

Light microscopic description and histopathological effects of *Eimeria* sp. (Protozoa: Apicomplexa) from the freshwater fish *Chrysichthys auratus*.

Aziza Marwan* and Bothaina Khidr

Zoology Department, Faculty of Science, Assiut University, Assiut, Egypt

ABSTRACT

A species of Coccidia, *Eimeria* was found infecting the Nile catfish *Chrysichthys auratus*. The parasite was found aggregated in mucosa, submucosa and connective tissue between muscle bundles of the intestine, and also in the parenchymal tissue of the liver and the pancreas. The recorded stages were the maturing oocysts, the sporogonial and gamogonial stages; no signs of merogony were recorded. This is the first Coccidian species described from *Chrysichthys auratus* in Egypt. Oocysts of *E. chrysichthii* are distinguished from other *Eimeria* spp. in size and shape of sporocysts, host and site of infection. Mature oocysts were spherical or subspherical, measuring 21.6-24 (22.65) μm . Sporocysts measured 6.5-8.4x 6-7.2 (7.5x6.75) μm . A stieda-like body was seen in some individuals. Several histopathological changes were observed in the intestine, liver and pancreas of the host as a result of eimerian infection. In many mucosal cells of the intestine, cytoplasmic vacuolations were detected, while in submucosa, there was a collection of mononuclear macrophages with very abundant brown cytoplasm and congested blood vessels. The muscle layer also was affected. The liver of the infected fish revealed cytoplasmic vacuoles and a great depletion of glycogen granules in its cells.

KEYWORDS: coccidia- *Eimeria*- sporogony- histopathology- catfish

INTRODUCTION

Several coccidian parasites of the genus *Eimeria* have been described from fish of different geographical regions of the world (Molnar & Pellerdy 1970; Molnar & Fernando 1974; Molnar & Hank 1974; Lom & Dykova 1981; Lukes 1993 and Sitja-Bobadilla *et. al* 1996). In Egypt, very little is known about coccidian parasites infecting freshwater fishes (El-Toukhy *et al.* 1994). El-Deep (1995) was the first to describe three isosporian and two Eimerian parasites from the faeces and intestine of *Oreochromis niloticus*, in Egypt and she also described two unidentified coccidian oocysts from the same fish. The present study is the first investigation of *Eimeria* in *Chrysichthys auratus*. *Eimeria* spp. are quite common in the intestine of marine and freshwater fish. In freshwater species the parasites have been detected either as intestinal or extra intestinal. *E. branchiphila* is an example of an extra-intestinal *Eimeria* from the kidney, gills, spleen, muscles and brain of *Rutilus rutilus* (Dykova & Lom 1983). The impact of the Eimerian parasites on host tissues is little un-resolved. Detailed data on histopathological changes are available only in carp-infecting species and one species infecting killifishes (Lom & Dykova 1981). In the present work the light microscopic description of the species and its histopathological effects are presented. Ultrastructure and cytochemistry data are presented in a companion paper.

MATERIALS AND METHODS

During a previous study (Khidr 1981) dealing with the digestive system of the catfish *Chrysichthys auratus*, the present parasite was detected as refractile aggregations in submucosa, serosa and connective tissue between muscle bundles of the intestine, and in the tissues of liver and hepatopancreas. The present study was carried out to describe the parasite and its histopathological effects. In the year 2000 twenty-five new specimens were added to the old samples, all specimens were from the River Nile at Assiut. The samples were

* Address for Correspondence

transported alive to the laboratory. Fresh faeces and scrapings of the intestinal wall were examined; 2.5 % potassium dichromate was added to all samples for at least 15 days. For histological studies, portions of intestine and liver were fixed in 10% formalin, paraffin sections of 4-6 microns thick were made and hematoxylin-eosin, Mallory triple stain, Perl's technique and PAS stains were used. Semi-thin sections were obtained from tissues fixed with 2.5% glutaraldehyde, embedded in Epon and stained with Toluidine blue for the fine investigation of the parasite and its stages. A camera lucida was used for the drawing of oocysts.

RESULTS

Description of the parasite: In spite of the very high intensity of infection in the histological sections, and very high prevalence (about 98%) of the parasite in tissues of the intestine, there were no oocysts detected in faecal samples even after 15 days post-treatment with potassium dichromate. The oocysts and some other developing stages were observed in intestinal scrapings 10-15 days after treatment.

