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PATHOLOGICAL STUDIES ON THE SWIMBLADDER OF EGYPTIAN EELS (*ANGUILLA ANGUILLA*) INFECTED WITH *ANGUILLICOLA CRASSUS*

(With 2 Tables and 27 Figures)

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دراسات باثولوجية على الحويصلة الهوائية فى أسماك الثعابين المصرية (أنجيولا أنجيولا) المصابة بالأنجيوليكولا كريسيس

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تـم تجميع عدد ٤٢ سمكة من أسماك الثعابين المصرية (أنجيولا أنجيولا) من كل من بحيرة المـنزلة بمحافظة الدقهلية (٢٧ سمكة) والأسواق المحلية بمحافظة الشرقية (١٥ سمكة) فى الفـترة من يونيو ٢٠٠٣ حتى فبراير ٢٠٠٤. وجد أن نسبة الاصابة حوالى ٥٧,١٤%، وتم وصـف الحويصلات الهوائية المصابة بالعين المجردة وأخذ العينات للفحص النسيجى. وجد أن جـدران الحويصـلة الهوائية سميك نتيجة للالتهابات الحادة والمزمنة فيه مع وجو الدودة البـالغة واليرقات داخل تجويف الحويصلة الهوائية. لوحظ فرط تنسج وتساقط وتقرح وزيادة حجـم الخلايـا الظاهرية المبطنة لجدار الحويصلة الهوائية مع وجود تغيرات بالنواه. ووجد تـنكس بالأعضـاء الداخلية نتيجة للأنيميا الحادة الموجودة. واستنتج أن الاصابة بالأنجيولا كريسـيس حوالـى ٥٧,١٤% فى الأسواق المصرية والتى تؤثر على انتاج أسماك الثعابين المصـرية وتسـبب الأنيمـيا والهزال بالإضافة إلى الالتهابات الحادة والمزمنة بالحويصلة الهوائية.

SUMMARY

Twenty-four (57.14%) out-of 42 Egyptian eels (*Anguilla anguilla)* were examined to *Anguillicola crassus*-infected swimbladder and collected from Manzala Lake, Dakahlia governorate (27 cases) and Zagazig markets, Sharkia governorate (15 cases) during the period from June, 2003 to Feb., 2004. The infected bladders were described grossly and specimens were taken for histopathological examination. The swimbladder-wall was markedly thickened by acute and chronic inflammatory reaction with the presence of the adult worms in the lumen

(1-8 worms/eel) and the larvae in the lumen and wall. Mucosal hyperplasia, desquamation and ulceration were seen beside hypertrophy of the lining epithelium with nuclear dysplasia. Mild degenerative changes were detected in the internal organs as a result of severe anemia. Finally, it could be concluded that the prevalence of *A. crassus* (57.14%) markedly impairs the Egyptian eel's production (anemia and emaciation) and produce intense acute and chronic inflammatory responses in the swimbladder.

Key words: *Fish, Eel, swim bladder, Anguillicola crassus*

INTRODUCTION

Angullicolosis is a disease caused by *Anguillicola crassus*. The latter parasitizes the swimbladder of eels originating from southeast Asia where it is a natural parasite of the Japanese type (Kuwahara *et al.*, 1974). It was brought into Europe in the early 1980, and then rapidly spread throughout the continent (Egusa, 1992). The parasite was firstly detected in German by Neumann (1985) and in Egypt by Abbas *et al.* (2001). The infective 3rd stage larvae develop in many non-crustacean intermediate hosts (copepods, cyclopedia or ostracoda) after ingestion of the 2nd stage larvae. Eels become infected by ingestion of the infected copepods, then the larvae penetrate to the swimbladder-wall where they develop to 4th stage larvae and pre-adult larvae (L_5), before its migration to the lumen of bladder (Molnar and Moravec, 1994; SzeKely, 1994 and Kirk, 2003).

The prevalence of anguillicolosis 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 10-29% among *Anguilla rostrata* in USA (Barse and Secor, 1999), 60-73% among *Anguilla Anguilla* in Portugal (Cardoso and Saraiva, 1998), 100% among *Anguilla rostrata* in Taiwan (Kean *et al.*, 1996) and 30% among *Anguilla anguilla* in Egypt (Abbas *et al.*, 2001).

