present study, schizonts of *Haemoproteus* were seen invading hepatocytes and also the epithelium lining of bile ducts replacing their nuclei. In addition, areas of hepatocyte degeneration, hepatocytes with dense pyknotic nuclei, apoptotic bodies, and fibrosis around blood vessels and portal areas were observed.

Regarding the degenerative changes in the hepatic parenchyma with *Haemoproteus* infection, it was previously noticed that hepatic cords which are the normal histological arrangement of the hepatic parenchyma disappeared in the affected areas7,37.

As *Haemoproteus* infection invades hepatocytes, infection with this genus is sometimes known as pseudomalaria because of its similarities with *Plasmodium* species. However in case of *Plasmodium* gametocytes are only present within erythrocytes38.

AS *Haemoproteus* infection in the liver follow the same way as *Plasmodium*, it is thought that the infected hepatocytes resist the host hepatocyte apoptosis to succeed in the intracellular replication within hepatocytes and then release new infectious forms into the blood. However, many hepatocyte with pyknotic

nuclei and apopototic bodies were observed in this study. Likely as in *Plasmodium* infection, it is thought that infected hepatocytes can lose their ability to resist apoptosis when anti-apoptotic mitochondrial proteins are inhibited, therefore, they follow the mitochondria-dependent apoptotic pathway39. It has also been shown that infected host cells undergo non-apoptotic cell death at the end of the liver stage development, presumably to foster the release of the infectious forms into the blood stream40.

V. Conclusions:

There is a high prevalence of *Haemoproteus columbae* in Qena, Egypt. The parasite can severely affect vital organs like the lungs and the liver that may cause from severe illness up to death of the pigeons.

References:

[1]. Shiff C. J., Cranfield M. R., Graczyk T.K., Extraction of Haemoproteus columbae (Haemosporina: Haemoproteidae) antigen from rock dove pigeons (Columba livia) and its use in an antibody elisa, J. Parasit., 80, 713-718 (1994)

[2]. Burry-Caines J. R. and Bennett G. F., The haemoproteidae (Apicomplexa: Haemosporina) of the avian families Fringillidae and Emberizidae sensulato, Can. J. Zool., 70, 1149-1160 (1992)

[3]. Adriano E. A. and Cordeiro N. S., Prevalence and Intensity of Haemoproteus columbae in Three Species of Wild Doves from Brazil, Mem. Inst. Oswaldo. Cruz., 96, 175-78 (2001)

[4]. Sá M. R., Studies of avian malaria and Brazil in the international scientific context (1907-1945), Hist. Cienc. Saude. Manguinhos. 18, 499-518 (2011)

[5]. Dey A. R., Begum N., and Khan M. A. ., "Haemoprotozoan infection in ducks: prevalence and pathology," Bangladesh, Journal of Veterinary Medicine, 6(1), 53–58 (2008)

[6]. Dey A. R., Begum, N., Paul, S. C., Noor, M. and Islam, K. M., Prevalence and pathology of blood protozoa in pigeons Reaered at Mymensingh District, Bangladesh, int. Bio. Res. 2(12), 25-29 (2010)

[7]. Mubarak M., and Abed G. H., "Pathological changes of lung tissues of pigeons (Columba livia domestica) infected with Haemoproteus columbae (Haemosporina: Haemoproteidae),"Journal of Biological Sciences, 5(5), 536–541 (2005)

[8]. Valkiūnas G., Avian malaria parasites and other haemosporidia, CRC Press, Boca Raton, Florida, pp 946, (2005)

[9]. Donovan T. A., Schrenzel M., Tucker T. A., Pessier A. P., and Stalis I. H., Hepatic hemorrhage, hemocoelom, and sudden death due to Haemoproteus infection in passerine birds: Eleven cases, J. Vet. Diagnostic Invest., 20, 304–313 (2008)

[10]. Garvin M. C., Homer B. L., Greiner E. C., Pathogenicity of Haemoproteus danilewskyi, Kruse, 1890, in blue jays (Cyanocitta cristata), J. Wildl. Dis., 39, 161-169 (2003)

[11]. Marzal A., De Lopes F., Navarro C., and Moller A. P., Malarial parasites decrease reproductive success: An experimental study in a passerine bird, Oecologia, 142, 541–545 (2005)

[12]. Soulsby E. J. L., Helminth, Arthropods and Protozoa of Domesticated Animals. 7th edition. Baillire, Tindall, pp 35-740 (1982)

[13]. Samani A. D., Kheirabadi K. P., Samani A. D., Prevalence and rate of parasitemia of Haemoproteus columbae in Columba Iivia domestica in Southwest of Iran (Short Communication) Iranian J. Parasitol, 8(4), 641-644 (2013)

[14]. Pidwirny M., Introduction to the Biosphere, Physical Geography, Chapter 9, (2008)

[15]. Cardona C. J., Ihejirika A., and Mcclella N., Haemoproteus lophortyx infection in bobwhite quail. Avian Diseases, 46, 249–255 (2002)

[16]. 16- Ferrell S. T., Snowden K., Marlar A. B., Garner M., and Lung N. P., Fatal hemoprotozoal infections in multiple avian species in a zoological park, J. Zoo and Wildlife Medicine, 38, 309–316 (2007)

[17]. Olias P., Wegelin M., Freter S., Gruber A. D., and Klopfleisch R., Avian malaria deaths in parrots, Europe, Emerging Infectious Diseases, 17, 950–952 (2011)

[18]. Nordljng D., Anderson M., Zohari S., and Gustafsson L., Reproductive effort reduces specific immune response and parasite resistance. Proceedings of the Royal Society of London, B 265, 1291–1298 (1998)

[19]. Valkiūnas G., and Iezhova T. A., Detrimental effects of Haemoproteus infections on the survival of biting midge Culicoides impunctatus (Diptera: Ceratopogonidae), Journal of Parasit., 90(1), 194-196 (2004)

[20]. Cable R. M. An Illustrated Laboratory Manual of Parasitology, 4th edition, Burges Publishing Co. Minneapolis 15, Minnesota, USA, pp 114-115, (1957)