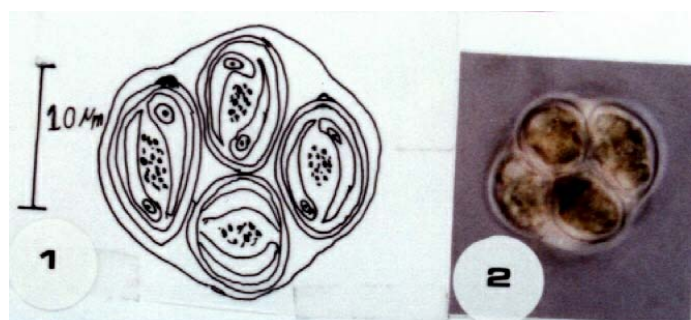
Mature oocysts: Mature oocysts (Fig. 1&2) were spherical or subspherical in shape. The oocyst wall is a thin transparent membrane in contact with the sporocysts. It maintains its spherical shape when deposited in the submucosa (Figs. 3a &3b). There is no oocyst residual body. Its dimensions are 21.6-24 μm (22.6) by 19-21.4 μm (20.8). From a lateral view, sporocysts are elliptical in shape and rounded from an apical view, 6.5-8.4 by 6-7.2 μm (7.5 x 6.75) in size. When visible, the Stieda body is knob like. The sporocyst residual body is oval in shape, measures about 2 x 3 μm and consists of moderate to large granules. The sporocyst has a double-walled envelope. The vermiform sporozoites have one pointed and one rounded end, measure about 10 x 2.5 – 11.5 x3 μm (11 x 2.7 μm). They lie lengthwise head-to-tail, or they overlap partially curled around each other in the sporocyst. Sections stained with hematoxylin-eosin revealed that the nucleus is located at the rounded end. Sporulating oocysts were encountered mostly in the submucosa, the connective tissue between muscle bundles and in serosa all over the intestine. They were less frequently in the parynchimal tissue of the liver and hepatopancreas (Figs 4, 19 & 20).

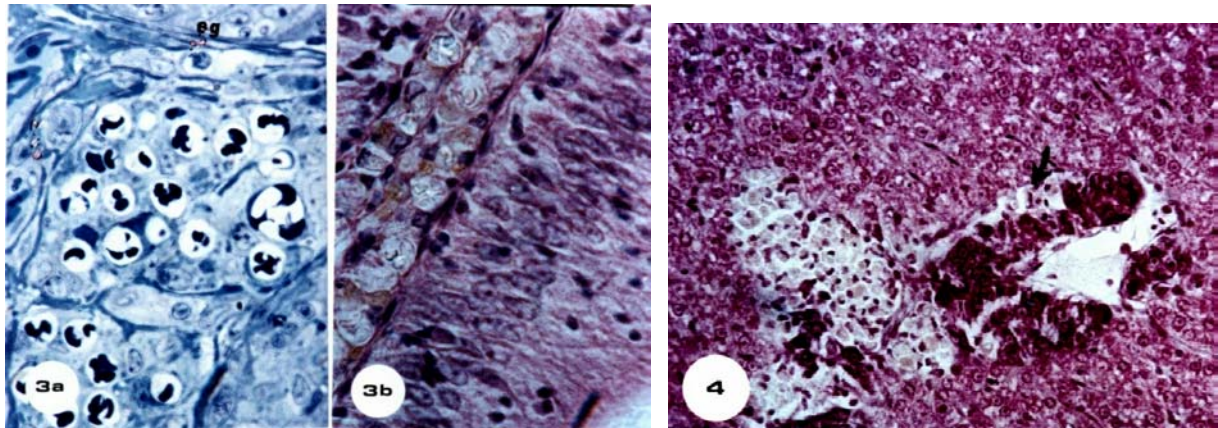
Developmental stages: Stages of gamogony were seen in the lamina propria and in the epithelial layer of the intestine, they were situated mostly below the level of the epithelial cell nucleus. The macrogametocytes measure about 9-10 μm in diameter and each has an eccentrically located nucleus and large spherical inclusions (Fig. 5). Microgametocytes with numerous nuclei are located at the periphery and measure about 7.5 μm in diameter, located in the lamina propria and in the bases of the epithelial layer (Fig. 6). The zygote stage was presented intracellular at the basal part of the epithelial tissue and in the submucosa (Fig. 7). It is spherical to sub-spherical measuring about 12 μm in diameter. No merogonial stages were detected in any of the examined tissue sections.

In scrapings of the intestinal tissue treated with potassium dichromate there were scarce structures that could be a meronts. Unsporulated oocysts (12 x15 μm) and oocysts with cleaving sporont (17-19 x 15-19.5 μm) were also seen (Fig. 8,9,10 and 11).

Fig. 1- Diagrammatic drawing of an oocyst of *Eimeria* sp.

Figs. 2- A photomicrograph of Mature oocyst showing four sporocysts. X1000.





Figs 3a & 3b: T. S. of intestine, showing maturing oocysts deposited in the submucosa and an early gamont 3a (eg) within a parasitophorous vacuole. TB stain. X 1000. in 3b refractile oocysts and mononuclear cells with brown cytoplasm. H. E. 1000.

