Pathological studies on some parasitic diseases of Eel
(Anguilla anguilla)
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SUMMARY

Sixty-five eels (Anguilla anguilla) collected in the period between January-May 2008, from Al Salam channel (15 cases), Zagazig markets (17 cases) and Al Manzala Lake (33 cases) were examined for pathological lesions induced by parasitic infestation with identification of the causative parasite.

The prevalence of pathogenic parasites (single or mixed infection) was 22 out of 65 eels (33.85%). The identified parasites were Anguillicola crassus (7 cases) (10.7 %), Dactylogyrus species (4 cases) (6.1 %), Pseudodactylogyrus species (5 cases) (7.7 %), Myxidium species (2 cases) (3.07 %), Trichodina species (3 cases) (4.6 %), and Proteocephalus species (one case) (1.5 %).

It could be concluded that the parasitic fauna markedly impairs the Egyptian eel’s production and produce mild to severe degenerative, necrotic and inflammatory changes in the affected organs.

INTRODUCTION

European eel (Anguilla anguilla) is one of the most important fish species cultured in southern Europe and the Mediterranean areas (Nielswen and Esteve-Gassent, 2006). Eel stocks underwent a significant reduction during the recent years which justified its classification in the red book of the threatened species (Muchiut et al., 2002). One of the principal factors responsible for this reduction is the presence of several pathogenic parasites that makes it the final or intermediate host (Elhilali 1998). Anguillicolosis is a disease caused by Anguillicola crassus which parasitizes the swim bladder of eels. Its prevalence depends mainly on the water salinity that limits the development
of the larval stages of the parasites (Pilcher and Moore, 1993). It was ranged from 11.7-66.5% among Anguilla anguilla in Egypt (Mohamed and Nouh, 2004 and Dosoky, 2007). The parasite sucks blood from the wall of the swim bladder, which gives them a dark brown to black coloration (Szekely, 2006). The wall of the swim bladder became markedly thickened, inflamed, hyperemic and showed epithelial hyperplasia and leukocytic infiltration together with focal hemorrhagic areas due to the presence of adult worms in the lumen and larvae in both lumen and wall (Hoglund et al., 1992). The most important monogenean gill worms affect eels are Dactylogyrus species and Pseudodactylogyrus species (Woo, 2006). The prevalence of Dactylogyrus species infection among Anguilla anguilla in Egypt was 7.5% (Dosoky, 2007). The parasite feed mainly on the superficial layers of the skin and gills. This feeding activity was irritating and thus often resulted in skin discoloration, erosions, ulcers, excess mucous production, epithelial hyperplasia and focal hemorrhages (Kennedy, 2001). In heavy infestations the parasite could kill eels specially small ones (Noga, 1996). Pseudodactylogyrus species was frequently found on European and Japanese eels and its prevalence among Anguilla anguilla in Egypt was 9.16% (Dosoky, 2007). The parasites attach themselves to the gills with a pair of hooks, causing severe irritation, excess mucous production, epithelial hyperplasia, erosions, ulcerations and hypere mia with leukocytic infiltrations (Craig et al., 2001). Myxidiosis is a one of the most important expanding protozoal diseases of cultured eels caused by Myxidium species (Le Breton and Marques, 1995). Its prevalence among Anguilla anguilla in Egypt was 2.9% (Dosoky, 2007). The parasite usually found in intestine but it can found in skin, gills, kidney and stomach where it forms white circular or oval spots with a size of 0.1-2.0 mm. (Pillay, 1990). These spots represent granulomatous reactions consisted of centrally located spore mass surrounded by mononuclear infiltrations and fibrous connective tissue proliferations (Copland, 1983). Trichodinosis is a disease caused by Trichodina species which is regularly encountered in wild and cultured eels (Buchmann and Bresciani, 1997). Its prevalence among Anguilla anguilla in Egypt was 5.36% (Dosoky, 2007). The parasite usually found on the gills but also can be found on the rest of the body, especially when the fish become weakened (Durborow, 2003). The parasite feed on the epithelial cells resulting in abrasions and erosions (Paperna,
Moreover, the eel became slimy, display lethargic behavior, weight loss and develops gill swelling (Buchmann and Bre- sciani, 1997). The cestode genus Proteocephalus occurred worldwide; however, very little is known about the mechanisms of distribution within the host range. It parasitizes the intestine of eel but the infections remained low and the pathology in the host was not detected (Zehnder and Mariaux, 1999). Moreover there is no data available on the prevalence or the pathogenicity of this parasite (Szekely, 2006).

