Ass. Univ. Bull. Environ. Res. Vol. 5 No. 2, October 2002

AUCES

PARASITOLOGICAL, HISTOPATHOLOGICAL AND ULTRASTURCTURAL STUDIES ON *HAEMOPROETUS COLUMBAE* IN PIGEONS IN ASSIUT GOVERNORATE

M. Mubarak*; M.I. Arafa and I.A. Fouad****

*Dept. of pathology, Faculty of Vet. Med. Assiut University

**Animal health research institute, Assiut lab.

ABSTRACT :

Examination of 220 blood samples of pigeons was done in Assiut Governorate for parasitological studies on *Haemoproteus columbae*. The incidence of infection with *H.columbae* was 35.45%. The rate of infection in domestic pigeons was 40.0% and in wild pigeons was 85.0% while it was not detected in squabs. The intensity of infection in apparently healthy pigeons was 2-6 parasites /1000 RBCs while it was 75-93 parasites/1000 RBCs in diseased pigeons. The morphological characters of different forms of *H.columbae* were described. Maturation process of gametocytes was done and the different changes of gametocytes were described.

Necropsy of highly infected pigeons revealed that the liver and kidneys were enlarged and dark in color. Histopathological examination of the viseral organs, brain and skeletal muscles was carried out. There was marked inflammatory reactions and degenerative changes in liver, lungs and kidneys caused by presence of *Haemoproteus* (Gametocytes, hemozoin pigmnet or schizonts). Ultrastructural examination of RBCs from selected cases was also carried out. The ultrastructural features of *Haemoproteus columbae* within RBCs were described.

INTRODUCTION :

Today birds represent one of the main source of animal protein allover the world. Pigeons are kept as a hobby in addition to their food value. The avain haemosporidian parasites are a diverse of some 250 species distributed throughout apicomplexan families: Leucocytozaidae, Plasmodiidae and Haemoproteidae (Bennett *et al.*, 1982). Nearly 90% of all records of mortality and pathogenicity due to avain haematozoa have been described from domestic birds (Bennet *et al.*, 1993). There are 128 species of *Haemoproteus* in birds all of which are host specific but few species are pathogenic (Bennett *et al.*, 1994). Earle *et al.*, (1993) mentioned that during 1990 a high mortality rate was recorded in doves in South Africa as a result of massive infection with *H.columbae.*

In Egypt, *H.columbae* was described from the Egyptian domestic pigeons by Farid (1933) and it was studied in detailes by several authors: Mohammed (1958), Ahmed & El-Sisi (1965),

Abd-El Salam (1978), El-Gwady (1983) and Abo-El-Magd *et al.*, (1988).

H.palumbis was discovered by Baker (1966) from the English wood-pigeons. Makhloof (1975) studied both sexual and asexual stages of *H.palumbis* in domestic pigeons in Assiut Governorate and considered them as a new host for this parasite. These results were confirmed by Abed (1991) in the same locality.

The aim of the present work was to study the incidence, intensity of infection and morphological characters of *H.columbae* in pigeons in Assiut Governorate in addition to identification of their histopathological changes and their ultrastructure.

MATERIAL AND METHODS :

Blood smears:

-Blood samples were collected from 220 pigeons out of which 150 adult (110 domestic pigeons and 40 wild pigeons) in addition to 70 domestic squabs. Most of these pigeons (200) were apparently healthy and 20 pigeons were diseased (weak, anemic, refused food and unable to fly)

-Thin blood films were made, fixed in methyl alcohol and stained with Giemsa's stain according to Soulsby (1982) then dried and examined microscopically.

-To estimate the parasitaema of *H.columbae*, fields with about 100 RBCs were selected and the number of parasites were counted according to Earle *et al.*, (1993).

-In order to determine maturity of gametocytes, exflagellation process was done in wet blood film according to Garnham (1966).

-Different forms of *H.columbae* detected by microscopic examination were measured with eye pice micrometer and microphotographed.

Gross pathology:

The sacrificed (highly infected with *H.columbae*) pigeons were examined for the existence of gross pathological changes in visceral organs, brain and skeletal muscles.

Histopathology:

Tissue specimens including liver, lungs, kidneys, heart, spleen, intestine, brain and skeletal muscles obtained from sacrificed pigeons were fixed in 10% neutral buffered formalin. Fixed tissues were processed routinely for paraffin embedding technique, sectioned at 4 μm and stained with hematoxylin and eosin (HE) according to Bancroft and Stevens (1982).

Transmission electron microscopy:

Blood samples from selected cases were collected with anticoagulant (heparin), fixed in 4% buffered glutaraldehyde (4°C) and 1mm thick slices of fixed blood with consecutive layers of leucocytes and erythrocytes were obtained according to procedure described by Anderson (1965). Blood slices were post-fixed in 1% osmium tetroxide, dehydrated in up-graded ethanol series and embedded in Epon 8/2. Ultra-thin sections were prepared, double stained with uranyl acetate and lead citrate (Johannessen, 1978) and examined under transmission electron microscope (TEM) (JEOL 100 CXII) operated at 80kv.