The eels of heavily parasitized bladder revealed reduction in the growth rate, emaciation, enlarged abdomen and mortality up to 65% (Sinderman, 1993 and Kirk *et al.*, 2000a), and they become more susceptible to bacterial infection (Hartmann, 1987 and Koie, 1991). The wall of infected swimbladders was greatly thickened, inflamed and showed focal hemorrahgic areas. Many round worms with chocolate-like material were seen in the lumina of such bladders (Beregi *et al.*, 1998 and Abbas *et al.*, 2001). Meanwhile, the histopathological changes

revealed parasitic aerocysititis, which varied according to the stage of infection. In acute cases, hyperemia, mucosal hyperplasia and desquamation, and hemorrhages were recorded (Molnar *et al.*, 1993), while, edema, fibrosis and mononuclear cells infiltration in the chronic cases (Wurtz and Taraschowshi, 2000). Migration of the larvae from the gut to the swimbladder cause irritation and inflammation of the surrounding organs (Koie, 1991). Fibrotic nodules containing larvae were reported in the intestinal submucosa and serosa. Molnar *et al.* (1993) reported that the thickening of the swimbladder-wall was associated with degenerative, inflammatory and proliferative changes, which were characterized by epithelial hyperplasia, hyperemia and granulomatous infiltrates around the larvae. Severe anemia and granulocytosis were reported during the course of infection with *A. crassus* (Hoglund *et al.*, 1992).

The objective of this work was to study the pathological changes of the swimbladder and internal organs associated with infection by larval and adult stages of *Anguillicola crassus* in eels form Manzala lake (Dakahlia governorate) and Zagazig markets (Sharkia governorate), Egypt.

MATERIALS and METHODS

Forty-two living eels, *Anguilla anguilla* weighting 250-400 gm and body length ranging between 22-76 cm were collected form Manzala lake, Dakahlia governorate (27 cases) and Zagazig markets, Sharkia governorate (15 cases). All obtained eels were immediately subjected to parasitological and postmortem examinations as described by Hoffman (1970) and Lucky (1977).

Ten random blood samples were collected from each apparently healthy and infected eels in vacutainer heparinized tubes for hematological studies. Erythrocytes, leukocytes counts and differential leucocytic count were carried out by standard clinical method (Nutt and Herrick, 1952 and Soliman 1986). Hematocrit (PCV) value was measured (Feldman *et al.*, 2000), while hemoglobin concentration was performed (Drabkin, 1949). The obtained data was statistically analyzed (Snedecor and Cochran 1982). Specimens from swimbladder 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 (HE.), Crossmon's trichrome stain for the connective tissue and PAS reaction for the parasitic elements and then examined microscopically (Bancroft *et al.*, 1996).

RESULTS

I-Prevalence and external examination:

All infected eels (57.14%: 19 out of 27 from Dakahlia governorate and 5 out of 15 from Sharkia governorate) were emaciated and showed enlarged abdomen with red and swollen anus. Meanwhile, the mean number of *A. crassus* in the swimbladder was 2.08 per eel (table, 1).

II-Pathological findings:

Swimbladder:

Macroscopically, the swimbladder-wall was markedly thickened, opaque and rarely fibrotic with narrowed or collapsed lumen. The latter was filled with adult worms, cloudy fluid or bloody contents containing larvae (L_2). In few cases, adhesions between the affected swimbladder and the surrounding organs were detected.