The objective of this work was to study the pathological changes associated with parasitic infection in Eels from different localities beside their prevalence.

MATERIALS AND METHODS

Sixty-five living eels (Anguilla anguilla) with body weight ranged from 125-680 gm and total length ranged from 27-78 cm were collected in the period between January to May 2008, from Al Salam channel at Hussenia district (15 cases), Zagazig markets (17 cases) and Al Manzala Lake (33 cases).

All obtained eels were immediately subjected to parasitological and postmortem examinations as described by Hoffman (1970) and Lucky (1977). Specimens from swim bladder, gills, skin, muscle, liver, kidney and intestine of infected eels were taken and immediately fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin sections were prepared, stained by Hematoxyline and Eosin and then examined microscopically (Bancroft et al., 1996).

RESULTS:

I-Nematodiasis (Anguillicolosis):

Macroscopically, the infected eels (7 out of 65, 10.7%) were emaciated and showed enlarged abdomen with red and swollen anus (Fig., 1 A). The mean number of Anguillicola crassus in the swim bladder was 1.85 per eel. The swim bladder wall was markedly thickened and the worms filled the lumen giving a picture of a case engorged with worms (Figs.1, B and C). Adhesions between the affected swim bladder and the surrounding organs were detected in two cases.

Microscopically, adult worms containing numerous larvae and their guts filled with blood were observed inside the lumen of the swim bladders accompanied with vacuolation of the epithelial lining and subepithelial edema (Figs., D and E). Acute and chronic inflammatory reactions were seen in the
swim bladder wall. The acute reaction represented by congested capillaries, extravasated erythrocytes, round cells infiltration and edema. The chronic reaction represented by fibrous tissue proliferation and round cell infiltrations besides coagulative necrosis of the epithelial lining (Figs., 2 A and B). The mucosa of some swim bladders showed hyperplasia of the lining epithelium, while in other cases showed desquamation of the lining epithelium accompanied with erosions and ulcers. Developing larvae were seen in the lumen, submucosa and serosa of the swim bladder accompanied with leukocytic infiltrations, hemorrhages and fibrosis (Fig., 2 C). Some of these larvae become degenerated, necrotic and surrounded by fibrous connective tissue.

II- Monogenean infection: (Dactylogyrus and Pseudoac-tylogyrus infections):

All infected eels (4 out of 65, 6.1 % for Dactylogyrus and 8.3%; 5 out of 65 for Pseudoactylogyrus) showed pale to grayish gills, swollen at the edges with presence of excessive amount of mucous (Fig. 2 D).

Microscopically, eels infected with either Dactylogyrus species or Pseudodactylogyrus species showed the same lesions which represented by presence of the mature and immature worms attached to the gill racher and gill filaments surrounded by mucous, desquamated epithelial cells and leukocytes besides, congestions, erosions and ulcers of the gill filament (Figs.3 A and B). The later revealed hyperplasia and fusion of the lamellar epithelium forming sheets of mucous secreting cells together with congestion of the brachial blood vessels and marked leukocytic infiltrations (Fig. 3 C). The gill racher showed edema, congestion, hyperplasia of mucous secreting cells (Fig. 3 D). The skin of gills showed erosions, ulcerations, spongiosis and goblet cell metaplasia. The subcutaneous and muscular layers of gill filaments revealed congestion, hemorrhages, edema and leukocytic infiltration.

III- Myxidiosis:

The infected eels (2 out of 65, 3.07%) showed distended abdomen, thickened intestinal wall with presence of white circular spots with a size of 0.1-2.0 mm in the intestinal mucosa which appeared edematous and congested (Fig. 4 A).