RESULTS :

Examination of 220 blood films of pigeons revealed that the rate of infection of *H.columbae* was 35.45% where it was detected in 78 pigeons. The incidence of *H.columbae* infection in adult pigeons was 52.0% while it was not detected in squabs. The wild pigeons are considered highly susceptible for infection where their incidence

was 85.0% while the incidence of infection in domestic pigeons was 40.0% (Table 1).

Parasitaemia:

In pigeons which were apparently healthy the parasitaemia was low 2-6 parasites/1000 RBCs, while the parasitaemia in diseased pigeons was high 75-93 parasites/1000 RBCs. Multiple invasions of host cell were common in diseased pigeons (plate I. 10, 11).

Morphological characters:

a-Merozoite: It was ring, ovoid or rod shaped, has a small chromatin granule. It mostly adherent to host cell membrane but in few cases the detected merozoites were adherent to both nucleus and host cell membrane (plate I 1, 2, 3).

b-Immature gametocyte: It was sausage shaped and it sometimes had irregular surface towards the host cell nucleus. It mostly had pigment granules but in some forms these granules were undistinct (plate I 4, 5).

c-Gametocytes: Macro and microgametes are mostly helter-shaped causing lateral displacement of host cell nucleus but some gametocytes occupied a pilar or an oblique position in the red blood cells so displace the host cell nucleus to the other pole or it may be expelled. Some gametocytes were highly vacuolated specially in diseased pigeons (plate I 6, 7, 8, 9). Pigment granules may be arranged in two or four groups specially in female gametocytes. All these forms were detected mainly in diseased pigeons, and caused a little hypertrophy of the infected RBCs. Measurements and other morphological characters were recorded in (Table 2).

Exflagellation: The process of maturation of micro and macrogametocyte of *H.columbae* was done on wet blood film through 15 minutes. Both gametocytes became active, smaller in size, ranged from 4.4-6.4μ (mean 5.4 μ) in length and produce one or more processes in both directions. The length of each process ranged from 2.4-4.8μ (3.5μ) (plate II 1,2). After 15 minutes these processes disappeared and the macrogmetocytes become rounded or ovoid in shape, their diameter ranged from 4.2-5.8μ (5.3μ) and their nucleus become eccentric. The nucleus of infected RBCs was pushed more peripherally (plate II 3). Some ookinetes were detected in EDTA blood samples after about 20 minutes from taking sample. It is composed from two parts, anterior elongated part with a narrow end and the posterior part which was swollen or spherical in shape. Their nucleus was located at the junction of the two parts. Pigment granules and small rounded vacuoles were scattered in the cytoplasm of the two parts. The measurements of the ookinetes were 10.4x3.5 – 12.6x4.8 μ (11.5 x 4.0μ) (plate II 4).

Table (1): Incidence of *Haemoproteus columbae* in pigeon in Assiut.

		No. of ex. Pigeon	No. of inf. Pigeon	%
Squabs	Domestic	70	-	-
Adult	Dombstic	110	44	40.00
	Wild	40	34	85.00
	Total of adult	150	78	52.00
Total		220	78	35.45

Table (2): Morphometeric parameters of *Haemoproteus columbae* in the present work. (all measurements are in microns).

	Range	Mean
Uninfected RBCs:		
Length	9.6-12.2	11.4
Width	4.0-4.8	4.4
Infected RBCs:		
Length	10.8-13.8	12.3
Width	4.8-5.6	5.3
Merozoites:		
Length	2-4.5	3.2
Width	1.6-2.8	2.2
Immature gametocytes:		
Length	6.5-10.5	9.2
Width	2.2-3.5	2.8
Macrogametocytes:		
Length	9.8-15.0	13.2
Width	2.5-3.5	3.0
Nucleus	1.2x1.8 – 1.6x2.4	1.6x2.0
Pig. granules	20-35 (number)	29
Vacuoles	3-9 (number)	7
Microgametocytes:		
Length	8.2-13.4	11.2
Width	1.6-2.8	2.4
Nucleus	1.6X2.4 - 2.4X2.8	1.8x2.5
Pig. granules	8-22 (number)	20
Vacuoles	2-5 (number)	3

Gross pathological findings:

The infected pigeons revealed enlargement of the liver and kidneys which appeared chocolate brown in color. Blood appeared thin and watery.

Histopathology:

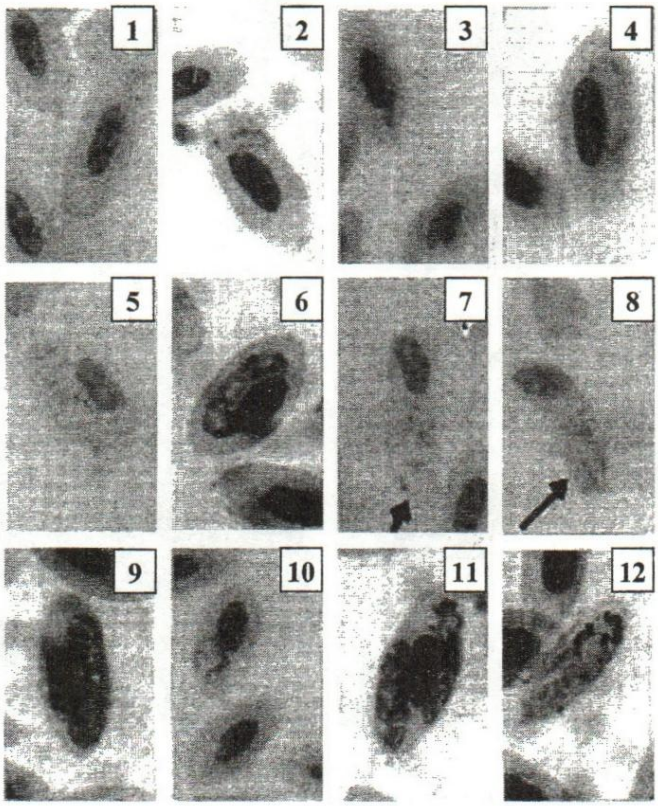
Microscopical examination of tissues obtained from infected pigeons, liver revealed presence of golden brown to dark brown pigment granules of different sizes (hemozoin) within macrophages and kupffer cells (plate III A). Gametocytes of *Haemoproteus* were frequently revealed within macrophages and kupffer cells in portal and periportal areas (plate III A). Marked aggregations of inflammatory cells were observed nearby or surrounding gametocytes and hemozoin and composed mostly of macrophages and lymphoid cells (plateIII B&C). Nodules composed of dense aggregations of mononuclear cells were observed in portal areas and hepatic parenchyma (plate IVB). The hepatocytes showed hydropic degeneration (plate III D).

Lungs revealed presence of gametocytes and hemozoin within macrophages (plate IV A). Gametocytes and schizonts were observed also in the pulmonary capillaries and arterioles (plateIVA). Schizonts appeared as rounded or oval structures containing darkly-stained bodies (merozoites). Periparabronchial inflammatory cell infiltrations composed of lymphoid cells, macrophages and heterophils were observed (plate IV B). The inflammatory cells were observed in parabronchial lumina (plate IV C). Sometimes the pulmonary tissue showed edema (plate IV D).

Kidneys revealed presence of gametocytes within blood vessels (plate V A). Hemozoin pigment could be observed in the interstitial tissue (plate V B). Perivascular and intertubular mononuclear cell infiltration was evident (plate V A&B). The kidney tubules were destroyed and replaced by the reacting mononuclear cells (plate V B).

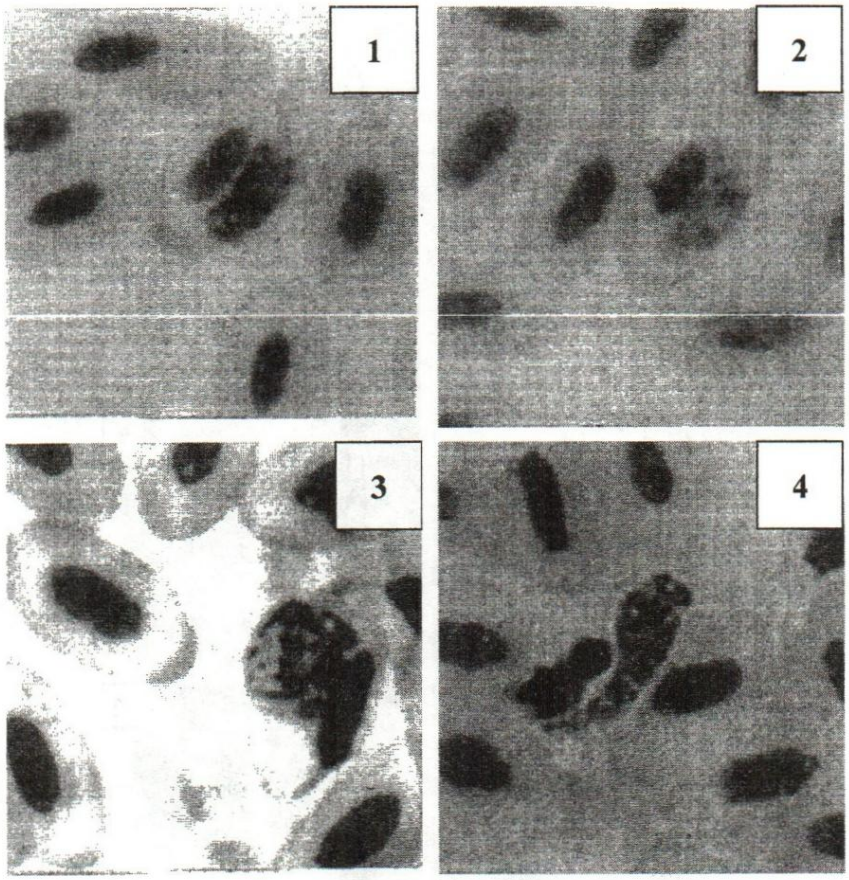
No histological changes could be observed in the other examined organs.