[21]. Levine N. D., Veterinary Protozoology, 1st edition, Iowa State University Press, Ames, pp 266-282 (1985)

[22]. Springer W. T., Other blood and tissue protozoa edited by Calnek, B.W; Barnes H.J.; Beard, H.J.; McDougald, L.R. and Saif, Y. M. In: Diseases of Poultry (10th edition) Iowa state University Press, U.S.A., pp 900-911 (1997)

[23]. Luna L. G., Manual of Histologic staining methods of the Armed forces Institute of Pathology. 3rd edition. Mc Graw Hill Book Co. New York, (1968)

[24]. Drury R. A. B., Wallington E. A., Carleton's Histology technique, 5th edition. Oxford University Press, Oxford, New York,Toronto, pp 241-242 (1980)

[25]. Friend M., Franson J. (eds), Parasitic diseases, In: Field manual of wildlife diseases: general field procedures and diseases of birds, US Geological Survey, Reston, pp 193–262 (1999)

[26]. Kruse W., Ueber Blutparasiten. Virchows. Arch. Path. Anat. Physiol., 121, 359–372 (1890)

[27]. Elahi R., Islam A., Hossain M. S., Mohiuddin K., Mikolon A., Paul S. K., Hosseini P. R., Daszak P., and Alam m. S., Prevalence and Diversity of Avian Haematozoan Parasites in Wetlands of Bangladesh, Hindawi Publishing Corporation, J. Parasit. Res., Article ID 493754, 12 pages (2014)

[28]. Jovani R., Amo L., Arriero E., Krone O., Marzal A., Shurulinkov P., Tomas G., Sol D., Hagen J., Lopez P., Martin J., Navarro C., Torres J., Double gametocyte infection in apicomplexon parasites of birds and reptiles, Parasitol. Res., 94,155–157, (2004)

[29]. Ishtiaq F., Gering E., Rappole J. H., Rahmani A. R., Jhala Y. V., Dove C. J., Milensky C., Olson S. L., Peirce M. A., Fleischer R. C., Prevalence and diversity of avian haematozoan parasite in Asia: a regional survey, J. Wildl. Dis., 43,382–398 (2007)
Senlik, B., Gulegen, E. and Akyol V., Prevalence and intensity of Haemoproteus columbae in domestic pigeons, IndianVeterinary Journal, 82(9), 998-999 (2005)

[30]. Tietz-Marques S. M., De Quadros R. M., Da Silva C. J., Baldo M., Parasites of pigeons (Columba livia) in urban areas of lages, Southern Brazil, Parasitol. Latinoam, 62, 183-187 (2007)

[31]. Mohammed A. H. H., Studies on the schizogony of Haemoproteus columbae Kruse, 1980, Procedings of the Egyptian Acadamy of Science, 19, 37 – 64 (1967)

[32]. Archawaranon M. and Subinprasert S., Bird-parasite Relations: A Hill Mynah Case Study, Journal of Entomology, 2, 112-116 (2005)
[33]. Mushi E. Z., Binta M.G., Chab R.G., Mathai M. and Ndedel R.T., Haemoproteus Columbae in domestic pigeons in Sebele, Gaborone, Botswana, Onderstepoort Journal of Veterinary Research, 66, 29-32 (1999)
[34]. Cottin V., Donsbeck A-V., Revel D., Loire R., and JEAN-FRANÇOIS Cordier J-F., Nonspecific Interstitial Pneumonia Individualization of a Clinicopathologic Entity in a Series of 12 Patients, 158(4), 1286-1293 (1998)
[35]. Korfei M., von der Beck D., Henneke I., Markart P., Ruppert C., Mahavadi P., Ghanim B., Klepetko W., Fink L., Meiners S., Krämer O. H., Seeger W., Vancheri C.,Guenther A. J., Comparative proteome analysis of lung tissue from patients with idiopathic pulmonary fibrosis (IPF), non-specific interstitial pneumonia (NSIP) and organ donors, J. Proteomics, 85,109-128 (2013)
[36]. Youssefi M. R., and Rahimi M. T., Haemoproteus Columbae in Columba livia domestica of Three Areas in Iran in 2010, Global Veterinaria, 7(6), 593-595 (2011)
[37]. 38- Gupta D. K., Jahan N., Gupta N., New records of Haemoproteus and Plasmodium (Sporozoa:Haemosporida) of rock pigeon (Columba livia) in India, J. Parasit. Dis., 35(2),155–168 (2011)
[38]. Kaushansky A., Metzger P. G., Douglass A. N., Mikolajczak S.A., Lakshmanan V., Kain H. S. and Kappe S. H. I., Malaria parasite liver stages render host hepatocytes susceptible to mitochondria-initiated apoptosis, Cell Death and Disease, 4, e762 (2013)
Sturm A., Amino R., van de Sand C., Regen T., Retzlaff S., Rennenberg A., Krueger A., Pollok J. M., Menard R., Heussler V. T., Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids, Science, 313, 1287–1290 (2006)
[39]. Kaushansky A., Metzger P. G., Douglass A. N., Mikolajczak S.A., Lakshmanan V., Kain H. S. and Kappe S. H. I., Malaria parasite liver stages render host hepatocytes susceptible to mitochondria-initiated apoptosis, *Cell Death and Disease*, 4, e762 (2013)
[40]. Sturm A., Amino R., van de Sand C., Regen T., Retzlaff S., Rennenberg A., Krueger A., Pollok J. M., Menard R., Heussler V. T., Manipulation of host hepatocytes by the malaria parasite for delivery into liver sinusoids, *Science*, 313, 1287–1290 (2006)