Microscopically, adult worms of *Anguillicola crassus* containing numerous larvae and erythrocytes were observed inside the lumen of the swimbladders (Figs. 1 and 2). Acute and chronic inflammatory responses were seen associating the presence of such parasites in the bladder and induced severe thickening of its wall. The acute inflammatory response was represented by congested capillaries, extravasated erythrocytes, round cells infiltration (mostly lymphocytes), and edema (Fig. 3). The latter was evident in the wall of bladder by widely separated fibers and eosinophilic homogenous materials (Fig. 4). While the chronic response was represented by granulation tissue containing numerous blood capillaries which greatly thicken the swimbladder-wall (Figs. 5 and 6). The collagen fibers, formed in the granulation tissue, was stained green by Crossmon's trichrome stain (Fig. 7). The lumens of some swimbladder showed numerous larvae, desquamated epithelium and inflammatory cells (Figs. 8 and 9) beside focal mucosal destruction or ulceration. The ulcerated areas were heavily infiltrated with lymphocytes, macrophages and eosinophilic granulocytes (Fig. 10). Some of these larvae were detected in the submucosal cavities and surrounded by numerous macrophages, lymphocytes and melanomacrophages (Fig. 11). Moreover, several granulomas without larvae were focally displaced the swimbladder-wall (Fig. 12) and mostly, consisted of aggregations of macrophages, lymphocytes and few eosinophilic granulocytes and enclosed in loose connective tissue (Fig. 13). Some granulomas showed central necrotic debris of eosinophilic and basophilic material, surrounded by fibrous connective tissue and infiltrated with round cells and few eosinophilic

granulocytes (Fig. 14). The parasites (adult and larvae) were positive for PAS-reaction (Fig. 15). Numerous melanomacrophages and hemosiderosis were also seen (Fig. 16). In some cases, mucosal epithelial hyperplasia, forming papillary projections inside the lumen of swimbladder, was observed. Some lining epithelium were hypertrophied with nuclear dysplasia. These nuclei were varied in sizes and shapes and appeared vesicular and hyperchromatic (Fig. 17a and b).

The pneumatic ducts revealed several vesicles among the lining epithelium, which contained light basophilic material (Fig. 18). The latter was positive for PAS-reaction (Fig. 19). The wall of such ducts was inflamed and thickened by edema, leukocytic aggregations and extravasated erythrocytes beside severely congested blood vessels. The surrounding adipose tissue was focally necrotic and showed several larvae without inflammatory reaction (Fig. 20).

Internal organs:

Macroscopically, the liver was pale with irregular congested areas particularly at the borders. The kidney was swollen and the intestine was apparently normal except for the adhesions with the infected swimbladders.

Microscopically, the liver showed diffuse hydropic degeneration, fatty changes (Fig. 21) and coagulative necrosis of individual hepatic cells, which evident by pyknotic or absent nuclei with an increase of the cytoplasmic eosinophilia. Few inflammatory cells mainly lymphocytes were detected around the portal blood vessels and among the hepatic cells. Melanomacrophages and congested blood vessels were focally seen throughout the hepatic tissue.

The posterior kidney revealed hypercellularity of the glomeruli, vacuolar degeneration and coagulative necrosis of the renal epithelia (Fig. 22). Hyaline casts inside the lumens of some renal tubules were observed. Few lymphocytes were seen among the renal tubules. The anterior kidney showed depletion of hemopoietic tissue and activation of melanomacrophages (Fig. 23). Perivascular edema and congestion of blood vessels were also seen.

The intestine showed focal intense inflammatory reaction and necrosis in the intestinal mucosa. The latter appeared ulcerated and infiltrated with round cells. The submucosa revealed congested capillaries, extravasated erythrocytes and lymphocytes infiltration (Fig. 24). Desquamated epithelia, fibrin casts and few leukocytic infiltrations were detected in the intestinal lumen (Fig. 25). Increased numbers of goblet cells were also noticed. Few lymphocytes infiltration in the

submucosa and lamina propria were seen in the remaining intestinal segment. Intermuscular edema and hyalinization of the muscular coat were also detected. Parasitic larvae were observed in the degenerated muscular coat (Fig. 26). The intestinal wall was thickened with fibrous connective tissue proliferation infiltrated with round cells particularly at the areas of adhesion (Fig. 27).

III-Hematological findings:

Table (2) illustrates normocytic normochromic anemia with significant reduction in the PCV, Hb%, RBCs count, with leucocytosis, lymphocytosis and monocytosis.

Table 1: Prevalence and intensity of *A. crassus* in the swimbladder.

