

Animal Health Research Institute
Assiut lab.

PARASITOLOGICAL AND PATHOLOGICAL STUDIES ON ATOXOPLASMOSIS IN PIGEONS IN ASSIUT GOVERNORATE

(With One Table and 15 Figures)

By

I.A. FOUAD and M.I. ARAFA

(received at 28/6/2004)

دراسات طفيلية وباثولوجية على مرض الاتوكسوبلازما في الحمام
في محافظة أسيوط

إبراهيم احمد فؤاد عبد الرازق ، محسن إبراهيم عرفة

أجريت هذه الدراسة لتحديد نسبة الإصابة لطفيل الاتوكسوبلازما وتأثيره علي الأنسجة المختلفة في الحمام في محافظة أسيوط. أشتمل البحث على فحص عينات الدم والبراز لعدد (300) من الحمام. وقد بلغت نسبة الإصابة بطفيل الاتوكسوبلازما (4.33%) حيث كانت في الحمام البالغ (1%) وفي زغاليل الحمام (11%). وقد تم دراسة كثافة الطفيل في الدم حيث كانت في معظم الحالات 1-2 طفيل/100 كرات دم بيضاء بينما كانت في ثلاث حالات 3-9 طفيل/100 كرات دم بيضاء. كذلك أوضح الفحص المجهرى لمسحات مباشرة من الكبد والطحال للحمام المصاب وجود الطفيل بنسبه عالية في الخلايا الليمفاوية. وقد تم الوصف المورفولوجي للأشكال المختلفة الموجودة في الدم وكذلك أكياس البيض الموجودة في عينات البراز للحمام المصاب. أظهرت الصفة التشريحية للزغاليل شديدة الإصابة بهذا الطفيل انتفاخ الأمعاء وامتلائها بمحتويات بيضاء واطهر الفحص الهستوباثولوجي للأمعاء تنكزز وتساقط لخلاياها الطلائية. كما وجدت أكياس البيض بداخل كثير من هذه الخلايا الطلائية. لوحظ وجود خلايا وحيدة النواة تتخلل النسيج الضام للأمعاء. ولوحظ تضخم الكبد والطحال والكلى وبسالفحص المجهرى لهذه الأعضاء أظهرت وجود عدد كبير من خلايا الالتهاب على هيئة حبيبات مكونه من خلايا وحيدة النواة وخلايا متعددة الصبغات. ولوحظ أيضا وجود طفيل الاتوكسوبلازما بداخل الخلايا وحيدة النواة.

SUMMARY

For parasitological and pathological studies of *Atoxoplasma* spp. of pigeons in Assiut Governorate, 300 blood and faecal samples were examined. The incidence of infection with *Atoxoplasma* spp. was 4.33 %. The incidence of infection was 11 % in squabs while in adults it was 1 %. In most infected pigeons the degree of parasitaemia was 1-2 parasite/ 100 WBCs while in 3 squabs it was 9-13 parasite/ 100 WBCs.

Examination of impression smears of liver and spleen revealed presence of high incidence of parasite in the lymphocytes. The morphological characters of different forms of *Atoxoplasma* were described. Necropsy of highly infected squabs revealed that intestine was distended and contained creamy white content. Microscopically, it revealed necrosis and desquamation of intestinal epithelium. Many epithelial cells contained oocysts. The lamina propria of intestinal mucosa and also submucosa contained moderate to marked mononuclear cell infiltration and the lamina propria contained schizonts. The liver, spleen and kidneys were enlarged and microscopically they revealed marked inflammatory reactions represented by granulomas composed of mononuclear cells and heterophils caused by presence of *Atoxoplasma* parasite in cytoplasm of mononuclear cells. It is considered the first report of the pathological effect of atoxoplasmosis in pigeons.

Key words: Pigeons, *Atoxoplasma*, granuloma

INTRODUCTION

Atoxoplasma spp. is a coccidian parasite primarily of passerine birds, specially canaries and sparrows. It has a prolonged life cycle that involves the reticuloendothelial system and intestinal epithelium (Cooper *et al.*, 1989). About 19 species of *Atoxoplasma* had been described in different birds, they are mostly host-specific (Box, 1970).