Fig. 4: A photomicrograph of section of the liver, showing developing stages in the parenchymal tissue and the pancreas (arrow). Note the vacuolar degeneration in hepatocytes. H.& E. X 400.

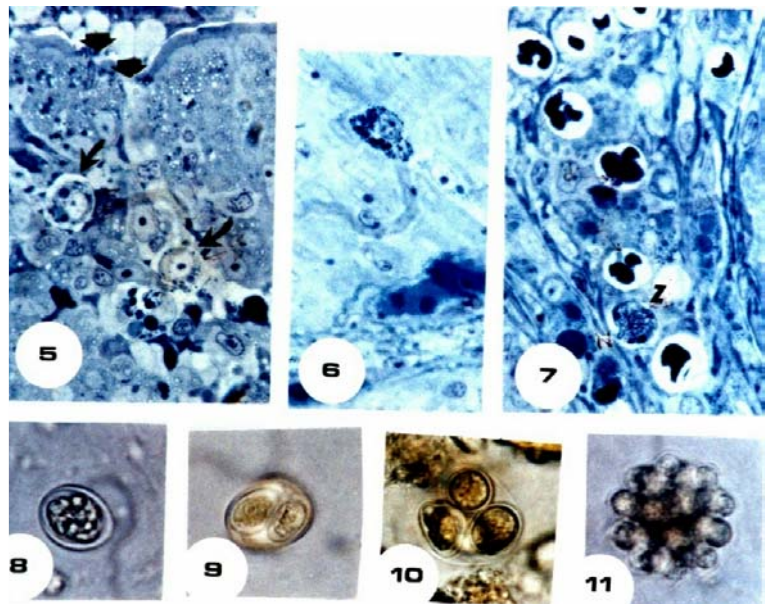
Fig. 5: T. S. of the intestine showing, macrogamonts present in the epithelial layer (arrows), note: cytoplasmic vacuolization and disintegration of brush border (head arrow). TB Stain. X 1000

Fig. 6: T. S. of the intestine showing, microgametocyte. X 1000.

Fig. 7: T. S. of the intestine showing, the zygote (z) and some maturing oocysts. TB X 1000.

Figs 8-11: A photomicrographs, showing some stages detected in tissue scrapings of the intestinal wall 15 days post treated with 2.5% potassium dicromate. Unstained X1000.

Fig. 8: Unsporulated oocyst. Fig. 9: Maturing oocyst showing two sporocysts. Fig. 10: Maturing oocyst showing three sporocysts. Fig. 11: Merogonial stage.



Histopathological effects:

Normal intestine: The intestine of *Chrysichthys auratus* consists of four main layers characterizing the vertebrate alimentary canal and arranged (from the outer layer) into the serosa, muscularis, submucosa and mucosa (Fig. 12). The serosa consists of simple squamous epithelium. The muscularis consists of an inner circular and outer longitudinal smooth muscle layers. The submucosa is formed of loose connective tissue richly supplied with blood vessels. The tunica propria is indistinguishable from the submucosa. The mucosa contains numerous folds formed of simple columnar epithelium comprised of goblet cells. The columnar cells are tall and their nuclei are basal in position. A striated border (brush border) covers their free surface. Numerous lymphocytes are encountered in the mucosa (Fig. 12).

Infected intestine: The presence of gamogony stages within the simple columnar cells caused vacuolar degeneration of the cytoplasm and disintegration of the brush border (Fig. 5). When the sporulating stages were found in the submucosa, histopathological lesions in the form of necrotic disintegrations with lymphatic infiltration were detected (Fig. 13). Another pathological change in the submucosa was manifested in the form of remarkable congestion of the blood vessels. In heavily infected specimens, there was severe haemorrhage in the

whole intestinal submucosa, extravasated red blood corpuscles and abundant mononuclear cells with brown cytoplasm (Figs. 3b & 14). The cells giving a positive reaction with Perl's technique indicate the presence of both intracellular and extracellular haemosiderin (Fig. 15). The degenerated submucosa was separated from the above mucosal epithelium, the epithelial lining at the top of the folds was disintegrated (Fig. 16) and shed epithelium could be observed in the lumen (Fig. 17).

Histological lesions, in the form of distortion of the fibre of the muscularis and collapse of muscle bundles, were due to the presence of sporulating stages (Fig. 18).