Season		Governorate	No. of eels	No. of infected eels	Prevalence (%)	Intensity of *A. crassus* (mean)
Summer	(June 28-Aug. 15, 2003)	Dakahlia	16	14	87.5	1-8 (2.92)
		Sharkia	8	4	50.00	1-3 (1.75)
Winter	Dec.9, 2003- Feb. 19, 2004)	Dakahlia	11	5	45.45	1-4 (2)
		Sharkia	7	1	14.28	2 (2)
Total examined eels			42	24	57.14	50 (2.08)

Table 2: Some hematological examination of healthy and infected eels

	RBCs 10^6/µl	Hb gm%	PCV %	TLC 10^3/µl	Absolute differential leucocytic count 10^3/µl			
					N	E	L	M
Control	2.08 ± 0.11	9.14 ± 0.49	22.54 ± 1.22	20.77 ± 1.12	1.45 ± 0.07	0.62 ± 0.03	17.03 ± 0.85	1.66 ± 0.08
Infected	* 1.62 ± 0.10	* 7.13 ± 0.45	* 17.58 ± 1.11	** 28.09 ± 1.77	1.40 ± 0.08	0.56 ± 0.03	** 23.60 ± 1.32	** 2.53 ± 0.16

DISCUSSION

In the present work, the prevalence of *A. crassus* (57.14%) showed seasonal dynamics and varied from 87.5 (summer) to 45.45 (winter) in Dakahlia governorate and from 50.0 (summer) to 14.28 (winter) in Sharkia governorate. The higher prevalence of infection in the summer (warm season) than in winter (cold one) may be attributed to the temperature is an important factor for hatching eggs, molting larvae and reproduction of intermediate hosts (Copepods). Thomas and Ollevier (1992) and Cardoso and Saraiva (1998) found that in the warm

period (from May to October) the prevalence of *A. crassus* nematodes was higher than in the cold period. Moreover, the intensity of the adult worms was ranged form 1-8/eel and Dakahlia governorate was greater intensity than Sharkia governorate. Our finding is in agreement with that obtained by Abbas *et al.* (2001) who found 60% of gill-harbored copepodes; intermediate host of *A. crassus*, in Manzala lake which rapidly spread the infection among the eels.

All infected eels were emaciated and showed enlarged abdomen with red and swollen anus. The enlargement in the abdomen was as a result of enlargement of the swimbladder, which filled with the adult worms (Kirk *et al.*, 2000a). While, the red and swollen anus may be due to 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 and Kirk *et al.*, 2000b). Van Banning and Haenen (1990) mentioned that the red and swollen anus of eels infected with *A. crassus* was due to bacterial lesions in the posterior regions of the abdomen. The migratory and blood-feeding activities of adult worms may be induced anemia and emaciation of infected cases (Boon *et al.*, 1990 and Sures *et al.*, 2001). These results were coincided with our clinicopathological parameters that revealed reduction in RBCs count and Hb%.

The lesions in the infected swimbladder with *A. crassus* were macroscopically represented by thickening, opaque and fibrotic wall with narrowed or collapsed lumen. The latter were as a result of fibrosis in the wall. Cloudy fluid or bloody contents containing larvae were detected inside the lumen beside the adult worms. The aforementioned results were similar to those obtained by Molnar *et al.* (1995), Haenen *et al.* (1994), Abbas *et al.* (2001) and Kirk (2003).

Microscopically, the swimbladder showed acute and chronic inflammatory responses associating the presence of adult worms inside the lumen and repeated invasion of the larvae into the wall (Wurtz and Taraschewshi, 2000). The acute inflammatory response was represented by congested blood vessels, hemorrhages, lymphocytes infiltration and edema; while the chronic inflammatory response was represented by granulation tissue that greatly thicken the swimbladder-wall. The edema is a general signs of anguillicolosis due to hypoproteinemia resulting from regular blood sucking of the adult worms (Molnar, 1994). The granulation tissue formation might be developed by injury to the connective tissue by larval migration. Molnar *et al.* (1991 & 1993) and Abbas *et al.* (2001) recorded similar findings.

