

Occurrence of *Anguillicola crassus* (Nematoda , Dracunculidae), a Parasite of European Eel (*Anguilla anguilla*) in Egypt

Saleh, G. Aly; El-Nobi, G. Ahmed and El-Dosoky, A. El-Sayed

Dept. of Fish Diseases and Management,
Fac. of Vet. Medicine, Zagazig University

ABSTRACT

A total of 568 eels (*Anguilla anguilla*) were collected from different localities during different seasons to study the prevalence of *Anguillicolla crassus* infection and its effect on health of eel. In addition, 180 eels infected with *Anguillicolla crassus* were used for treatment trials using levamisole and humate substance.

The results revealed that, the prevalence of *Anguillicolla crassus* infection in examined eels was 75.2% with mean intensity of 6.15 worms per infected eel. The highest prevalence (82.8%) was observed in Al-Salam channel which supplied by fresh water. Also, the highest prevalence (91.06%) was recorded during summer seasons. Small sized eel (less than 40 cm) recorded a highest prevalence of infection (90.47%). Infected eel showed abnormal behaviour by hanging near the water surface, belly sometimes swollen, pink or red coloration of anal opening , thickening the wall of swim bladder with fibrosis and engorgement with worms giving a picture of sausage like. There were a marked changes in hematological and histopathological findings in infected eel. Levamisole (10-20 mg/L/24 hrs) and humate substance (2 g/kg food /10 months) were effective in treatment of infected eel.

INTRODUCTION

Eel is suitable species for culture because of its relative rusticity and various other advantages such as its adaptability to artificial food, tolerance of variation in temperature and salinity and high stocking densities. However, several limiting factors as the eels are sensitive to environmental stresses (chemical, physical and biological, parasitic, fungal, bacterial and viral diseases) all of which are responsible for lower survival, lower growth, lower commercial value and for higher costs in preventive and curative (1). The European eels, *Anguilla anguilla* are wide spread and common species of the European and North African Coasts. All works have showed that this fish is not as resistant as it generally accepted.(2).

Parasitic nematode are remarkable by the complexity of their cycle which often imply a migration inside the body of the host, and by their capacity to produce eggs in great quantity (3). Mainly the nematode

Anguillicolla crassus, which attacks the swim bladder will causes significant mortalities in the eel population in aquaculture or in the natural environment (4).The damage to eels caused by the parasite is considered to be related mainly to the blood – sucking activity especially the pre- adult stages of the nematode in the swim bladder (5). Recently, it was suggested that infection with this parasite may soon be widespread in natural basins in Europe and North Africa (6,7).

Although *Anguillicolla crassus* was first recorded in 1985 (8), its wide spread distribution in different eel populations in the Netherlands at that time indicated that introduction could have already taken place 2 or 3 years earlier. *Anguillicolla* species were the most important fish parasites in the tropics (9).

Therefore this work was performed to study the prevalence of *Anguillicolla crassus* infection in eels in relation to locality and water quality, seasons and fish size. Also, the

effect of *Anguillicola crassus* infection on health of eels and trials for treatment.

MATERIAL AND METHODS

A total of 568 eels (*Anguilla anguilla*) with body weight ranged from 26-1100 g and total length from 28-82 cm were collected from 4 different localities during the years 2003-2006 for clinical and parasitological examinations (Table 1). In addition, 180 eels infected with *Anguillicola crassus* were used for treatment trials. Water samples were collected from the 4 different localities at the same time of fish collection.

Examination of water

Dissolved oxygen was measured by oxygen-meter Jenway model 9070 (U.K). The pH value of the water determined by Mv 870 Digital pH-Meter (Germany). Total salinity was estimated (10).

Clinical examination of eels

The eels were anaesthetized with tricaine methane sulphonate (Ms 222, 100 mg/L for 2 hours) to prevent struggling (11) prior to clinical, postmortem, parasitological and haematological examinations. The collected eels were examined clinically using the previously described methods (12,13).