The taxonomic position of *Atoxoplasma spp.* have been the subject of much discussions, where it was formerly known as *Lankesterella* or *Isospora spp.* Now it was currently differentiated from *Lankesterella* according to the route of transmission, where *Atoxoplasma spp.* is transmitted via fecal-oral route and not by mites as *Lankesterella* (Box, 1981). Also it differentiated from *Isospora spp.* according to the site of asexual amplification which takes place in reticuloendothelial system in addition to intestinal epithelium (McName *et al.*, 1995; Little *et al.*, 2001).

The clinical signs of atoxoplasmosis are non specific and include diarrhea, anorexia, ataxia, very bad reproduction, respiratory and nervous symptoms. It may cause mortalities which may approach 80% in young birds while adults were generally a asymptomatic carriers (Ritchie *et al.*, 1994). *Atoxoplasma* has been implicated as a cause of "going light" syndrome in young greenfinches (Cooper *et al.*, 1989)

The present work was conducted for studying the prevalence and morphological characters of *Atoxoplasma spp.* in pigeons in addition to studying their pathological effect in different organs.

MATERIALS and METHODS

Blood and faecal samples were collected from 300 pigeons out of which 200 adults and 100 squabs. Most of these pigeons were apparently healthy and some of them (13 cases) were suffered from weakness, anemia, 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.

The number of parasitized leucocytes (WBCs) were counted to estimate the parasitaemia of *Atoxoplasma* (Earle *et al.*, 1991). Faecal examination was done with flotation technique (Levine, 1985). Oocysts were collected and sporulated in 2.5 % potassium dichromate solution. Impression smear was done from internal organs of sacrificed pigeons, dried, fixed, stained with Geimsa and examined microscopically.

Different forms of *Atoxoplasma* detected by microscopic examination were measured with eye piece micrometer and microphotographed.

Gross pathology:

Squabs infected with *Atoxoplasma* were sacrificed and examined for the existence of gross pathological changes in intestine, liver, spleen, kidneys, lungs, brain and skeletal muscles.

Histopathology:

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

RESULTS

Parasitological studies:

Incidence:

Out of three hundred pigeons examined, 13 (4.33 %) were harbored the infection with *Atoxoplasma Spp.* The incidence of infection was 11 % in squabs while in adults it was 1 % (table 1). The infected adult pigeons were infected also with *Haemoproteus columbae* (fig.4).

parasitaemia:

In most infected pigeons the degree of parasitaemia was low (1-2 parasite/ 100 WBCs) while in 3 squabs it was high (9-13 parasite/ 100 WBCs) which was characterized by multiple invasion of the host cell with merozoites.

Morphological characters:

Merozoites were detected with high incidence in cytoplasm of monocytes and lymphocytes of heart blood than peripheral blood. It appeared as a spherical or ovoid bodies red in color and they may be vacuolated. These bodies were surrounded with very thin faintly staining boundaries. It was mostly lied in an indented notch in the host cell nucleus. One or more merozoites of variable size could be detected in the cytoplasm of infected host cell (fig. 1, 2, 3). The spherical merozoites measured 1-2.5 μm in diameter (1.2 μm), while ovoid merozoites measured 1.6 X 0.8-3.4X 2.5 μm (2.2 X1.4 μm).

Examination of impression smears revealed intensive tissue infection (liver and spleen) of three squabs with the parasite. Different forms of merozoites were detected specially in lymphocytes of these organs but they were slightly smaller in size (fig. 5, 6, 7). Free circulating sporozoites were detected in-between cells of liver and spleen. They were spindle-shaped had one sharp end and another blunt one, in addition to prominent centric or eccentric nuclei. It measured 8.2 X2.5-12.5 X2.0 μm (9.5 X2.2 μm) (fig. 7).