Microscopically, the intestine showed necrotic hemorrhagic enteritis with presence of large number of different developmental stages of the parasite in the epithelial lining (Fig. 4 B). The intestinal mucosa appeared ulcerated and infiltrated with round cells. Focal
granulomatous reactions represented by focal aggregations of lymphocytes and epitheloid cells surrounded by mononuclear infil-
trations fibrous connective tissue were also seen in the intestinal mu-
cosa (Figs. 4 C and D). The sub-
mucosa revealed congested capil-
laries, extravasated erythrocytes and lymphocytic infiltration (Fig. 5 A). Desquamated epithelia, cellu-
lar debris and few leukocytes were seen in the intestinal lumen. Hy-
perplasia of goblet cells together with leukocytic infiltrations and congestion of the lamina propria were also noticed (Fig. 5 B). Inter-
muscular edema and hyalinization of the muscular coat were also de-
tected. The intestinal wall was thickened with fibrous connective tissue proliferation infiltrated with round cells.

IV- Trichodinosis:

The infected eels (3 out of 65, 4.6 %) were emaciated and showed slimy skin and swollen gills.

Microscopically, mucous plug (pale bluish substance containing desquamated epithelial cells and few leukocytes), was seen between gill filaments, besides unilateral sloughing of the secondary lamel-
lae (Fig. 5 C). The gill racher showed round cell infiltrations with hyperplasia of the mucous se-
creting cells (Fig. 5 D). The mus-
cular and subcutaneous layers of the skin reveled marked round cell infiltration (Figs. 6 A and B).

V- Cestodiasis (Proteocephalus species infection):

The infected eel (one out of 65, 1.5 %) were apparently normal but the parasites appeared upon cutting of the intestine.

Microscopically, the intestine of eel showed mild catarrhal enteritis represented by congestion of the mucosal blood vessels, goblet cell metaplasia and mononuclear cell infiltration together with presence of the parasite in the intesti-
nal lumen.

VI- Mixed infection:

There were 2 cases of mixed infection with both Trichodina species and Dactylogyrus species and one case of Trichodina species and Anguilllicola species.
Figs. (1 A-E):
A- Eels infected with Anguillicola crassus showing distended abdomen and red swollen anus (arrow).
B & C- Eels infected with Anguillicola crassus showing swim bladder filled with the parasites (arrows).
D- swim bladder of Anguillicola crassus infected eels showing adult worm containing numerous larvae (arrow) and erythrocytes (arrow head) in the lumen, HE. x300.
E- swim bladder of Anguillicola crassus infected eels showing adult worm containing numerous larvae (arrow) and erythrocytes (R) in the lumen with vacuolation of the epithelial lining (arrow head) and subepithelial edema (E), HE. X1200.
Figs. (2 A-D):

A- swim bladder of Anguillicola crassus infected eels showing thickened wall by fibrous tissue proliferations and leukocytic infiltrations (arrow) besides coagulative necrosis of the epithelial lining (arrow head) H&E.X120.

B - High power of the previous figure to show the fibrosis (arrow), leukocytic infiltrations (arrow head) and the coagulative necrosis of the epithelial lining (N) H&E.X300.

C- swim bladder of Anguillicola crassus infected eels showing developing larvae in the wall (arrow) with hemorrhages (H) and fibrous tissue proliferations(F) H&E.X300.

D- Gill of Dactylogyrus infected eels showing paleness and swollen edges (arrow).
Figs. (3 A-D):

A - Gill of Dactylogyrus infected eels showing mature worm (arrow) attached to the gill filament, surrounded by mucous and desquamated epithelium (arrow heads) besides congestion of the blood vessels (C) H&E.X120.

B - Gill of Pseudodactylogyrus infected eels showing mature worm attached to the gill rachor (arrow) with congestion of the blood vessels(arrow head) H&E.X120.