Several granulomas were detected throughout the bladder-wall consisting of aggregations of macrophages, lymphocytes and few eosinophilic granulocytes or central necrotic debris of eosinophilic and basophilic material, surrounded by fibrous connective tissue and infiltrated with round cells . These results are in accordance to those obtained by Molnar (1994) and Monlaer *et al.* (1995) who recorded granulomatous reaction surrounding the larvae within the wall of swimbladder of eels infected with *A.crassus*. Numerous melanomacrophages and hemosiderosis were also observed. These cells may play an important role in the non-specific cellular immunity of fish; thus they were detected around the larvae to elicit its damages. Ferguson (1989) mentioned that the presence of melanomacrophages is the prominent feature of chronic inflammation in fish.

The intestine showed focal intense inflammatory reaction, necrosis and ulceration with congested capillaries and round cells infiltration. Mucosal epithelial hyperplasia and hypertrophy of some lining epithelia with nuclear dysplasia were detected. These lesions could be attributed to the mechanical injury of the parasite to the epithelial lining of infected swimbladder. Our results are in partial agreement with Chunge *et al.* (1996) and Abbas *et al.* (2001) found epithelial hyperplasia and desquamation in the infected swimbladder with *A. crassus*.

The lesions in the liver and kidney were mild and included degenerative and rarely necrotic changes. The latter were secondary to the structural and inflammatory changes in the infected swimbladder. These findings can significantly alter the function of the swimbladder and consequently induce hypercapnia, which leads to degeneration and necrosis in the parenchymatous organs (McKenzie *et al.*, 2003). Our results don't agree with those obtained by Lefebvre *et al.* (2004) who described liver and spleen masses in eels infected with *A. crassus*.

In the present study, the hematological parameters revealed significant decrease in the RBCs-count, Hb%, PCV and granulocytes beside an increase of lymphocytes and monocytes. The decrease in the RBCs-count resulting from the blood sucking activation of the nematodes (Boon *et al.*, 1990) and increased number of the lymphocytes and monocytes may be due to activation of the immune response (Palikova and Navratil, 2001). Our findings are in partial agreement with Hoglund *et al.* (1992) who found significant decrease in the RBCs-count and increase of granulocytes in infected eels with *A .crassus*.

Finally, it could be concluded that the prevalence of *A. crassus* (57.14%) markedly impairs the Egyptian eel's production (anemia and emaciation) and produce intense acute and chronic inflammatory responses in the swimbladder.

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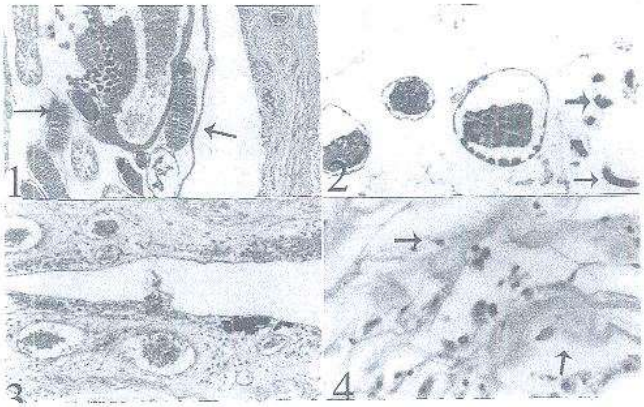
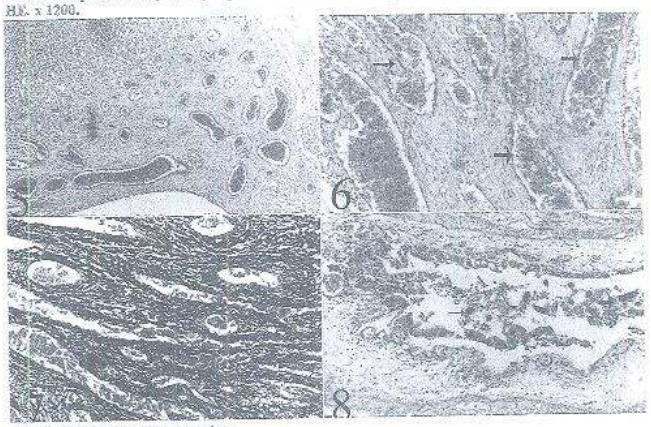
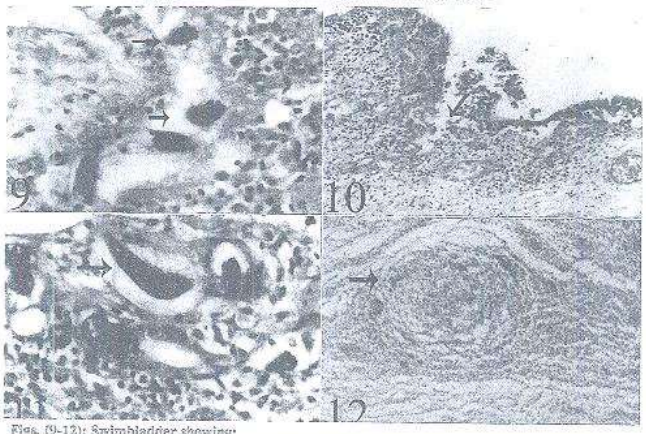