Oocyst:

Isospora-like oocysts were detected in faecal samples of some positive cases in squabs but not detected in adult pigeons. It appeared as transparent elliptical oocyst with distinct thick wall but it had no micropolar cab but had small rounded steady body and measured 19 X15.5-22 X16.8 μm (20 X16.0 μm). It contained two ovoid sporocysts each one measured 10 X6-12 X7.5 μm (10.8 X 7.0m). It sporulated after 3- 4 days (fig. 8).

Table 1: Incidence of *Atoxoplasma spp.* in pigeons in Assiut Governorate.

Squabs			Adults			Total		
Examin. birds	Infected birds	%	Examin. Birds	Infected birds	%	Examin. birds	Infected birds	%
100	11	11%	200	2	1%	300	13	4.33%

Pathological examination:

The intestine was distended and contained creamy white content. Microscopically, it revealed necrosis and desquamation of intestinal epithelium. Many epithelial cells contained oval-shaped dense eosinophilic bodies identified as oocysts. Some of oocyst-bearing epithelial cells were ruptured and thus oocysts could be observed on the epithelial surface or in the lumen (fig.9). The lamina propria of intestinal

mucosa and also submucosa contained moderate to marked mononuclear cell infiltration. The lamina propria occasionally contained schizont appeared as ovoid or spherical in shape. It mostly surrounded with thick wall that composed of two layers: outer thin deeply eosinophilic layer and inner thick homogenous lightly-stained layer. It mostly appeared overcrowded with multinucleated bodies varied in size and shape without definite arrangement (fig.10).

The liver was pale in color with small multiple white foci. Microscopically, it revealed presence of multiple granulomas composed of macrophages, lymphoid cells and heterophils (fig.11). Mononuclear cell infiltration was also observed inbetween hepatic cords. Some mononuclear cells mainly macrophages or lymphocytes in hepatic sinusoids, granulomas or nearby granulomas had peripherally-located nuclei or nuclear indentation in which one or more eosinophilic protozoal-like bodies were observed in nuclear notch. These bodies might be identified as merozoites of *Atoxoplasma spp.* (fig.12)

The spleen was congested and enlarged. Microscopically, it revealed presence of small granulomas composed mainly of macrophages and lymphocytes. Some of mononuclear cells in the granulomas or those presented in sinus areas revealed presence of intracytoplasmic eosinophilic bodies might be merozoites similar to that observed in the liver (fig.13, 14).

The kidney was enlarged. Microscopically, it revealed presence of multiple focal areas of inflammatory cell infiltration composed mainly of macrophages and lymphocytes. The renal tubules were obscured by reacting cells. Many of these cells specially macrophages and lymphocytes contained merozoites (fig.15).

Other organs revealed no evidence of remarkable lesions.

DISCUSSION

Atoxoplasma has been recorded as a subclinical infection in some species of birds in Western Europe (Poelma *et al.*, 1971). It has been also associated with clinical signs in greenfinches (Cooper *et al.*, 1989 and Ball *et al.*, 1998), canaries (Quiroga *et al.*, 2000) and in house sparrows (Lianson, 1959 and Box, 1967). The present study described the incidence, morphological and pathological characters of *Atoxoplasma* infection in pigeons. Examined blood smears of pigeons revealed that 13 (4.33 %) were infected with *Atoxoplasma spp.* This result is apparently similar to that obtained by Mandour *et al.* (1986) who detected *Atoxoplasma* infection in 5.7 % of examined pigeons.

Concerning to the age susceptibility and shedding of the oocysts, the present work cleared that squabs were more susceptible (11.0 %) to *Atoxoplasma* infection than adults (1.0 %) while the oocysts detected in the faecal sample of infected squabs only. Rosskopf and Woerpel (1996) found that the clinical atoxoplasmosis was common in nestlings and young birds. Also they mentioned that most *Atoxoplasma* infection had a limited period of oocysts output. The characteristic site of the dense red colored merozoites within the indented notch of the host lymphocytes and phagocytic cells in both blood and tissue smears in addition to detection of isospora-like oocyst in faeces of infected birds confirmed the diagnosis of *Atoxoplasma* infection in pigeons. On the another hand, *Isospora* infection had not described previously in pigeons according to the available literatures, while the *Lankesterella* infection appeared as non pigmented parasite in infected leucocytes. The same morphological characteristic forms of *Atoxoplasma* (merozoites, sporozoites and oocysts) had been described by several authors in sparrows and canary (Box, 1967; McNamee *et al.*, 1995; Martinez and Munoz, 1998 and Quiroga *et al.*, 2000). Non specific name was given to the detected parasite in the present work. The identification of complete life cycle and cross transmission was needed for accurate determination of new species.