Plate (I) : Blood smears of infected pigeons showing different forms of *H.columbae.*



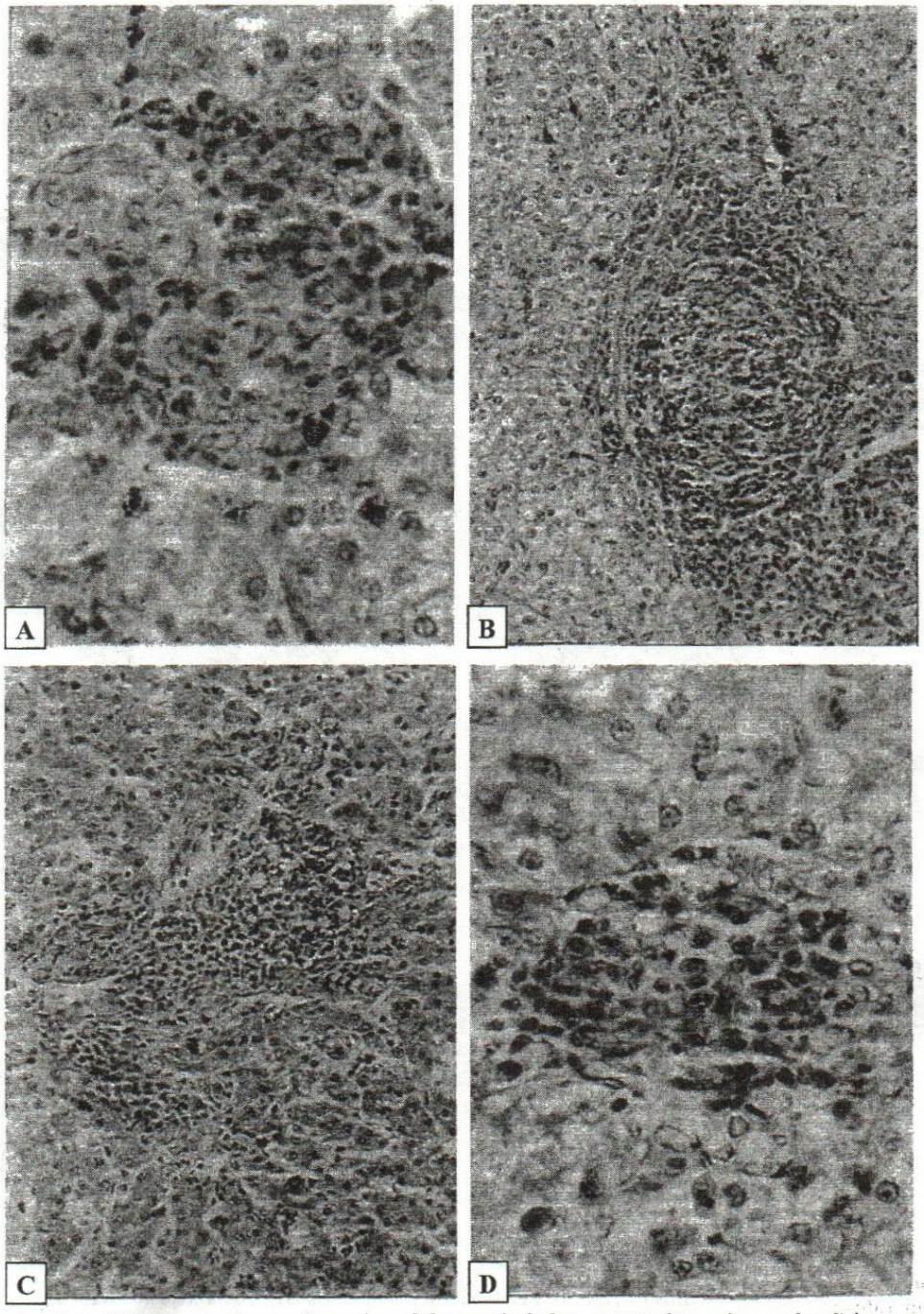
(1, 2, 3) : Different forms of merozoites X 1000.
(4, 5) : Immature gametocytes X 1000.
(6, 7) : Microgametocytes X 1000.
(8, 9) : Macrogametocytes X 1000.
(10, 11) : Multiple infection of *H.columbae* X 1000
(12) : RBCs completely occupied with microgametocytes X 1000.

Plate (II): Maturation of gametocytes



1- Exflagellation of macrogametocyte X 1000.
2- Exflagellation of microgametocyte X 1000.
3- Complete maturation of macrogametocyte X 1000.
4- Developmental ookinete X 1000.

Plate (III) : Liver of infected pigeons.