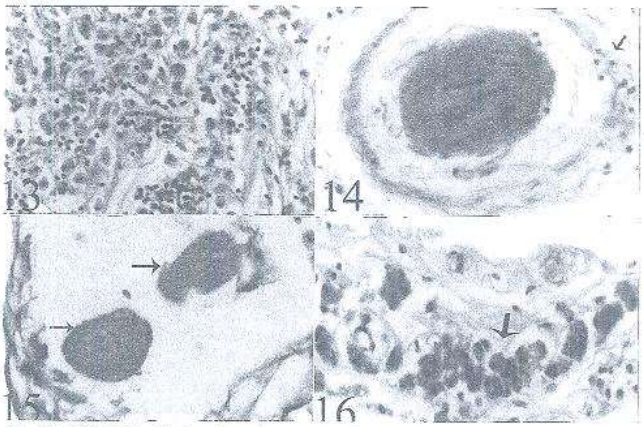
Figs. (1-4): Swimbladder showing
1-Adult Worm of *A. crassus* (arrows) inside the lumen. HE. x 120.
2-Numerous larvae (arrows) and erythrocytes inside the worm, HE. x120.
3-Thickening of the wall with congested capillaries, extravassted erythrocytes, round cells infiltration and edema. HE. x300.
4-Edema represented by widely separated fibers with eosinophilic homogenous material (arrows). HE. x 1200.



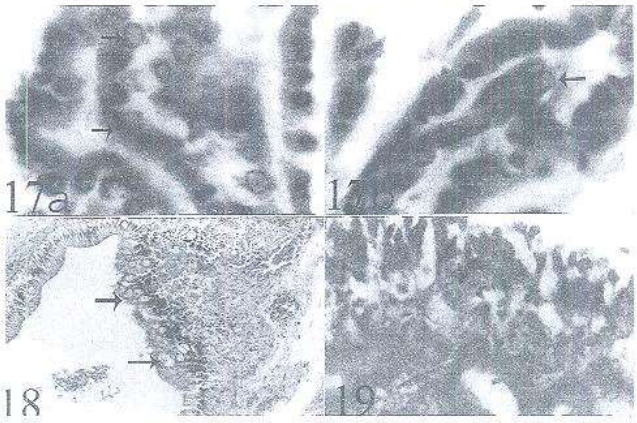
Figs. (5-8): Swimbladder showing:
5-Granulation tissue replacing the wall, HE. x 120.
6-Numerous blood capillaries (arrows) in the granulation tissue. HE. x 300.
7-The collagen fibers were stained green with Crossmon's trichrome stain x 300.
8-Numerous larvae (arrows), desquamated epithelium and inflammatory cells inside the lumen, HE. x 120.