Parasitological examination

The nematode after being collected from swim bladder were washed in saline solution and kept in refrigerator for killing and stretching. The worms were treated with 70% alcohol and 5% glycerol. After that, for best clearing they were put in lactophenol for 24 hours and then mounted in polyvinyl alcohol as best clearing and mounting agent, then microscopically examined for their morphological characteristics (14). Also, the mean number of worms per infected eel (mean intensity) was recorded.

Haematological and histopathological examinations

Blood samples were drawn from the caudal blood vessels (12). Erythrocytes (RBCs) and

leukocytes (WBCs) counts (15), hematocrit (PCV) (16), hemoglobin concentration (Hb) (17) using Sahli's method and the blood indices (16) were carried out. Specimens from infected swim bladder and liver were prepared and examined histopathologically (18).

Treatment trials of eels infected with *Anguillicola crassus*

A cement ponds, each 2.5 meter width, 4 meter length and 1.5 meter depth, filled with water up to one meter height were used for treatment trials. The ponds were irrigated by fresh water and drained daily. The stocking density of fish was 8 fish /m³.

Anguillicola crassus in infected eels was recognized by clinical and parasitological examinations of random samples.

Levamisole

A total of 30 eels infected with *Anguillicola crassus* were divided into 3 equal groups. The first group remained as a control. The second and the third groups were treated for 24 hours by levamisole bath 10 mg/L and 20mg/L respectively. Levamisole hydrochloride in the form of levamisole 7.5% produced by ADWIA Company, Egypt, was used.

Humate substance

A total of 150 eels infected with *Anguillicola crassus* were divided into 2 equal groups. The first group remained as a control. The second group was treated by humate substance as medical feed by ratio of 2g /kg food for 10 months. Humate substance (Natural material) in the form of Biofarm dry, produced by Pharmavet Company, Turkey, was used.

Diet and feeding were carried out (19). Fish were observed daily, evaluation of health condition and mortality were recorded (12).

Statistical analysis

The results were statistically analyzed using analysis of variance procedure in SAS (20).

Table 1. Eels collected from different localities during different seasons .

Locality and water supply	Season				Total
	Summer	Autumn	Winter	Spring	
Al-Salam channel Hussinia district (Fresh water)	56	43	49	38	186
Al-Gohhr area Manzala lake (Brackish water)	69	44	57	34	204
Al-Gameel area West Port -Said (Marine water)	31	16	38	21	106
Bahr El-Bakar Shader Azzam*	23	10	22	17	72
Total	179	113	166	110	568

* The water supply to this area is polluted with sewage.

RESULTS AND DISCUSSION

1. Prevalence and intensity of infection with *Anguillicola crassus* in relation to different localities and water quality

Our results revealed that, the prevalence of *Anguillicola crassus* infection in examined eels was 75.2% with mean intensity of 6.15 worms per infected eel (Table 2). These results are nearly similar to that obtained in Morocco with prevalence of 70% and mean intensity of 5.33 worms per infected fish (21). Also, in Turkey the prevalence was 82.86% and mean intensity of 3.31 (22). The highest prevalence (82.8%) was observed in Al-Salam channel, which supplied by fresh water with mean intensity of 7.48 worms per infected eel. While the lowest prevalence (60.4%) was observed in Al-Gameel area which supplied by marine water with mean intensity of 4.20 worms per infected eel (Table 2). This may be due to the direct effect of salinity on the rate of *Anguillicola crassus* infection. Similar findings were previously recorded (23-25).

2. Seasonal prevalence and intensity of *Anguillicola crassus* infection in examined eel

It was observed that, the highest prevalence was in summer (91.06%) followed

by autumn (80.53%), while the lowest prevalence of infection was in winter (55.42%). Also, there were variation in the mean intensity of worms per fish, where it was 8.02 worms per fish in summer and 3.81 worms per fish in winter (Table 3). These may be attributed to that, the eel feed little during winter. Also, it seemed probable that, the recruitment of this parasite was confined to the warmer months of the year. These findings were in agreement with those previously reported (4, 22, 26, 27).