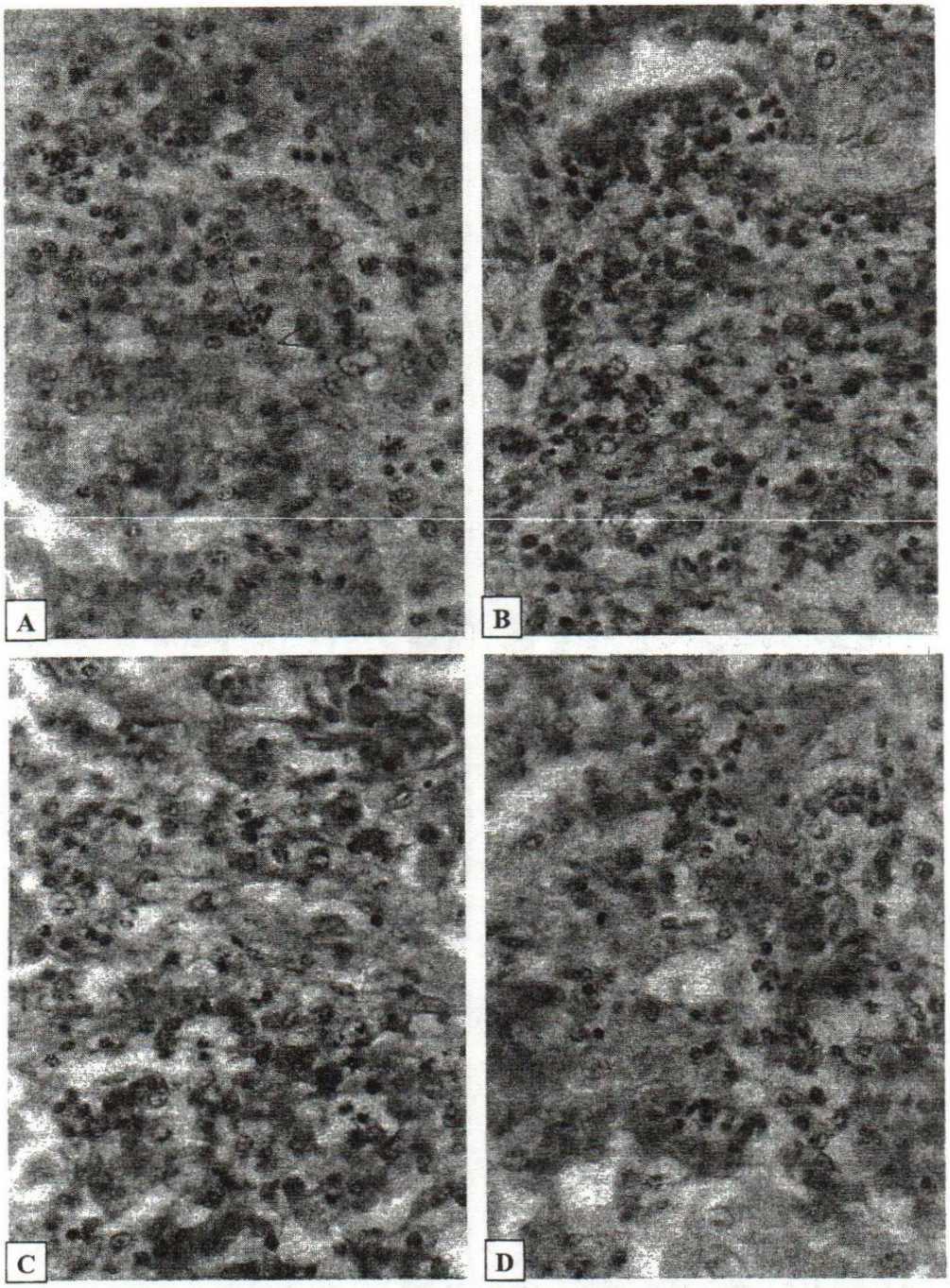
(A):Gametocytes within macrophages (arrow) and hemozoin-laden macrophages (arrow head) in a portal and periportal areas surrounded by aggregations of macrophages and lymphoid cells. HE. Oil immersion X 1000.

(B): A nodule composed of dense aggregations of mononuclear cells HE. X400.

(C): Mononuclear cell infiltration in a portal and periportal areas HE. X 400.

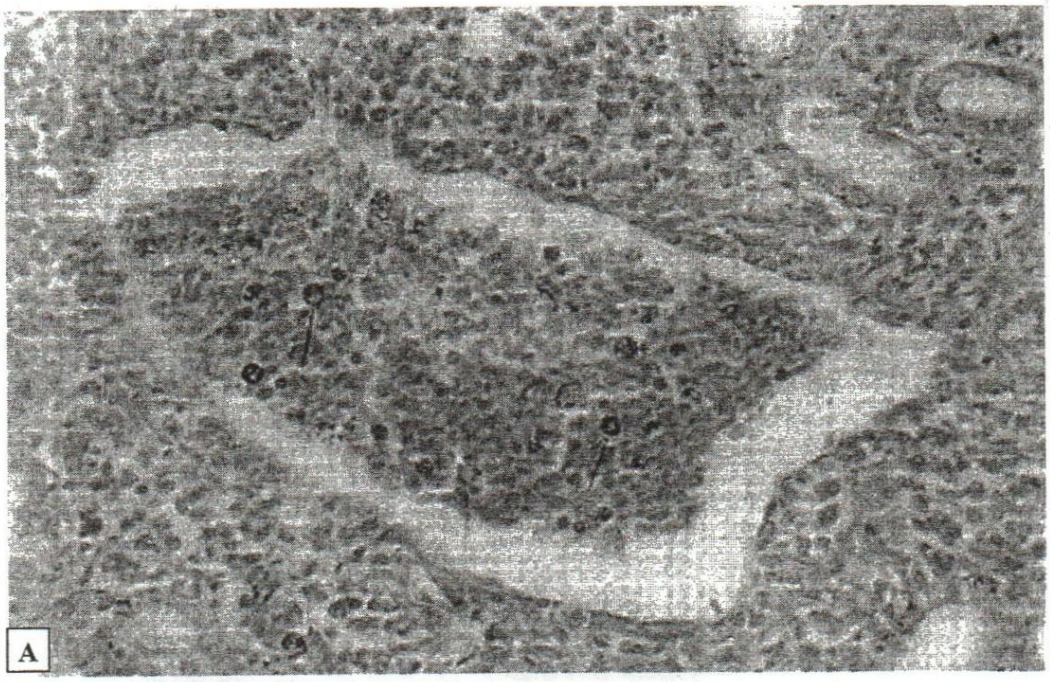
(D): Mononuclear cell aggregations in a focal area, some macrophages filled with hemozoin. The hepatocytes showing hydropic degeneration HE. Oil immersion X 1000.