C - Gill of Dactylogyrus infected eels showing hyperplasia and hypertrophy of the lamellar epithelium forming sheets of mucous secreting cells (arrow) besides congestion of the brachial blood vessels (arrow head) and leukocytic infiltrations (L) H&E.X120.

D - Gill of Pseudodactylogyrus infected eels showing edema (E), congestion (arrow) and hyperplasia of mucous secreting cells of the gill rachor (arrow head) H&E.X120.
Figs. (4 A-D):

A- Intestine of Myxidium species infected eels showing white circular spots with a size of 0.1-2.0 mm in the intestinal mucosa (arrows).

B - Intestine of Myxidium species infected eels showing the parasite in the intestinal epithelium. (arrows) H&E.X1200.

C-Intestine of Myxidium species infected eels showing granulomatous reaction represented by focal aggregation of lymphocytes and epitheloid cells surrounded by fibrous connective tissue (arrow) H&E.X120.

D-High power of the previous figure to show lymphocytes, epitheloid cells and the fibrous connective tissue H&E.X 300.
Figs. (5 A-D):

A- Intestine of Myxidium species infected eels showing congested capillaries (arrows), lymphocytic infiltrations and extravasated erythrocytes (arrow head) H&E. X 1200.

B- Intestine of Myxidium species infected eels showing hyperplasia of goblet cells (arrow) with congestion (arrow head) and leukocytic infiltrations (L) of the lamina propria H&E. X 300.

C- Gill of Trichodina species infected eels showing mucous plug (P) and unilateral sloughing of the secondary lamellar epithelium (arrow) H&E. X 300.

D- Gill rachor of Trichodina species infected eels showing round cell infiltrations (arrow) and hyperplasia of the mucous secreting cells (arrow heads) H&E. X 300.
Figs. (6 A-B):
A- Muscle of Trichodina species infected eels showing marked round cell infiltrations (arrow) H&E. X 300.
B- subcutaneous tissue of Trichodina species infected eels showing round cell infiltrations (arrow) H&E. X 300.
DISCUSSION

In the present work, the identified parasites and their prevalence were; *Anguillicolosis* (10.7%) *Dactylogyrus* species infection (6.1%), *Pseudodactylogyrus* species infection (7.7%), *Myxidiosis* (3.07%), *Trichodinosis* (4.6%), and *Proteocephalus* species infection (1.5%). These results were nearly similar to those obtained by Dosoky (2007) but it was lower than those obtained by Mohamed and Nouh (2004) who reported that the prevalence of *Anguillicola crassus* infection among *Anguilla anguilla* in Egypt was 66.5%. This difference could be due to the seasonal variations as this work was done during winter. The higher prevalence of infection in the summer than in winter may be attributed to the temperature which is an important factor for hatching eggs, molting larvae and reproduction of intermediate hosts (Thomas and Ollevier 1992 and Cardoso and Saraiva 1998).

All infected eels with *Anguillicolosis* were emaciated and showed enlarged abdomen with red and swollen anus. The emaciation was due to the blood-feeding activities of adult worms (Sures et al., 2001). The enlarged abdomen might be a result of filling of the swim bladder with the adult worms (Kirk et al., 2000a). The red and swollen anus could be due to the release of eggs and larvae from swimbladder to the intestine via the pneumatic duct and then to the water through the anus inducing severe inflammation (De Charleroy, et al., 1990), or due to bacterial lesions in the posterior regions of the abdomen (Van Banning and Haenen 1990). The swim bladder wall was markedly thickened and showed acute and chronic inflammatory responses together with presence of adult worms in the lumen and of larvae in the lumen and wall. These results are in agreement with (Hoglund et al 1992). The thickening in the swim bladder wall was due to the fibrosis induced by the chronic inflammation (Abbas et al., 2001). The inflammatory reactions were induced by the irritation induced by the feeding activity of the parasite and the larval migrations (Molnar, 1994). The edema was due to hypoproteinemla which resulted from regular blood sucking of the adult worms (Molnar et al., 1993).