In the present study, hepatosplenomegaly and small white foci of the liver were the most pronounced changes detected in infected pigeons specially in squabs which had high parasitaemia. These features were coincided with those described in different birds by Partington (1989) and McNamee (1995). The histopathological changes observed in intestine, liver and spleen were more or less similar to those reported in canary by Quiroga *et al.* (2000). However, kidney lesions observed in pigeons in the present study had not been reported in canary by Quiroga *et al.* (2000) or in captive bullfinch by McNamee *et al.* (1995). The histopathological changes in internal organs pointed to the serious effect of *Atoxoplasma spp.* in pigeons. This observation has an important value since the pathogenicity of *Atoxoplasma spp.* in pigeons had not been discussed in the available literatures.

Generally, the occurrence of atoxoplasmosis has been associated with poor hygienic measures specially contamination of drinking food and water with sporulated oocyst (Samour, 2000). Much more researches are needed to determine *Atoxoplasma* infection in other wild and domestic birds.

REFERENCES

- Ball, S.J.; Brown, M.A.; Daszak, P. and Pittilo, R.M. (1998): *Atoxoplasma* (Apicomplexa: Eimeriorina: Atoxoplasmatidae) in the greenfinch (*Carduelis chloris*). *J. Parasitol.*, 84: 813-817
- Bancroft, D. and Stevens, A. (1982): Theory and practice of histological techniques, 2nd Edn. Churchill Livingstone (Edinburgh, London, Melbourne).
- Box, E. (1967): Influence of *Isospora* infections on palency of avian *Lankestrella* (*Atoxoplasma*, Garnham, 1950) *J. Parasitol.*, 53: 1140-1147.
- Box, E. (1970): *Atoxoplasma* associated with an isosporan oocyst in canaries. *J. Protozool.*, 17: 391-396.
- Box, E. (1981): *Isospora* as an exraintestinal parasite of passerine birds. *J. Protozool.*, 22: 165-169
- Cooper, J.E.; Gschmeissner, S. and Greenwood, A.G. (1989): *Atoxoplasma* in greenfinches (*Carduelis chloris*) as a possible cause of "going light". *Vet. Rec.*, 124: 334-344.
- Earle, R.A.; Horak, I.G.; Huchzermeyer, F.W.; Bennet, G.F.; Braack, L.E. and penzhorn, B.L. (1991): The prevalence of blood parasite in helmeted guinea fowl, *numida meleagris*, in the kruger national park. *Onder. J. Vet. Res.* 56: 145-147
- Levine, N.D. (1985): Veterinary Protozoology. 1st ed. Iowa State University, Press. Ames.
- Lianson, R. (1959): *Atoxoplasma* Garnham, 1950, as a synonym for *Lankesterella* Labbe, 1899. Its life cycle in the English sparrow (*passer domesticus domesticus*, Linn.) *J. protozool.* 6: 360-371.
- Little, S.E.; Kelly, L.S.; Norton, T.M. and Terell, S.P. (2001): Developing diagnostic tools to further our understanding of *Atoxoplasma* species. *Proc. Assoc. Avian Vet.*, 157-159.
- Mandour, A.M.; Abdel-Rahman, A.M. and Abdel-Salam, F.A. (1986): *Atoxoplasma* (*Lankestrella*) *columbae* sp. Nov. in the domestic pigeon *Clumbae livia* in Assiut province. *Assiut Vet. Med. J.*, 34: 95-98.
- Martinez, F. and Munoz, E. (1998): *Atoxoplasma* spp. In a hybrid passerine (*Serinus Canarius* X *Carduelis cannabina*) *Avian pathol.* 27, 420-422.
- McName, P.; Pennyoott, T. and McConnell, S. (1995): Clinical and pathological changes associated in a captive buffinch (*Pyrrhyula pyrrhula*). *Vet. Rec.*, 136, 221-222.