Plate (IV): lung of infected pigeons.



(A): Gametocytes (arrow) and Schizonts, (arrow head) within blood vesels HE Oil immerssion X1000.
(B): Periparabronchial mononuclear cell infiltration composed of lymphoid cells and macrophages. HE. Oil immerssion X 1000.
(C): Lymphoid cells, macrophages and heterophils in the lumina of parabronchi. HE. Oil immerssion X 1000.
(D): Edema and perparabronchinal mononuclear cell infiltration HE. Oil immersion X 1000.

Plate (V): Kidney of infected pigeons.



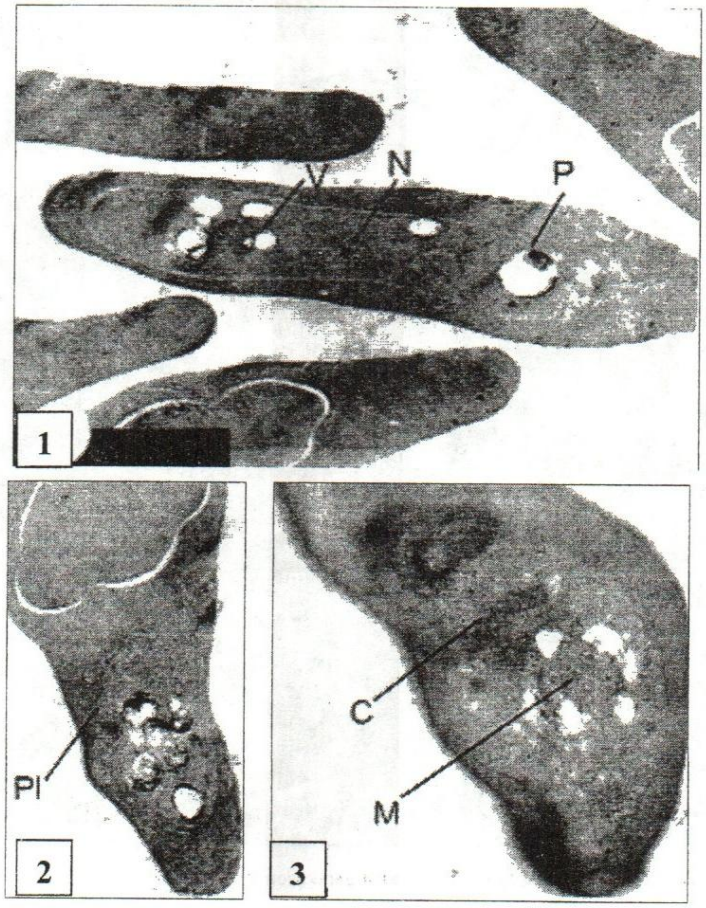
(A):Gametocytes (arrow) within a blood vessel with perivascular mononuclear cell infiltration HE. Oil immersion X 1000.

(B):Marked mononuclear cell infiltration in intertoubular tissue and replacing the destroyed renal tubules. Haemozoin pigment present in interstitial tissue. (arrow head) HE. X400.

Ultrastructural examination of *H.columbae*:

Examination of sections in some infected RBCs with electron microscope revealed that: (plate VI 1) shows a longitudinal section of gametocytes has a central spherical nucleus, with peripheral small nucleolus. There is a large pigment granule in each pole. These granules were vacuolated and surrounded by an electron dense area. Peripherally, there were also four vacuoles scattered in the cytoplasm of gametocytes. (plate VI 2) shows a highly magnification of one pole of gametocytes, clear in which that the pellicle (outer covering) consists of two layers with a distinct space between them, while the pigment granules appeared either vacuolated or as an electron dense bodies. (plate VI 3) shows a high magnification of premature gametocyte, clear in which the cytostome (micropyle) appeared as a deep invagination in the pellicle near the inner pole of the premature gametocyte. Section in mitochondria was located at the center surrounded by several vacuoles while pigment granules were not present.

Plate (VI): Transmission electron micrographs.



1-RBCs containing gametocytes. notice the Nuclulous (N), Pigment granules (P) and Vacuoles (V) X 10500.
2-One pole of gametocytes. Notice the double layers of the pellicle(Pl) X 10500.
3-Immature gametocytes: Notice Cytostome (C) , Mitochondria (M) X 20500.

DISCUSSION :

The present study revealed that the incidence of infection with *H.columbae* was 35.45%, this result is considered lower than that detected by Abd-El Salam (1978), Gad (1982) and Abo-El Magd *et al.*, (1988). This difference between the results might be attributed to the hygienic measures or current usage of insecticides which play a role in control of the vector (*Pseudolynchia canariensis*).

The rate of infection of *H.columbae* in wild pigeons was higher (85.0%) than that of domestic pigeons (40.0%) this might be due to the breeding program of the domestic pigeons which were kept indoor for long time.