Eels infected with *Dactylogyrus* and *Pseudodactylogyrus* species showed pale to grayish gills, swollen at the edges with presence of excessive amount of mucous. These results are in agreement with Pillay (1990). The gill surface and filament of *Dactylogyrus* and *Pseudodactylogyrus* infected eels usually contain sections of the mature and immature
worms surrounded by hyperplastic lamellar epithelium abundant mucous and desquamated epithelium. The gill filament showed edema, congestion, leukocytic infiltrations and hyperplastic proliferations of the lamellar epithelium. The skin of gills showed erosions, ulcerations, spongiosis and goblet cell metaplasia. The same results were obtained by (Aguilar et al., 2005 and Dosoky 2007). Paperna 1991 mentioned that the attachment of Dactylogyrus and Pseudodactylogyrus species on the gills and their feeding activity on the epithelial cells resulted in severe irritation which induces erosions, ulcerations, excess mucous production, inflammatory reactions, hemorrhages, and hyperplasia of the epithelium of the gills and destruction of the gill filaments.

Eels infected with Myxidium species showed distended abdomen, thickened intestinal wall with presence of white circular spots with a size of 0.1-2.0 mm in the intestinal mucosa. This result was in agreement with (Pillay 1990). The distended abdomen could be a result of the severely congested, edematous and thickened intestinal wall due to enteritis induced by the parasite (Rodjuk and Sheleenkova 2006). The white circular spots in the intestinal mucosa were focal granulomas (Copland 1983). The intestine revealed severe enteritis with presence of Myxidium species in the enterocytes. The intestinal mucosa and submucosa appeared edematous, congested, hemorrhagic, ulcerated and infiltrated with round cells. The intestinal lumen contained desquamated epithelial cells, erythrocytes, myxozoan spores, cellular debris and few leukocytes. These finding were in agreement with Borgsteede et al., (1999). Woo (2006) mentioned that the proliferation of Myxidium species in the intestinal mucosa resulted in mechanical destruction of the enterocytes, with the result of inflammatory reactions, hemorrhages and epithelial desquamation.

Eels infected with Trichodinosis were emaciated and showed slimy skin and swollen gills. These results were nearly similar to those obtained by Buschman and Berisciani (1997) and Madsen et al., (2000). Trichodina species, desquamated epithelial cells, leukocytes and mucous plug were seen between gill filaments which revealed edema, congestion, hemorrhages, erosions, ulcerations and unilateral sloughing of the secondary lamellae. Trichodina species damage the epithelial tissue through adhesion and crawling actions (Paperna, 1996). They feed on the epithelial cells through sucking of the cellular contents and the host responds by increased mucous secretion, epithelial hyper-
plasia, cellular destruction and inflammation (Woo et al., 2002).

Eels infected with Proteocephalus species were apparently normal but the intestine showed mild catarrhal enteritis. This result was in agreement with Zehnder and Mariaux (1999). The enteritis might be due to the feeding activity of the parasite.

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دراسات باثولوجية على بعض الأمراض الطفيلية في ثعابين الماء

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الملخص العربي
أجريت هذه الدراسة على 65 ثعابین ماء وذلك لدراسة الإصابات المرضية الناتجة عن الإصابة بالطفيليات الممرضة مع تصنيف هذه الطفيليات. تم تجميع هذه الأسماك من ترعة السلام وبحيرة المنزلة وبعض محلات بيع السمك في مدينة الزقازيق في الفترة من يناير- مايو 2008. أظهرت الدراسة أن معدل الإصابة بالطفيليات الممرضة هو 33.85% و وذلك نتيجة الإصابة بالباختیولا کروز (7.7%) والداتفیکول (6.1%) والپولیدم (7.7%) والپولیکول (6.1%) والتربیوکسیفسل (5%). وقد تبين من خلال الدراسة أن هذه الطفيليات تحدث العديد من الإصابات المرضية منها ضعف وحزال وتكسم ونخروا انزوفه في العديد من أعضاء هذه الأسماك طبقاً لطبيعة الإصابة مما يؤدى إلى ضعف في أنتاجية هذه الأسماك.

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