- Partington, C.J. (1989): Atoxoplasmosis in Balimynahs. *J. Zoo Wildl. Med.* 20: 328-335.
- Poelma, F.G.; Zewart, P. and Strik, W.J. (1971): *Lankesterlla* (*Atoxoplasma*, Garnham, 1950) infection in birds in the Netherlands. *Netherland J. Vet. Sci.*, 4: 43-50.
- Quiroga, M.I.; Aleman, N.; Vazquez, S. and Nieto, J.M. (2000): Dignosis of atoxoplasmosis in canary (*Serinus canaries*) by histopathologic and ultrastructural examinations. *Avian Dis.*, 44: 465-469.
- Ritchie, B.W.; Harrison, G.J. and Harrison, L.R. (1994): *Avian Medicine Principles and Applications*. 1st ed., Wingers Publishing, Inc.
- Rosskopf, W.J. and Woerpel, R.W. (1996): *Diseases of cage and avian birds*. 3rd ed., Williams & Wilkins A Waverly company, USA.
- Samour, J. (2000): *Avian Medicine*. 1sted., Harcourt Publishers Limited-London.
- Soulsby, E.J.L. (1982): *Helminth, Arthropods and protozoa of Domesticated animals* 7th ed., Bailliers Tindall.

LEGENDS OF FIGURES

- Fig. 1, 2, 3:** Single and multiple infection of *Atoxoplasma* merozoites of variable sizes in monocyte of blood film of pigeons. Note marked indentation of host cell nuclei and faintly stained peripheries of the parasite. Geimsa X1500.
- Fig. 4:** Mixed infection of *Atoxoplasma* and *Haemoprotus* (exoflagellated macrogametocytes). Geimsa X1500.
- Fig. 5:** Impression smear of pigeons liver showing multiple infection of *Atoxoplasma* merozoites in monocyte Geimsa X1500.
- Fig. 6, 7:** Impression smear of pigeons spleen showing multiple infection of *Atoxoplasma* merozoites in lymphocytes (arrow head) and free sporozoites (arrows). Geimsa X1000.
- Fig. 8:** Sporulated oocysts of *Atoxoplasma spp.* In fecal sample of a pigeon. X100.
- Fig. 9:** Micrograph showing necrosis and desquamation of intestinal epithelium, presence of oocysts in epithelial cells and epithelial surface (arrowes). Note the mononuclear cell infiltration in lamina propria of intestinal mucosa and in submucosa (arrows). HE. X250.

- Fig. 10:** Micrograph showing presence of a schizont in the lamina propria of intestinal mucosa surrounded by connective tissue capsule and mononuclear cells. HE. X400.
- Fig. 11:** Liver micrograph of a pigeon showing presence of a granuloma composed of macrophages, lymphoid cells and heterophils. HE. X400.
- Fig. 12:** Liver micrograph of a pigeon showing presence of intracytoplasmic eosinophilic bodies causing nuclear indentation (arrows) in mononuclear cells in hepatic sinusoid and inbetween hepatic cords. HE. X1000.
- Fig. 13:** Spleen of a pigeon showing presence of a granuloma composed of macrophages and lymphocytes. Some of these cells containing one or more intracytoplasmic eosinophilic bodies (arrows). HE. X1000.
- Fig. 14:** A sinus area of the spleen of a pigeon showing presence of merozoites in cytoplasm of macrophages (arrows). HE. X1000.
- Fig. 15:** Kidney of a pigeon showing presence of focal mononuclear cell infiltration. Some of these cells containing one or more merozoites (arrows). The renal tubules are obscured by the inflammatory cells. HE. X1000.

Fig. 10: Micrograph showing presence of a schizont in the lamina propria of intestinal tissue surrounded by connective tissue (100X).