Squabs were found free from *H.columbae*, This result agrees with results of previous authors and this might be attributed to the length of the incubation period of *Haemoproteus* which ranged from 28-30 days (Calnek *et al.*, 1997).

The morphological characters of most forms which were detected in the present work agree with that of *H.columbae* which were described by Makhloof (1975), Abd-El Salam (1978) and Abed (1991). In some cases the detected forms were typical to that of *H.palumbis* but according to the recent literatures *H.palumbis* is considered as a synonym for *H.columbae* (Bennett *et al.*, 1992; and Edson & Nelson 2001) so all detected forms in the present work were identified as *H.columbae*.

The parasitaemia was high in diseased pigeons, while low level of parasitaemia was detected in most infected pigeons, which were apparently healthy. Lack of clinical signs or pathological changes for *H.columbae* in most infected pigeons are unknown, but as suggested by Bennett *et al.*, (1992) might be interpreted as a long-term evolutionary association of *Haemoproteus* parasite with pigeons to establish such a complementary equilibrium status.

Investigation conducted on maturation of both gametocytes in wet blood film showed macro and microgametocytes complete their maturation and ookinetes were detected in a short time not more than 15 minutes. These results were nearly similar to that obtained by Makhloof (1975).

In the present study, the gross lesions associated with the infection are in line with those observed by Atkinson *et al.*, (1995) who mentioned that infected blood cells being destroyed and removed from circulation so the blood appeared thin and watery. Dark coloration of organs may attributed to deposition of hemozoin produced by *Haemoproteus* (Levine, 1985; Atkinson *et al.*, 1995). The histopathology of infected pigeons in the present study were represented by inflammatory processes with a marked degree of tissue damage in the liver, kidneys and lungs. These marked changes associated with presence of gametocytes and/or hemozoin. Schizonts were seen only in the lungs. These findings were in agreement with Ahmed & Mohamed (1977) who observed the formation of schizonts in lung tissue only but contrary to findings noted by Earle *et al.*, (1993) who detected schizonts in various tissues, including striated muscle. The presence of megaloschizonts in different organs and striated muscles had been regarded by Earle *et. al.*, (1993) as an indication of the severity of the infection rather than the limitations of *Haemoproteus species* to form schizonts in different tissues. However, in the present study marked inflammatory response and tissue damage evoked by gametocytes and hemozoin pigment with or without presence of schizont were enough to indicate the severity of the infection.

The electron microscopical examination of some infected RBCs threw light on the fine structure of *H.columbae*. Some of our present findings are coincided with that reported by Abed (1991), but the difference being in the description of the cytostome, where it was detected in the present work as a narrow invagination in the wall, while Abed (1991) described it as a flask- shape. This difference appeared to be related to the activity of that organelle, where its function is mostly feeding purpose (Scholtyseck, 1979).

Based on the present study we can conclude that *H.columbae* under certain conditions increase in number and invades large numbers of RBCs that affects their efficiency and induce pathological changes in different organs specially in liver, lung and kidney. Functional disturbances of these organs reflect on the general condition of infected pigeons and may lead to death.

REFERENCES :

1-Abdel-Salam, F.A. (1978): The role of soft ticks in transmission of parasitic disease in domestic birds in Assiut Governorate. M.V.Sc Thesis, Fac. of Vet. Med., Assiut Univ., Egypt.

2-Abed, G.H (1991): Studies on Protozoan Parasites which may infect some birds in Assiut Governorate. Ph. D. Thesis, Fac. of Sci., Assiut Univ., Egypt.

3-Abo-El-Magd, M.M., El-Badri, A.A. and Selim, M.K. (1988): Observations on pigeon blood parasites at Qena province. Assiut Vet. Med. J., 20(39) 199-202.

4-Ahmed, A.A.S. and El-Sisi, M.A. (1965): Observations on diseases affecting pigeons in Egypt and their incidence with special reference to ornithosis, paratyphoid and trichomoniasis. Egypt. Vet. Med. J. (Giza), 11, 319.

5-Ahmed, F.E. and Mohammed, A.H.H. (1977): Schizogony in *Haemoproteus columbae* Kruse. J. Protozology, 24, 389-393.

6-Anderson D.R. (1965): A method of preparing peripheral leucocytes for electron microscopy. J. Ultrostru. Res. 13, 263.

7-Atkinson C.T., K.L. Woods, R.J. Dusek, L. Sileo and IKO, W.M. (1995): Wildlife, disease and conservation in Hawaii pathogenicity of avian malaria (*Plasmodium reticulum*) in experimentally infected 'I'iwi (*Vestiaria coccinea*). Parasitology 111: S 59–S 69.

8-Baker, J.R. (1966): *Haemoproteus palumbis* sp. nov. of the english wood-pigeon *Columba p. palumbus*, J. protozoology, 13 (3), 515-519.

9-Bancroft, D. and Stevens, A. (1982): Theory and practice of histological techniques, 2nd Edn. Churchill Livingstone (Edinburgh, London, Melbourne).

10-Bennett, G.F., Earle, R.A., Hester Du toit and Huchzermeyer, F.W. (1992): A host-parasite catalogue of the haematozoa of the sub-Saharan birds. Onderstepoort. J. Vet. Res., 59, 1-73.