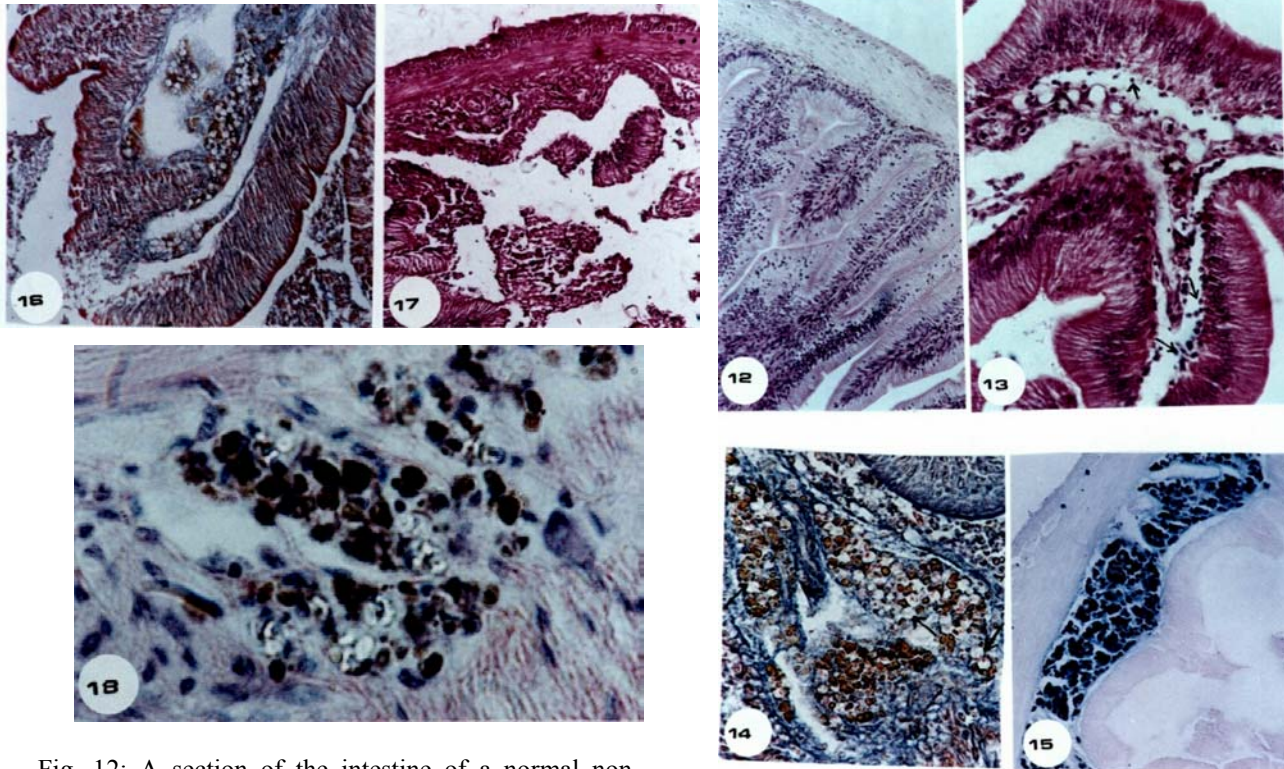


Fig. 12- A section of the intestine of a normal non-infected fish, showing the general histological structure of intestine. H. E. X 200.

Fig. 13- A magnified part of the infected intestine, showing disintegration of submucosa and lymphatic infiltration (arrows). H. E. X 400.

Fig.14: A magnified part of the submucosa in the heavily infected fish, showing the abundant mononuclear cells with brown cytoplasm & numerous parasites (arrows). Mallory's Triple Stain X 400.

Fig. 15: A section of the infected intestine, showing haemosidrin granules. Perl's technique X 200.

Fig. 16- A magnified part of infected intestinal fold, showing disintegration of the epithelium lining at the top of the fold. Mallory's Triple Stain X 200.

Fig. 17- A photomicrograph of a section of the infected intestine, showing the presence of mucosa sloughs in the lumen. H. & E. X 200.

Fig. 18- A magnified part of muscle layers of infected fish, showing distortion and collapse of muscle fibers. H. & E. X 1000.

Normal liver: The arrangement of the hepatic parenchymal cells in *Chrysichthys auratus* differs from that of mammalian lobules in the absence of the connective tissue that delineate the hepatic cell mass (Fig. 19). The hepatic cell mass is interrupted by blood vessels and sinusoids. Those cells are mostly polygonal with central nuclei. Dispersed in the hepatic tissue are bile ducts and ductules.

Infected liver: Examination of infected liver, showed cytoplasmic vacuolation of liver cells (Fig. 4). Sometimes, irregularity shaped necrotic areas were shown infiltrated by leucocytes (Fig. 20). In the liver cells of normal fish a considerable amount of polysaccharide material was observed in the cytoplasm as shown by the PAS technique (Fig. 21).

In the infected fish, a considerable depletion of polysaccharides was observed in hepatocyte cytoplasm but the developing stages revealed a strong reaction (Fig. 22).