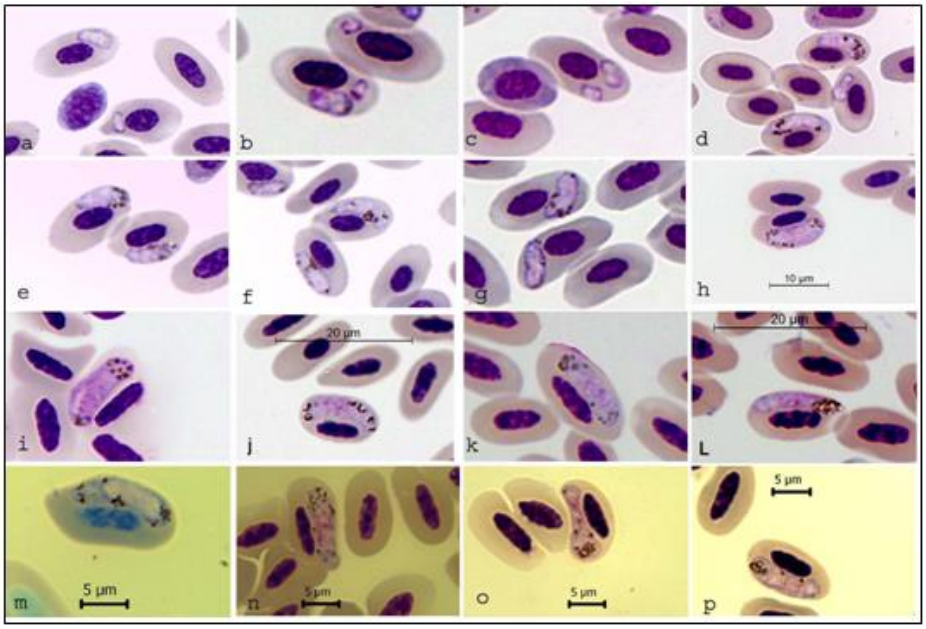

Fig 1 Microphotographs of immature stages of *Haemoproteus columbae* (a-c) in addition to young microgametocytes (d-h) and fully formed microgametocytes (i-p), The scale bar of (a-i) is the same as j.

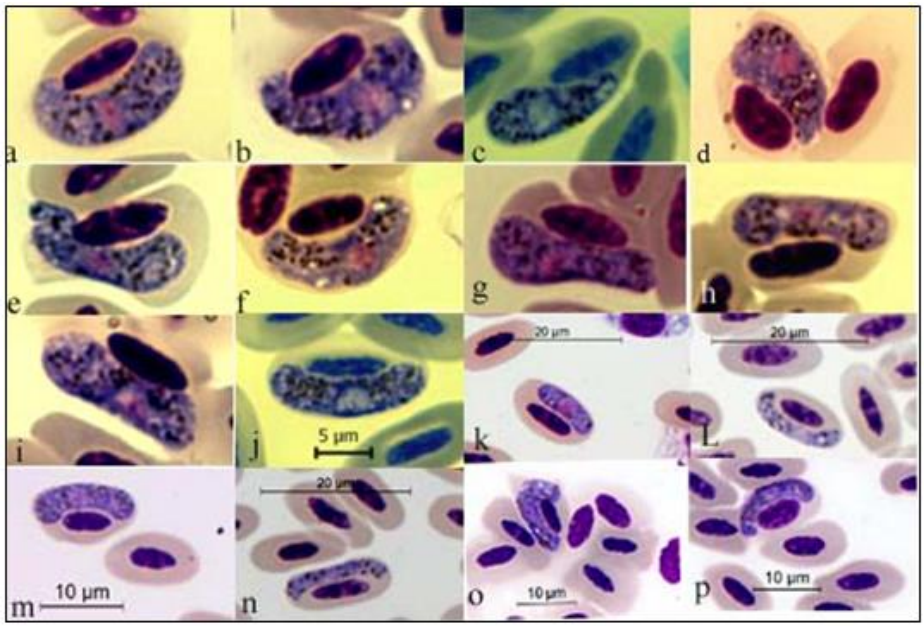

Fig 2 Microphotographs of fully grown macrogametocytes of *Haemoproteus columbae* (x1000): a, b, e, f, j, L, m, n, o, p: Curved around the host cell nucleus. c ,d: Broad at one end and narrow at the other, c: The host cell nucleus displaced towards the pole. b, d, f, o, p: Host cell cytoplasm completely filled with macrogametocyte.

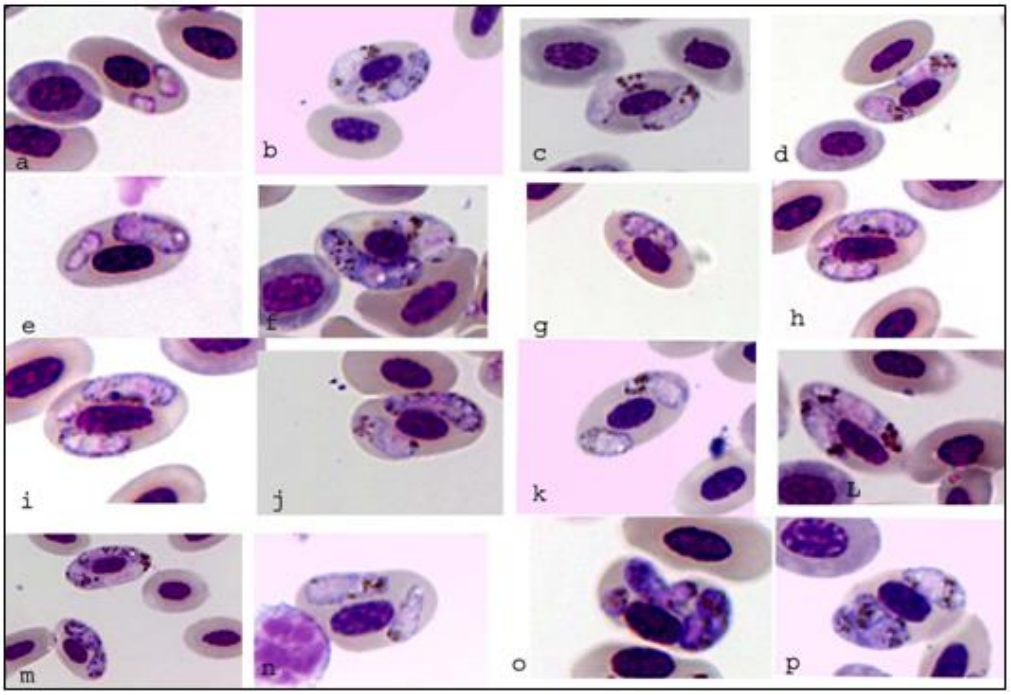

Fig 3 Microphotographs of more than one stage of *Haemoproteus columbae* in each RBC