11-Bennett, G.F., Peirce, M.A., Ashford, R.W. (1993): Avian haematozoa mortality and pathogenicity. J. Nat. Hist., 27: 993-1001.

12-Bennett, G.F. Peirce, M.A., Earle, R.A. (1994): An annotated checklist of the valid avain species of *Haemoproteus*, *Leucocytozoon* (Apicomplexa: Haemosporida) and *Hepatozoon* (Apicomplexa: Hemogregarinida). Systematic parasitology, 29: 61-73.

13-Bennett, G.F., Whiteway, M. and Woodworth-lynas, C.R. (1982): Host-parasite catalogue of the avain Haematozoa. Memorial University of New Foundl and Occasional papers in Biology, 5, 1-243.

14-Calnek, B.W., John Barnes, H, Beard, C.W., McDougaid, L.R. and Saif, Y.M. (1997):

Diseases of poultery 10th ed., Mosby International, Lynton House, London.

15-Earle, R.A., Stella, S.Poastianello, Bennett, G.F., and Krecek, R.C. (1993): Histopathology and morphology of the tissue stage of *Haemoproteus columbae* causing mortality in Columbiformes. Avain pathology, 22, 67-80.

16-Edson, A.A. and Nelson, S.C. (2001): Prevalence and intensity of *Haemoproteus columbae* in three species of wild doves from Brazil. Ins. Oswaldo cryz, 96 (2) 175-178.

17-El-Gwady, H.M.A. (1983): Some studies on blood parasites (*Haemoproteus columbae*) of domestic pigeons. Ph. D. thesis, Faculty of Vet. Med. Zagazig University, Egypt.

18-Farid, A. (1933): Veterinary service annual report minist. Agric. Egypt. Cairo, Govt. Press 17, (Cited from Abed, G.H., 1991).

19-Gad, N.A.A. (1982): Studies on some blood protozoa and ectoparasites of birds with special reference to method of control. Ph. D. Thesis, Fac. Vet. Med. Assiut Univ. Egypt.

20-Garnham, P.C.C. (1966): Malaria parasites and other Haemosporidia. Blackwell scientific publication oxford.

21-Johannessen, J. (1978): Instructions and techniques. In: Electron Microscopy in Human Medicine, Vol. 1. Mcgraw – Hill international book company.

22-Levine D.N. (1985): Apicomplexa: *Plasmodium*, *Haemoproteus*, *Leucocytozoon* and Related protozoa. In: Veterinary Protozology, 1st edition, P. 265-290.

23-Makhloof, L.M. (1975): Studies on the role playcd by some insects in transmitting some parasitic diseases in Assiut Province. M.D. Thesis, Fac. Med. Assiut Univ. Egypt.

24-Mohammed, A.H., (1958): Systemic and experimental studies on protozoal blood parasites of Egyption birds vol. I, II Faculty of Science publication.

25-Scholtyseck, S. (1979): Fine structure of parasitic protozoa. Springer – verlag Berlin Heidenlberg.

26-Soulsby, E.J. L (1982): Helminths, Arthropodes and profozoa of Domesticated animals. Englich language book society Bailliere tindall.

Ass. Univ. Bull. Environ. Res. Vol. 5 No. 2, October 2002

دراسات طفيلية وهستوباثولوجية والتركيب الدقيق للهيموبروتيس كولمبى فى الحمام فى محافظة أسيوط

محمد مبارك محمد*، محسن إبراهيم عرفه**، إبراهيم أحمد فؤاد عبد الرازق**

* قسم الباثولوجيا – كلية الطب البيطرى – جامعة أسيوط

** معهد بحوث صحة الحيوان – معمل بيطرى أسيوط

تم فحص ٢٢٠ عينة دم من الحمام فى محافظة أسيوط لعمل دراسة طفيلية عن الهيموبروتيس كولمبى. وقد بلغت نسبة الإصابة به ٣٥,٤٥٪ حيث بلغت نسبة الإصابة فى الحمام المستأنس ٤٠٪، وفى الحمام البرى ٨٥% ولم توجد عدوى بالهيموبروتيس فى زغاليل الحمام. وقد تم دراسة كثافة الطفيل فى الدم حيث تراوحت فى الحمام السليم ظاهريا من ٢-٦ طفيل/١٠٠٠ كرات دم حمراء بينما بلغت ٧٥-٩٣ طفيل/١٠٠٠ كرات دم حمراء فى الحمام الذى كان يحمل أعراض مرضية. وقد تم الوصف المورفولوجى لجميع الأطوار الموجودة بالدم. كذلك تم إجراء تجربة تحويل الجاميتوسيتات إلى جاميتات، وتم وصف التغيرات التى تحدث خلال عملية التحويل.

كما أظهرت الصفة التشريحية للطيور المصابة اللون الغامق والتضخم للكبد والكليتين، تم إجراء الفحص الهستوباثولوجى للأعضاء الداخلية والمخ والعضلات الذى أظهر إلتهابات شديدة فى الكبد والرئتين والكليتين بسبب وجود طفيل الهيموبروتيس بمراحله المختلفة، بالإضافة إلى ذلك تم فحص بعض العينات المصابة بواسطة المجهر الإلكترونى. وتم وصف التركيب الدقيق للأطوار التى وجدت داخل كرات الدم الحمراء.