Normal pancreas: In normal pancreas, the pancreatic tissue surrounds branches of the hepatic portal vein within the liver. The exocrine portion consists of a large number of acini. Each acinus is made up of glandular cells, which may be polygonal or conical in shape. Each glandular cell has an eccentric, deeply staining nucleus (Fig. 19).

Infected pancreas: Examination of infected pancreas revealed degeneration of some glandular cells and presence of few lymphocytes around the acini (Fig. 20).

With the PAS technique, while the carbohydrate contents of normal exocrine pancreas were in the form of few stained fine granules (Fig. 21), no granules were observed in the infected fish. However, the developing stages had a high degree of carbohydrate (Fig. 23).

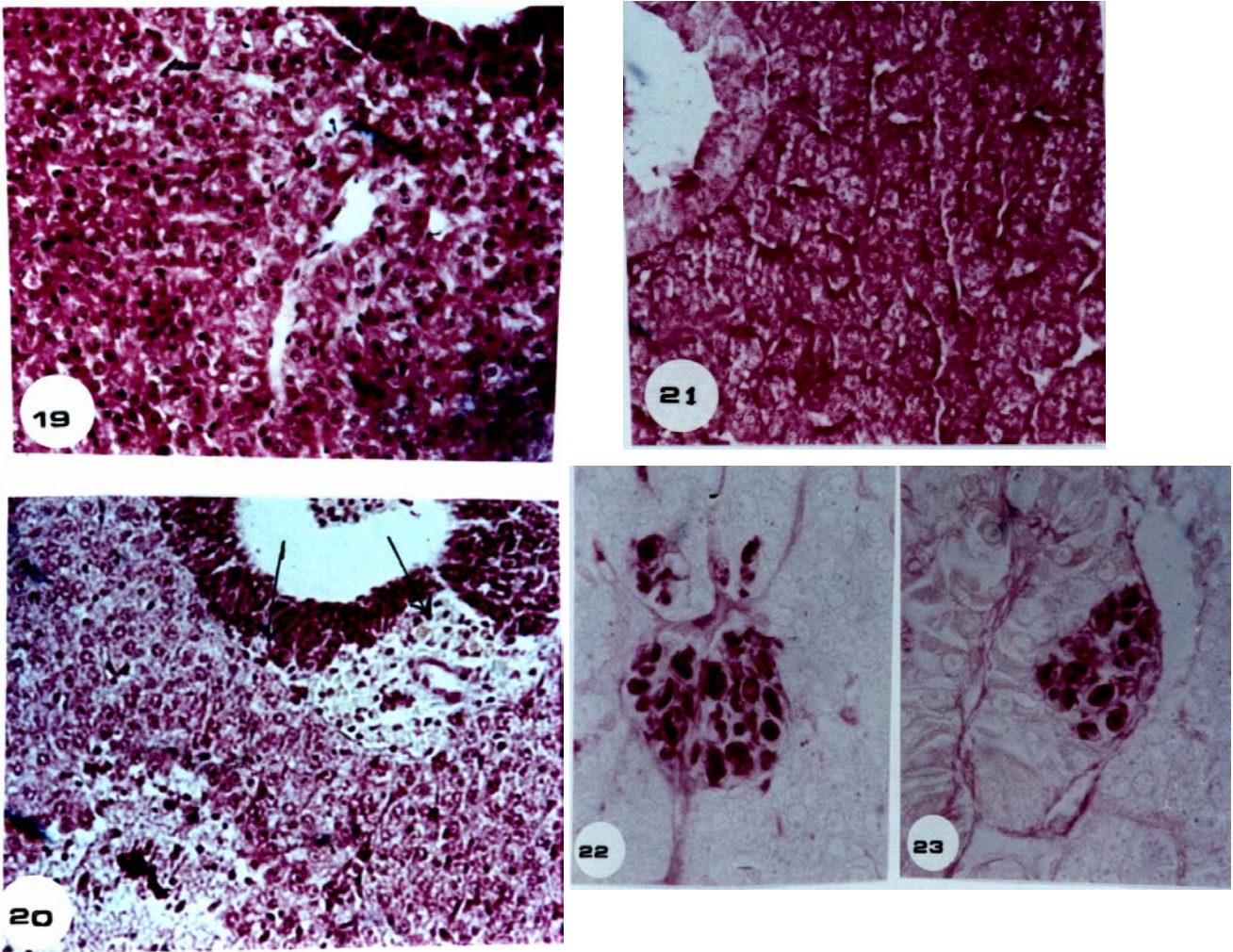


Fig.19: A section of the liver of non-infected fish, showing the polygonal hepatic cells and exocrine pancreas. H. & E. X 400.

Fig. 20: A section of infected liver, showing necrotic area and degeneration of some glandular cells in infected pancreas (arrows). H. & E. X 400.