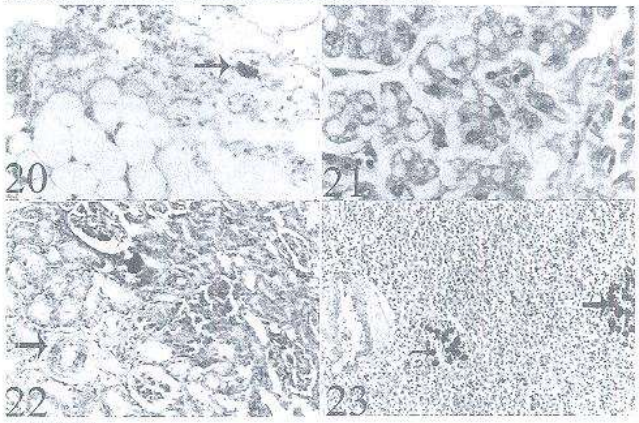
Figs. (9-12): Swimbladder showing:
9-Larvae (arrows) and leukocytes (macrophages and lymphocytes) inside the lumen, HE.x1200.
10-Mucosal ulceration with intense leukocytic infiltration (arrow), HE. x 300.
11-Numerous larvae inside submucosal cavities, which surrounded by macrophages, lymphocytes and melanomacrophages. HE x 1200.
12-Granuloma displacing the wall (arrow), HE. x 120.



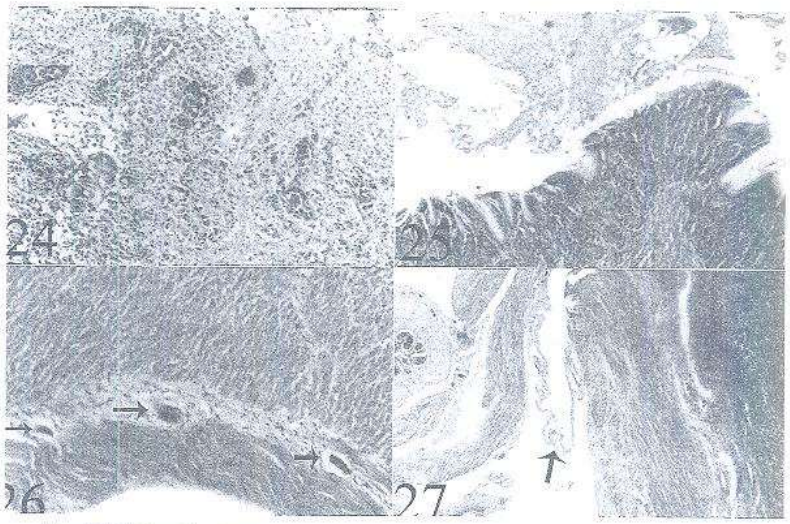
Figs. (13-16): Swimbladder showing:
13-Macrophages, lymphocytes and eosinophilic granulocytes in the constituent of the granuloma. HE. x 1200.
14-Necrotic debris of eosinophilic and basophilic material in the center of granuloma. HE. x 1200.
15-Positive PAS-reaction for larvae (arrows). HE. x 1200.
16-Numerous melanomacrophages in the wall. HE. x 1200.



Figs. (17-19):
Figs. (17 a & b): Swimbladder showing mucosal epithelial hyperplasia and hypertrophy with nuclear dysplasia (arrows), HE. x 3000.
Figs. (18 and 19): Pneumatic duct showing:
18-Several vesicles (arrows) among the epithelial lining, HE. x 300.
19-Positive PAS-reaction for vesicular content (mucus), HE. x 1200.



Figs. (20-23):
20-The adipose tissue, surrounding the pneumatic ducts, showing focal area of necrosis and larva (arrow), HE. x300.
21-Liver showing vacuolations and necrosis (absent nuclei) in some hepatocytes, HE. x 1200.
22-Posterior kidney showing focal area of coagulative necrosis (arrow), HE. x 300.
23-Anterior kidney showing depletion of the constituent cells and activation of melanomacrophages (arrows), HE. x 300.



Figs. (24-27): Intestine showing:
24-Focal intense inflammatory reaction and necrosis in the mucosa, H & E. x 300.
25-Desquamated epithelia, fibrin cast (arrows) and few leukocytes inside the lumen, HE. x 300.
26-Larvae in the hyalinized muscular coat (arrows), HE. x 300.
27-Fibrous C.T. proliferation at the site of adhesion with the swimbladder (arrows), HE. x 120.