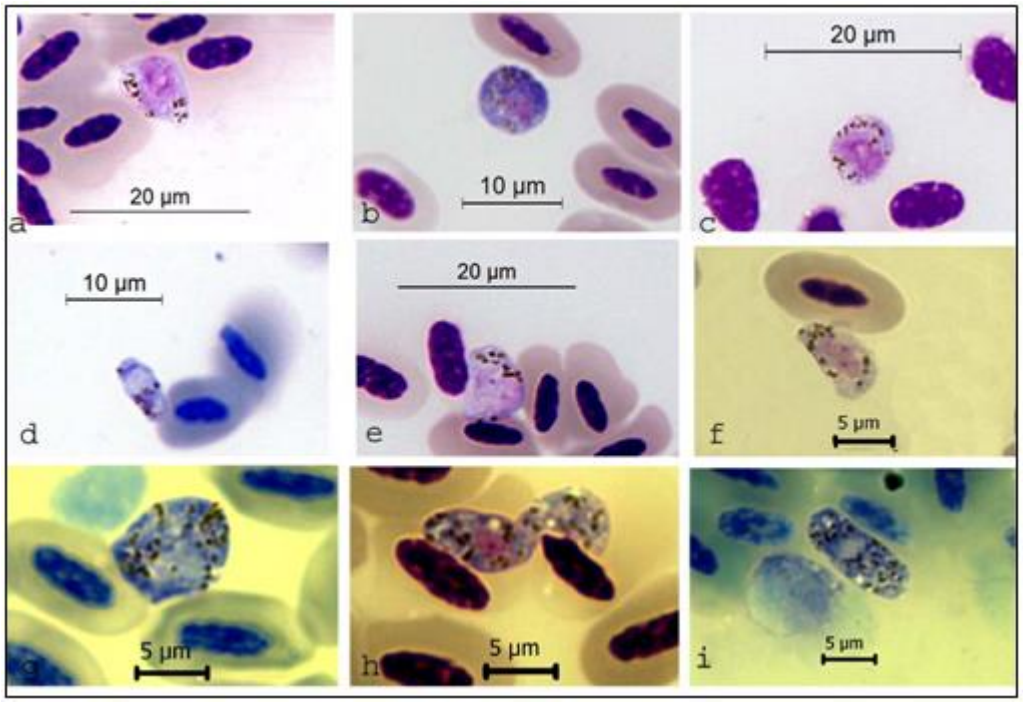

Fig 4 Microphotographs of extracellular forms.