Fig. 21: A section of the liver of non-infected fish, showing the hepatic cells and the exocrine pancreatic cells reaction towards PAS test. X 400.

Fig. 22: A section of the infected liver, showing a considerable decrease in polysaccharides in the hepatocytes and positively stained parasites. PAS reaction X 1000.

Fig. 23: A section of the infected pancreas, showing depletion of polysaccharides in pancreatic cells and positively stained parasites. PAS reaction X 1000.

DISCUSSION

The present parasite is a member of the genus *Eimeria* because of the morphology of the oocyst, with four sporocysts bearing a Stieda-like body and lacking a suture which is characterizing the genus *Goussia*. In recent years in many geographical regions, great progress has been made in research into coccidian parasites, especially of those infecting economically important warm-blooded hosts. According to Dykova & Lom (1981), coccidian species infecting fish have not been paid much attention until recently, they also mentioned that most of the species of fish coccidia have been left within the genus *Eimeria* Schneider 1875 until new data make possible their correct classification. More than 130 species of *Eimeria* have been described from a wide range of fish hosts and geographic locations (Davies & Ball 1993).

In Egypt, very little is known about coccidian parasites infecting fishes. To our knowledge only two species related to *Eimeria* and three to *Isospora* have been detected in the faeces and intestine of *Oriochromis niloticus* as the first record of these parasites in Egypt (El-Deep 1995). El-Toukhy *et al.* (1994) were the first to describe *Goussia* sp. as record of this species from marine fish in Egypt.

The present parasite was detected mainly in sections of intestine and liver; while no oocysts were detected in fresh faeces or epithelial scrapings, while the oocysts and some developing stages were detected in both but only after 15 days post treatment with 2.5% potassium dicromate. Gamogonial and sporogonial stages were recorded, the former concentrated in the bases of the epithelial cells and the lamina propria, while sporogonial stages were restricted to the submucosa. These observations differ from the two species described by El-Deep (1995) who detected oocysts and other developing stages in both fresh faeces and tissue scrapings; and also found merogonial, gamogonial and sporogonial stages in intestinal sections in mucosa, lamina propria and submucosa. El-Deep (1995) has not indicated the site of infection inside the epithelial cells relative to the nucleus. But, in the parasite under discussion it was detected in subnuclear position. The present parasite differs from many other species (e.g. *Eimeria ivanae* Lom & Dykova 1981; *Eimeria sparis* Sitja-Bobadilla *et. al* 1996) in being present only in the basal parts of epithelial cells. The present parasite differs also in the absence of merogonial stages in examined tissues. It may be present in organs other than the intestine, liver and pancreas or it may be exogenous (the merogomeal stage was detected in tissues treated with potassium dichromate). Dykova & Lom (1981) mentioned that in most species of *Eimeria* infecting fish, little more than the oocysts and the site of infection in their hosts have been described. Merogony and gamogony stages are mostly unknown. A complete account of the life cycle exists for only a few species like *E. anguillae* (Hine 1975) and *E. carpelli* (Leger & Stankovich, 1921).

Most species of fish coccidia invade the digestive tract, but a great proportion also develops in other organs. The infected organs may be liver parenchyma, pancreas, kidney, spleen adipose tissue, air- and gall-bladder, serosa, ovary and testes. The parasite may develop in more than one organ. In the case of *E. brevoortiana*, merogony and gamogony take place in the epithelium of the pyloric caeca while sporogony is found only in the testes. In the development of *E. sardinae*, sporogony is known to occur in the testes, while the site of merogony and gamogony has not been recorded (Dykova & Lom 1981). The same authors summarized six patterns of life cycles of fish coccidia, the patterns are: 1- intracytoplasmic merogony, gamogony and sporogony stages. 2- Intranuclear merogony, gamogony and sporogony. 3- Endogenous merogony and gamogony with sporogony partly endogenous, partly exogenous. 4- Intracytoplasmic merogony and gamogony with exogenous sporogony. 5- Epicellular merogony and gamogony and an intracytoplasmic sporogony. 6- Epicellular merogony and gamogony and an exogenous sporogony.

The oocysts walls of most fish coccidia are extremely thin. This agree with the wall of the present oocysts. The thin oocyst wall may not be very persistent so that mostly free sporocysts are found in the faeces of the infected fish. Concerning the size of the oocysts, the present oocysts are smaller than the two species described by El-Deep (1995) and also the sporocysts and sporozoites are smaller, but they are similar to them in having a thin single oocyst wall and a two-layered sporocyst wall. The data on pathogenicity of fish coccidia are very few. Fish coccidia have received special attention in the last decade, mainly due to the pathogenic possibilities of some species (Dykova & Lom 1981; Mackenzie 1981; Kent & Hedrick 1985; Costa & Mackenzie 1994; Jendrysek *et al.* 1994).