3. Prevalence and intensity of *Anguillicola crassus* infection in relation to the size of eels

It was found that, the highest prevalence of infection (90.47%) was recorded in small sized eel (less than 40 cm). While the lowest prevalence (31.42%) was observed in larger sized eel (more than 60 cm) (Table 4). These may be related to the feeding behaviour, where the primary food of small eels are annelids, small shell fish and cyclops which contain the infective stage of the parasite. In addition, the significant mortality of eels caused by this parasite decreases the number of parasitized eel that arrive the adult stages. These results were similar to that previously recorded (4, 28-30).

4. Clinical signs and post mortem findings

Eels infected with *Anguillicola crassus* showed anorexia, reduced swimming ability and some hanging near the water surface, belly sometimes swollen, reduction in growth rate and increase the mortality rate. The anal opening showed yellow–orange, pink or red colouration (Fig. 1-C,D). These findings may be due to the general weakness and anemia in infected eels caused from sucking of blood by the parasite. These results were in agreement with that previously obtained (4, 31,32).

The swim bladder of eel infected with *Anguillicola crassus* showed narrowed or collapsed lumen and the worms appeared from outside. The worms filled the swim bladder giving a picture of a case engorged with worms (sausage like) (Fig. 2- A,C,D,E,G). The swim bladder wall in some infected eels showed thickening the wall of swim bladder which was sometimes fibrotic and adhesive to adjacent organs as kidney and intestine. The liver sometimes enlarged and pale in colour. The spleen showed enlargement especially when the swim bladder filled with large number of worms (Fig. 2-A). Inflammation of the pneumatic duct was observed (Fig. 2- C, E). Rupture of some mature female, *Anguillicola crassus*, in swim bladder of eel leaving eggs, larvae and alimentary canal contents, mainly blood of infected eel, which looks like dissolved chocolate (Fig. 2-E). The swim bladder wall ruptured due to imposition of internal pressure as an excessive number of parasites (which reach up to 35 parasites) then the parasites over flow within the abdominal cavity of infected eels (Fig. 2-B, H). These results are in accordance with the previous investigation (11).

5. Hematological changes

Our results revealed that, there was microcytic hypochromic anemia in the eels infected with *Anguillicola crassus* which represented by a reduction in erythrocytic count, hemoglobin concentration and packed cell volume (PCV). In addition there was increase of mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and mean corpuscular

volume (MCV) (Table 5). This may be due to *Anguillicola crassus* suck the blood from blood vessels of swim bladder of infected eels. Our results agree with the results previously recorded (33,34).

6. Histopathological findings

The swim bladder of eels infected with *Anguillicola crassus* containing numerous worms which their guts filled with blood and surrounded by thickened swim bladder wall (Fig. 3 – A,B, C). The mucosa of the swim bladder showed either hyperplasia and proliferative changes inside the lumen of the swim bladder or desquamated forming erosive or ulcerative lesions, oedema, hemorrhage and congested blood vessels beside few leukocytes could be seen in the remaining layer of swim bladder (Fig. 3-D). Moreover, mononuclear cells and eosinophilic granular cells and proliferative capillaries could be seen in all coats of the swim bladder wall. Sometimes fibroblasts proliferation could be seen particularly in the muscular coat (Fig. 3-E). Moreover, developing larvae usually seen in swim bladder lumen, submucosa and serosa. Some larvae became degenerated and necrotic and surrounded by fibrous tissue. Single or multiple cysts could be recognized within the wall of swim bladder. The liver of infected eels showing degenerated hepatic cells (Vascular and hydropic) beside mild perivascular fibrosis (Fig. 3-F). These findings are nearly similar to that previously reported by several investigators (27, 31,35,36).