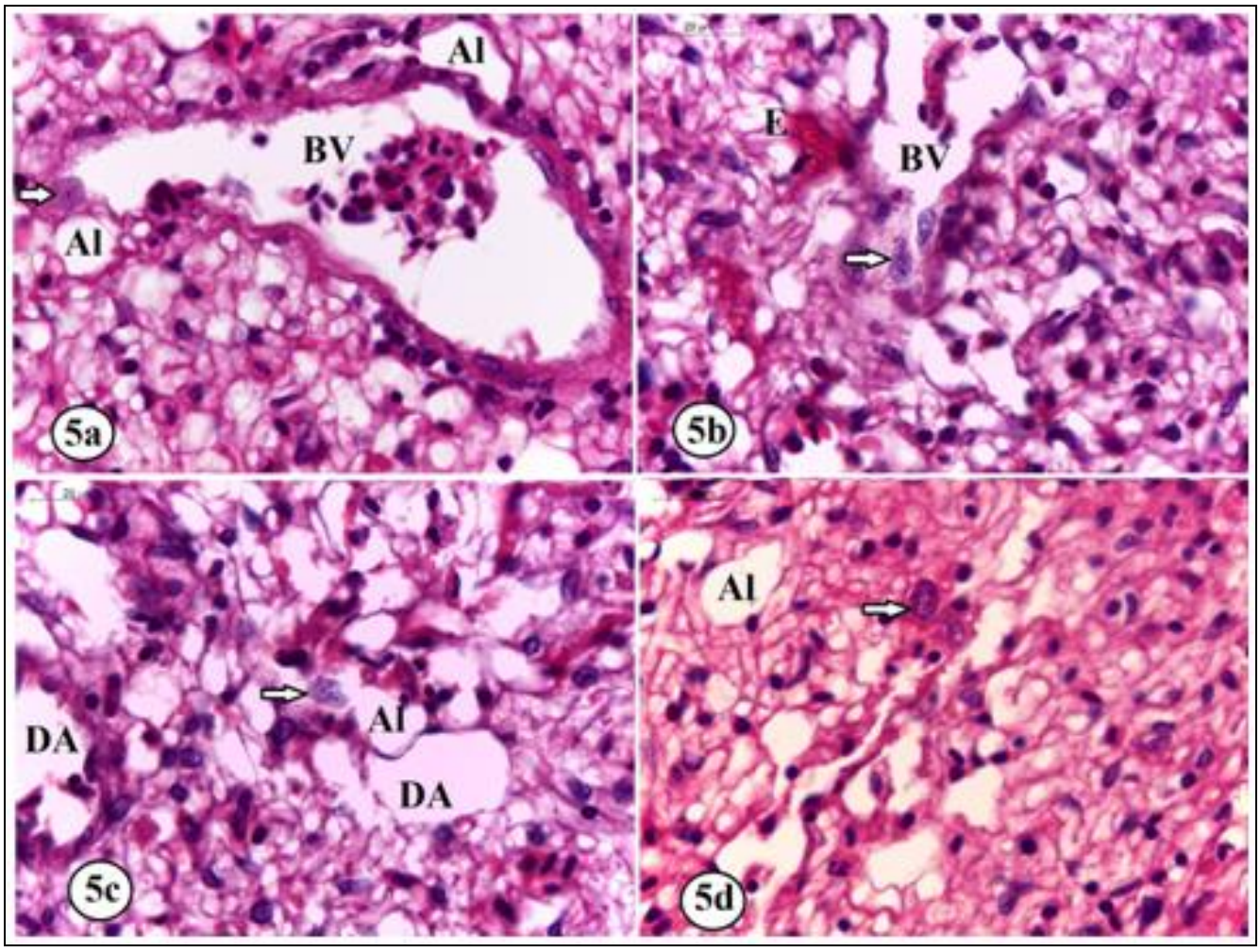

Fig. 5 Photomicrographs of the lung of a severely infected pigeon. (a): shows schizont (arrow) invading the endothelium of a pulmonary blood vessel (BV) which appears dilated and congested. Notice the cellular infiltration of the pulmonary interstitium and that most of the alveoli are collapsed while few are dilated (Al). (H&E, X 1000). (b): shows schizont (arrow) eroding a pulmonary blood vessel (BV) with lysis of its endothelium. Notice the presence of an exudate (E) around the blood vessel. (H&E, X 1000). (c): shows a schizont (arrow) eroding a pulmonary alveoli (Al). Notice that most alveoli are abnormally dilated and destroyed (DA) with lacking of their endothelium (H&E, X 1000). (d): shows schizont (arrow) within the pulmonary interstitium which shows cellular infiltration. Notice that some alveoli (Al) are dilated with lysis of their endothelial lining. (H&E, X 1000).

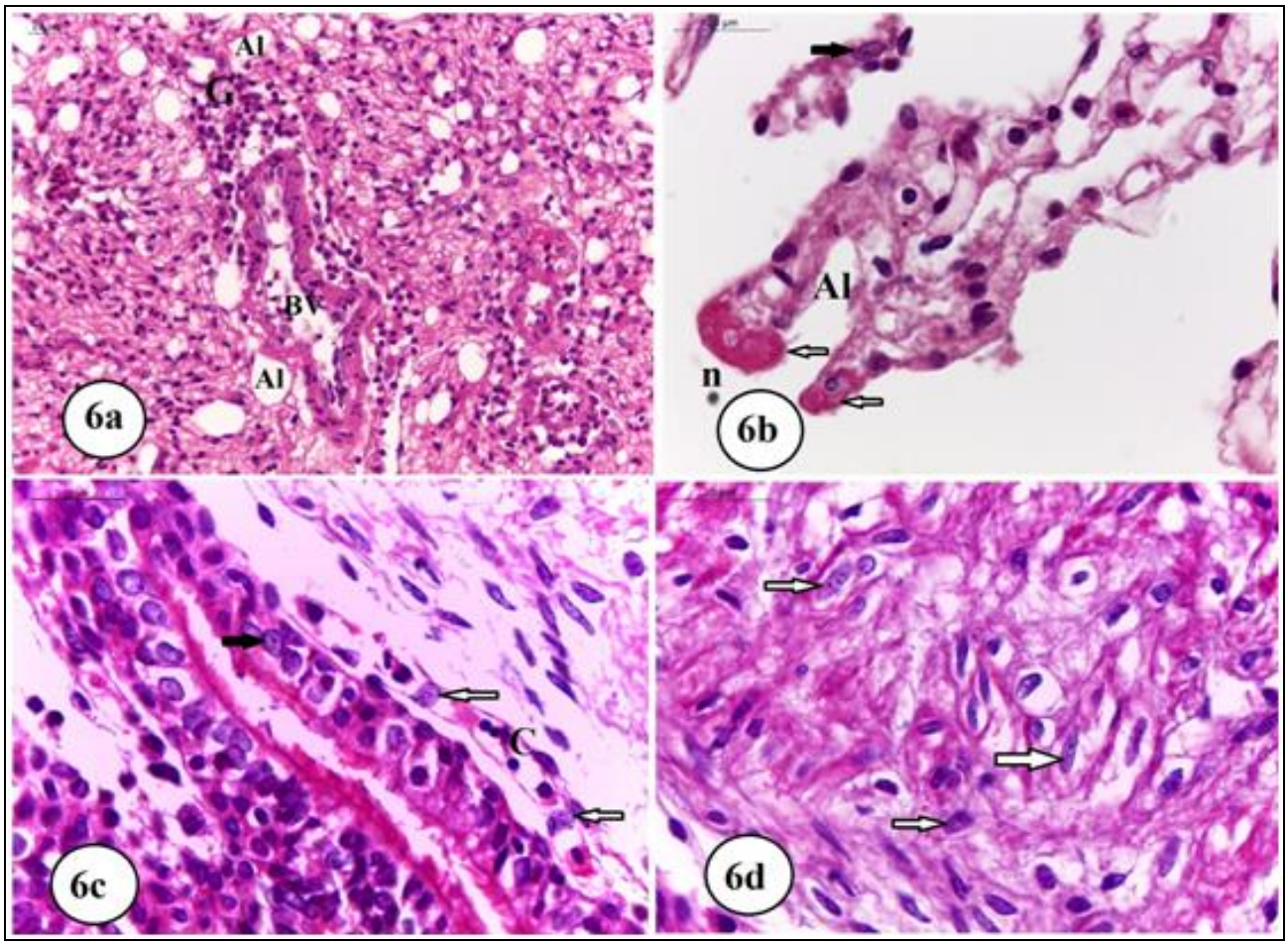

Fig. 6 Photomicrographs of the lung of a severely infected pigeon. (a): shows a round cells granuloma (G) around a thickened dilated blood vessel (BV). Notice the presence of dilated alveoli (Al) that looked lacking their endothelium. (H&E, X 400). (b): shows a nearly destroyed part of the lung with alveoli that lack their endothelium (Al). Notice the presence of prominant type II pneumocytes (white arrows). One of these pneumocytes shows an extruded nucleus (n) leaving an empty space. Schizonts are also observed within the pulmonary interstitium (back arrow). (H&E, X 1000). (c): shows an intrapulmonary bronchus with schizonts (black arrow) replacing the nuclei of their epithelial lining. They also can be detected (white arrows) within a blood capillary (C) underlying the epithelium. (H&E, X 1000). (d): shows the musculosa of an intrapulmonary bronchus with schizonts (arrows) replacing the nuclei of its smooth muscle fibers. (H&E, X 1000).