The results of the present investigation revealed vacuolization in the intestinal mucosa. Similar results were reported by Jendrysek *et al.* (1994) on carp, El- Shershaby *et al.* (1998) on *Columba livia domestica* and by Al-Hoot (2000) on *Falco tinnunculus tinnunculus* infected by *Goussia carpelli*, *Eimeria labbeana* and *Isospora mohammedi* respectively. Such vacuolation was also observed by El-Banhawy *et al.* (1993), who related the presence of these vacuoles under pathologic conditions to the collection of injurious substances. In the present investigation, the presence of extravasated red blood corpuscles were due to lesions in the blood vessels. Additionally, accumulation of haemosiderin in the submucosa of infected fish could be attributed to the destruction of the erythrocytes. According to Tizard *et al.* (1978) toxic factors produced by trypanosomes or released during trypanolysis involve in the destruction of the red cells.

A shed epithelium could be observed in the lumen of the infected intestine. Fernando (1982) stated that villous atrophy was a feature of intestinal coccidiosis in lambs, chickens and man. However, Hemmer *et al.* (1998) observed an increased number of mitotic enterocytes at the base of the mucosal folds infected by *Goussia carpelli* in *Cyprinus carpio*. This finding suggests a high regeneration capacity of the carp intestine, which could explain the mild clinical symptoms in fish affected by *Goussia carpelli* coccidiosis. The cellular degeneration observed in the hepatocytes was probably due to toxic factors produced by coccidia. Awadalla *et al.* (1975) stated that the decreased level of enzyme activity in liver tissue of mice infected with *Schistosoma mansoni* might either be related to the release of enzymes from the necrotic tissue or attributed to increased cell membrane permeability as a result of relative anoxia, and irritation due to toxic or metabolic products of the parasites.

The present study showed a high polysaccharide content of normal fish hepatocytes, while examination of sections from infected fish indicated depletion of polysaccharides in the cytoplasm of the hepatocytes. Similar results were reported by Farmer (1980) and by Peeters *et al.* (1984) in rabbits infected by *Eimeria stiedae* and by *Eimeria intestinalis* respectively. Farmer (1980) stated that the infection of rabbits with *Eimeria stiedae* caused dysfunctional in the liver. Thus, reduction in liver glycogen may be attributed to liver dysfunction. According to Peeters *et al.* (1984), the eimerian parasite caused a marked decrease of food intake and consequently energy intake. Hoppe & Chapman (1947) suggested that pathogenic trypanosomes consume so much sugar in the blood stream that they cause exhaustion of the carbohydrate reserves of the host. However, no change was found in liver glycogen of chickens infected with *Eimeria tenella* (Freeman 1970) and *Eimeria bruntti* (Ruff *et al.* 1981). From these studies, we come to the following conclusion; taking into consideration the sites of infection, pattern of life cycle, size and host, we suggest that the present coccidian parasite is a new species, but this needs more detailed study. The extent of damage caused by the parasite depends not only on the intensity of the infection but also on how deep the developmental stages of coccidia reach within the intestinal wall.