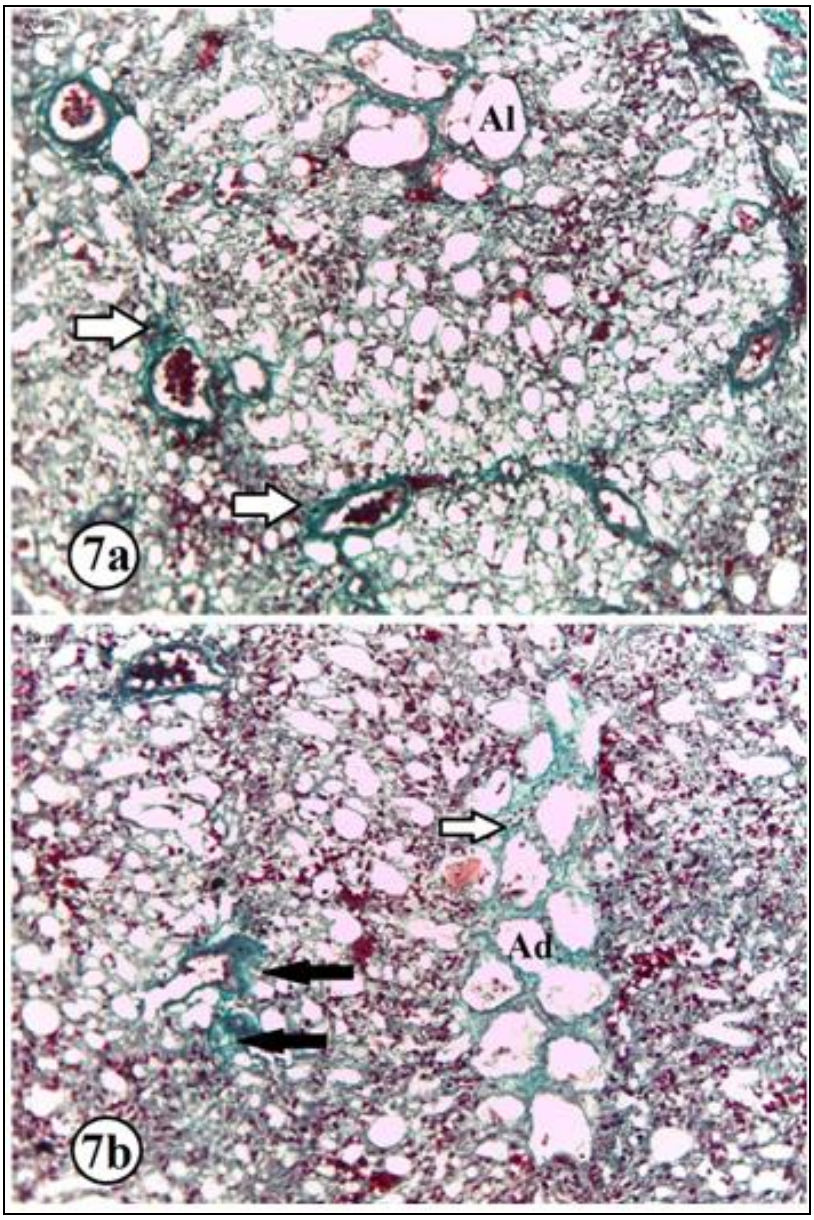

Fig. 7 Photomicrographs of the lung of a severely infected pigeon. (a): shows a relatively thick layer of collagen fibers around most pulmonary blood vessels (arrows). Notice that some alveoli are destructed and dilated (Al). (Masson trichome, X 400). (b): shows collagen fibers (arrow) surrounding an alveolar duct (Ad) forming a honeycomb fibrosis and within the pulmonary parenchyma around destructed alveoli (black arrows). (Masson trichome, X 400).

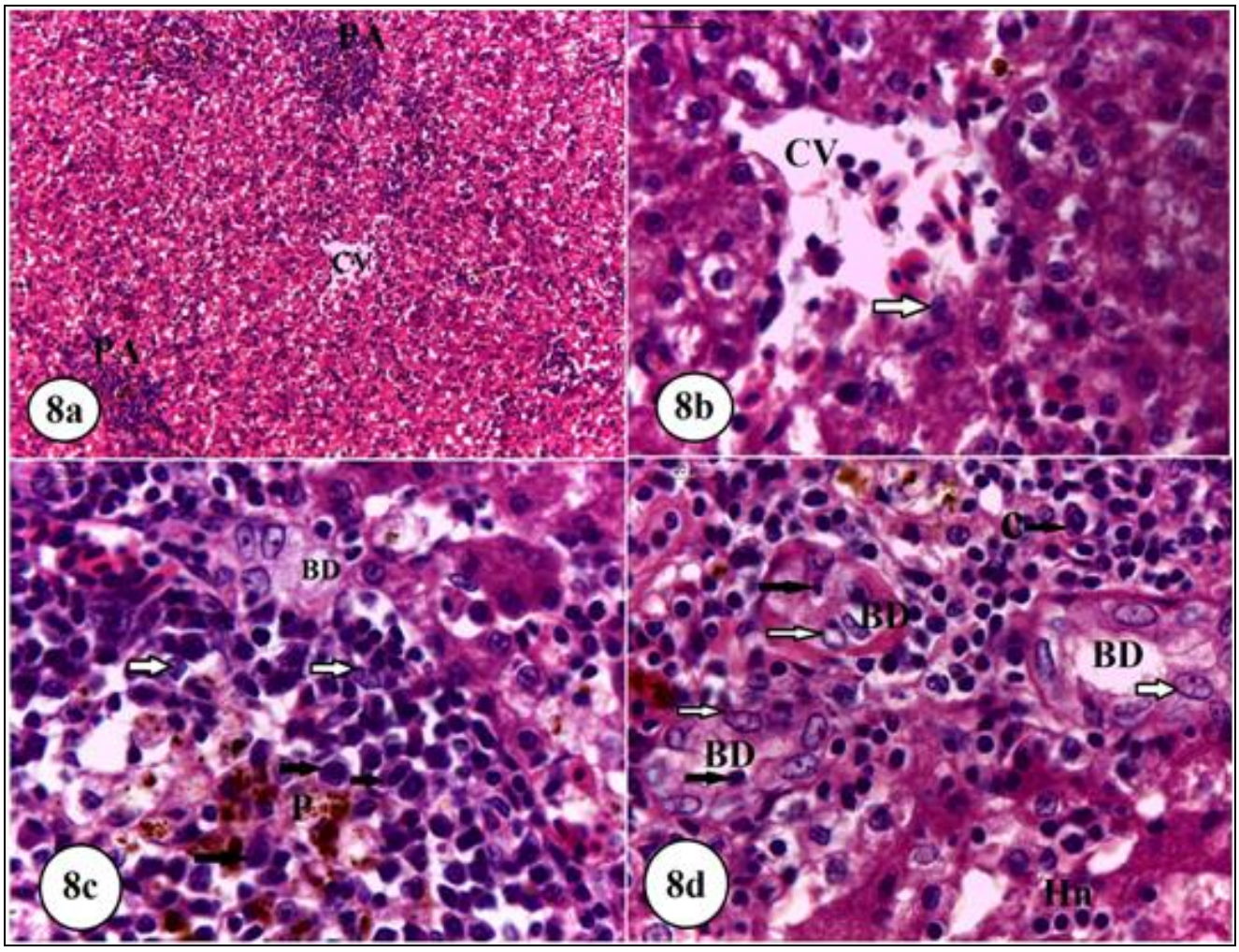

Fig. 8 Photomicrographs of the liver of an infected pigeon (a): shows round inflammatory cell infiltration of the hepatic parenchyma that is mainly concentrated in the portal area (PA), CV (central vein). (H&E, X 200). (b) a magnification of the central vein (CV) shows absence of its endothelial lining and its invasion by schizonts of the parasite (arrow). (H&E, X 1000). (c): a magnification of the portal area shows massive infiltration by round inflammatory cells (lymphocytes), many schizonts (white arrows) and many plasma cells (black arrows) are seen. P (brown haemozoin pigment), BD (bile duct) within the portal area with the nuclei of its epithelium are replaced by schizonts of the parasite. (H&E, X 1000). (d): shows three bile ducts (BD) within an infiltrated portal area with complete replacement of the nuclei of their lining by schizonts (white arrow) of the parasite, black arrows (indicating the extruded nuclei of the epithelial lining of the bile ducts). Notice that most hepatocyte nuclei are dark pyknotic nuclei (Hn). (c) a plasma cell among the round inflammatory cells. (H&E, X 1000).