REFERENCES

- Al-Hoot A A (2000) Light and electron microscopic studies of the endogenous stages of *Isospora mohammadii* Al-Hoot infecting the kestrel (*Falco tinnunculus tinnunculus*) with reference to pathogenic effects on the host. *Egypt. J. Zool.* 35: 111-133.
- Awadalla HN, Sherif AF, Shafei AZ, Khalh HA & Giurgis FK (1975) Enzyme level in homogenate of liver from mice infected with *Shistosoma mansoni* and from uninfected mice. *Int. J. Parasitol.* 5 : 27-31.
- Costa G & Mackenzie K (1994) Histopathology of *Goussia clupearum* (Protozoa: Apicomplexa: Coccidia) in marine fishes from Scotch water. *Dis. Aqua. Organ.* 18 (3): 195-202.
- Davies AJ & Ball SJ (1993) The biology of fish coccidia. *Adv Parasitol.* 32: 293- 366.
- Dykova I & Lom J (1981) Fish coccidia: Critical notes on life cycles, classification and pathogenicity. *J. Fish Dis.* 4: 487-505.
- Dykova I & Lom J (1983) Fish coccidia: An annotated list of described species. *Folia Parasito. (Praha)* 30: 193-208.
- El-Banhawy MA, Sanad SM, Sakr SA, El-Alaimy IA & Mahran HA (1993) Histopathological studies on the effect of the anticoagulant rodenticide “Brodifacoum” on the liver of rat. *J. Egypt. Ger. Soc. Zool.*, 12 (c): 185-227.
- El-Deep N (1995) Studies on Protozoa infecting some Nile fishes in Egypt. M. Sc. Thesis Girl’s Collage Ain Shams Univ.
- El-Shershaby AM, Elewa FE, Amer MA & Abdel-Aziz AM (1998) Histopathological and histochemical effects of eimerian infection on the duodenal mucosa of the pigeon. *Egypt J. Zool.* 31: 31-51.
- El-Toukhy A, Abdel Aziz A & Abdel Gawad M (1994) *Goussia* sp. from the liver of *Scomber japonicus* in Egypt. *J Egypt. Ger. Soc. Zool.* 14(D): 315-322.
- Farmer JN (1980) The Protozoa “ Introduction to Protozoology” The c. V. Mosby Company St. Lovis. Toronto, London.
- Fernando NA (1982) Pathology and pathogenicity. In the Biology of the Coccidia (ed.PL Long). Pp.187-327. London. Edword Arnold (Publishers) Ltd.
- Freeman BM (1970) Carbohydrate stores in chickens infected with *E. tenella*. *Parasitology* 61:245-251.
- Hemmer N, Steinhagen D, Drommer W & Korting W (1998) Changes of intestinal epithelial structure and cell turnover in carp *Cyprinus carpio* infected with *Goussia carpelli* (Protozoa: Apicomplexa). *Dis. Aquat. Organ.* 34: 39-44.
- Hine PM (1975) *Eimeria anguillae* Leger & Hollande, 1922 Parasitic in New Zealand eels. *New Zealand Journal of Marine and Freshwater Research* 9: 239-243.
- Hoppe JO & Chapman CW (1947) Role of glucose in acute parasitemic death of the rat infected with *Trypanosoma equiperdum*. *J. Parasit.* 33(6): 509-516.
- Jendrysek S; Steinhagen D; Drommer W & Korting W (1994) Carp coccidiosis: intestinal histo-cytopathology under *Goussia carpelli* infection. *Dis. Aquat. Org.* 20: 171-182.
- Kent ML & Hedrick RP (1985) The biology and associated pathology of *Goussia carpelli* in goldfish *Carassius auratus*. *Fish pathol.* 20: 485-494.
- Khidr BM (1981) Studies on the food, feeding habits and the morphology of the digestive system of the Nile catfish *Chrysichthys auratus*. M. Sc. Thesis. Assiut Univ.
- Leger L & Stankovich S (1921) Sur la coccidiose des alevins de la carpe. *Copte Rendu des Seances de la Societe de Biologie (Paris)* 173 : 742.
- Lom J; & Dykova I (1981) New species of the genus *Eimeria* (Apicomplexa: Coccidia) from marine fish. *Z.Parasitenkd* 66: 207-220
- Lukes J (1993) A coccidian (Apicomplexa: Eimeriidae) with extracytoplasmally located stages, in the kidney tubules of golden carp (*Carassius auratus gibelio* L.)(Cyprinidae). *Folia Parasitologica* 40: 1-7.
- Mackenzie K (1981) The effect of *Eimeria* sp. infection on the condition of blue whiting, *Micromesistius poutassou* (Risso). *J. Fish Dis.* 4: 473-486).
- Molnar K & Fernando CH (1974) Some new *Eimeria* (Protozoa, Coccidia) from fresh water fishes in Ontario, Canada. *Canad. J. Zool.* 52: 413- 419.
- Molnar K & Hanek G (1974) Seven new *Eimeria* spp. (Protozoa. Coccidia) from freshwater fishes of Canada. *J. Protozool.* 21 :489-493
- Molnar K & Pellerdy L (1970) Further studies on coccidia of freshwater fishes in Hungary. *Acta Veter Acad Sci Hung* 20:45-55.
- Peeters JE, Charlier G, Antoine O & Mammerickx M (1984) Clinical and pathologiccal changes after *Eimeria intestinalis* infection in rabbits. *Zent. Vet. Med.* 31(1): 9-24.
- Ruff MD, Allen PC & Chute MB (1981) Composition of heart, liver and skeletal muscle from broilers with coccidiosis. *Poult. Sci.* 60: 1807-1811.

Sitja-Bobadilla A, Palenzuela O & Alvarez-Pellitero P (1996) Light microscopic description of *Eimeria sparis* sp. nov. And *Goussia sparis* sp. nov (Protozoa: Apicomplexa) from *Sparis aurata* L. (Pisces: Teleostei). *Parasit. Res.* 82 (4): 323-332.

Tizard I, Nielsen KH, Seed JR & Hall JE (1978) Biologically active products from African trypanosomiasis. *Microbiological Review* 42:661- 681.

(:)

-

-

-

.() , ,

15

x 7.5) 7.2-6 x 8.4-6.5

.(22.65) 24-21.6

.(6.75

()