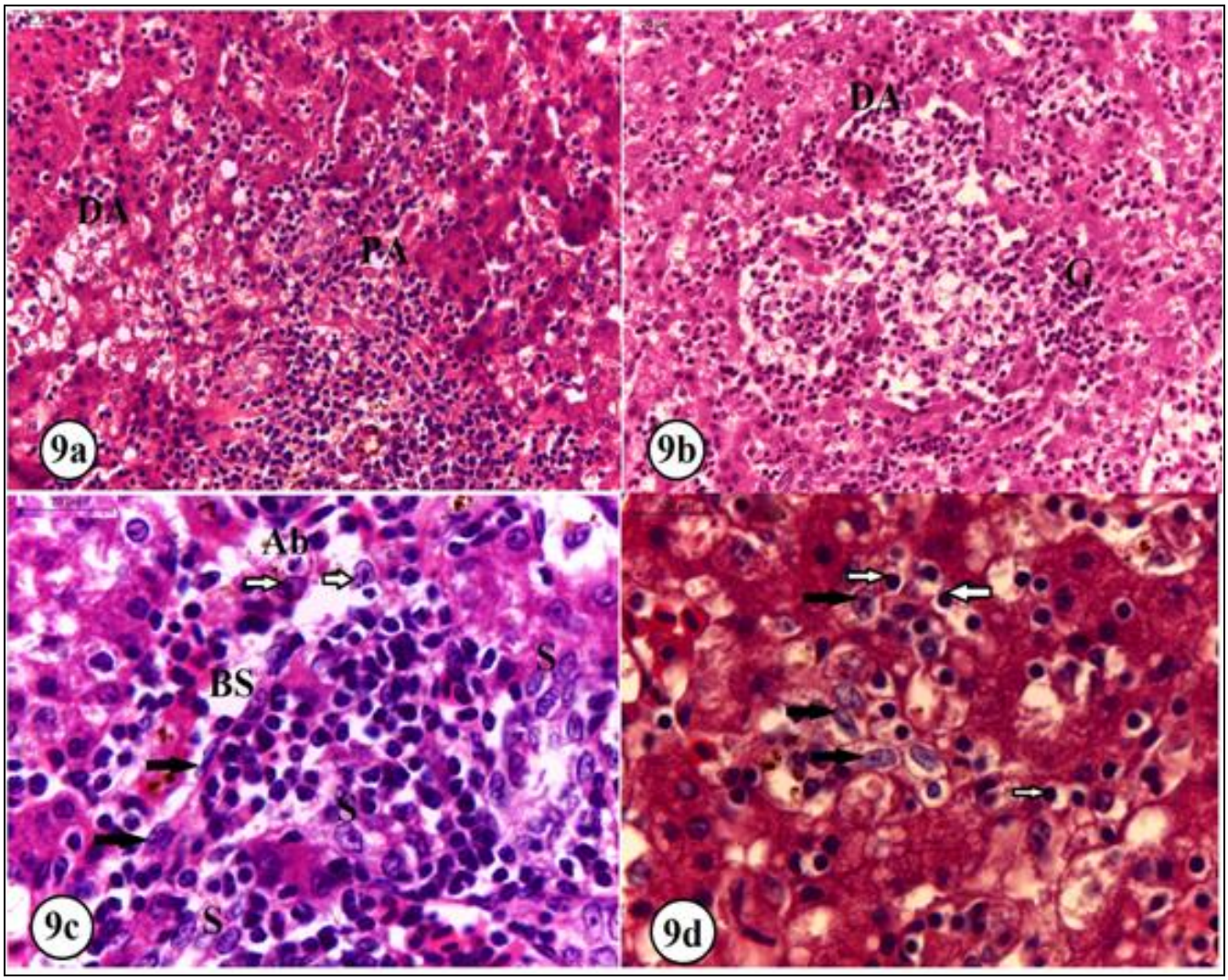

Fig. 9 Photomicrographs of the liver of an infected pigeon (a): shows an area of degenerated hepatocytes (DA) with an obviously vacuolated cytoplasm just adjacent to a highly inflammatory cell infiltrated portal area (PA). (H&E, X 400). (b): shows a well-circumscribed area of degenerated hepatocytes (DA) infiltrated at its boundary by round inflammatory cells that forms a small granuloma (G). (H&E, X 400). (c): shows a hepatic sinusoid (BS) which is eroded at sites of invasion by schizonts (arrows) where there is an absence of its endothelium, a nearby apoptotic body (Ab) can be detected. Remnants of the endothelium of the blood sinusoid can be observed (black arrows). Notice: the infiltration by round inflammatory cells with many schizonts in between (S) around the blood sinusoid. (H&E, X 1000). (d): shows a part of the liver parenchyma with most hepatocytes have a degenerated cytoplasm and a small dense "pyknotic" nucleus (white arrows), in some hepatocytes schizonts replace their nuclei (black arrows). (H&E, X 1000).

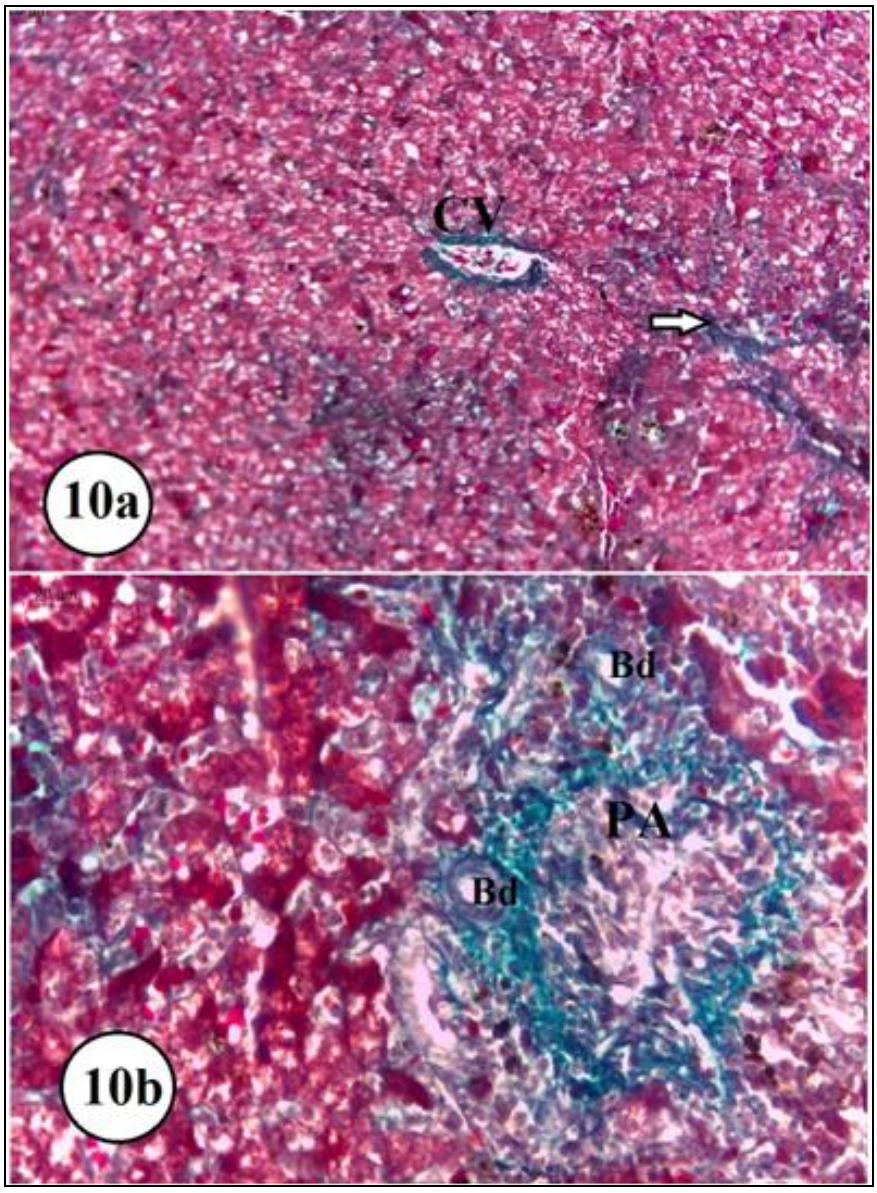

Fig. 10 Photomicrographs of the liver of an infected pigeon (a): shows deposition of the green stained collagen fibers around a central vein (CV) and in between the hepatic lobules (arrow). (Masson trichrome, X 200).
(b): shows marked deposition of the green stained collagen fibers in and around the portal area (PA) but lacking at its center, Bd (bile duct). (Masson trichrome, X 400).