7. Treatment trials

7.1. Treatment of eels infected with *Anguillicola crassus* using levamisole

Our results revealed that, adult *Anguillicola crassus* nematode showed paralysis and death 24 hours after medical bath with 10mg/L and 20mg/L levamisole. While L₃ larvae still alive in the swim bladder wall (Table 6). Our results come in accordance with previously recorded (37-39). Levamisole kills *Anguillicola crassus* adult in swim bladder of infected eels, but not kill the L₃ larvae which found in swim bladder wall (13).

7.2. Treatment of eels infected with *Anguillicola crassus* using humate substance

It was observed that, the survivability of humate treated group all over the experiment was 93.3% while that of untreated group was 84.0%. The prevalence of *Anguillicola crassus* of humate treated groups was 18.6% while that

of untreated groups was 100 %. The intensity of infection of humate treated group was 2.3 worms per infected eel, while the intensity of infection in infected untreated group was 8.5 worms per infected eels (Table 7). These results indicated the curative ability of humate substance in treatment of *Anguillicola crassus* infection in eels (40).

Table 2. Prevalence and mean intensity of *Anguillicola crassus* infected eels at different localities.

Parameters Locality and water supply	Water quality			No. of examined eels	No. of infected eels	Prevalence (%)	Mean intensity	Total number of parasites
	Average pH	Average salinity (ppt)	Average Dissolved O ₂ (mg/l)					
Al-Salam channel Hussinia district (Fresh water)	6.9	0.2	8.1	186	154	82.8	7.48	1152
Al-Gohhr area Manzala lake (Brackish water)	7.5	5.4	4.1	204	152	74.5	5.34	812
Al-Gameel area West Port –Said (Marine water)	7.7	39.8	2.14	106	64	60.4	4.20	269
Bahr El-Bakar Shader Azzam*	8.1	1.2	5.6	72	57	79.2	6.88	392
Total				568	427	75.2	6.15	2625

* The water supply to this area is polluted with sewage.

Table 3. Seasonal prevalence and mean intensity of *Anguillicola crassus* in infected eels.

Items	Summer	Autumn	Winter	Spring
No. of examined	179	113	166	110
No. of infected	163	91	92	81
Prevalence %	91.06	80.53	55.42	73.63
Mean intensity	8.02	5.15	3.81	6.13
Total number of parasites	1308	469	351	497

Table 4. Prevalence and mean intensity of *Anguillicola crassus* infection in relation to the size of eels.

Items	Length (cm)				
	Less than 30	30-40	40-50	50-60	More than 60
No. of examined	62	315	113	43	35
No. of infected	44	285	68	19	11
Prevalence %	70.96	90.47	60.17	44.19	31.42
Mean intensity	2.40	6.29	6.57	8.52	10.45
Total number of parasites	106	1795	447	162	115

Table 5. Some hematological changes in the eels infected with *Anguillicola crassus*.

Group	Parameters	RBCs	Hb	PCV	MCV	MCH	MCHC
		10 ⁶ /μl	gm/dl	%	FI	Pg	%
Control		2.18±	9.18±	23.85±	109.40±	42.11±	38.49±
		0.10	0.41	0.94	4.72	1.89	1.54
Infected		**	**	*	*	*	*
		1.41±	7.94±	17.86±	126.67±	56.31±	44.46±
		0.07	0.40	0.98	5.81	3.10	2.18

* Significant at P <0.05

** Significant at P <0.01

Table 6. Treatment of eels infected with *Anguillicola crassus* using levamisole.

Group N=10	Item	Worms survivability and condition	
		Adult worms	L ₃ larvae (infective stage)
Control (untreated)		Live	Live
Levamisole water bath (10 mg/L/24hrs)		Paralysis and death	Live
Levamisole water bath (20 mg/L/24hrs)		Paralysis and death	Live

Table 7. Survivability, prevalence and mean intensity in humate treated eels.

Group N=75	Item	No. of eel at the end of experiment	Survivability %	No. of infected eel at the end of experiment	Prevalence %	Mean intensity
		Control (untreated)	63	84.0	63	100
Treated eels (2g/kg food/10 months)	70	93.3	13	18.6	2.3	

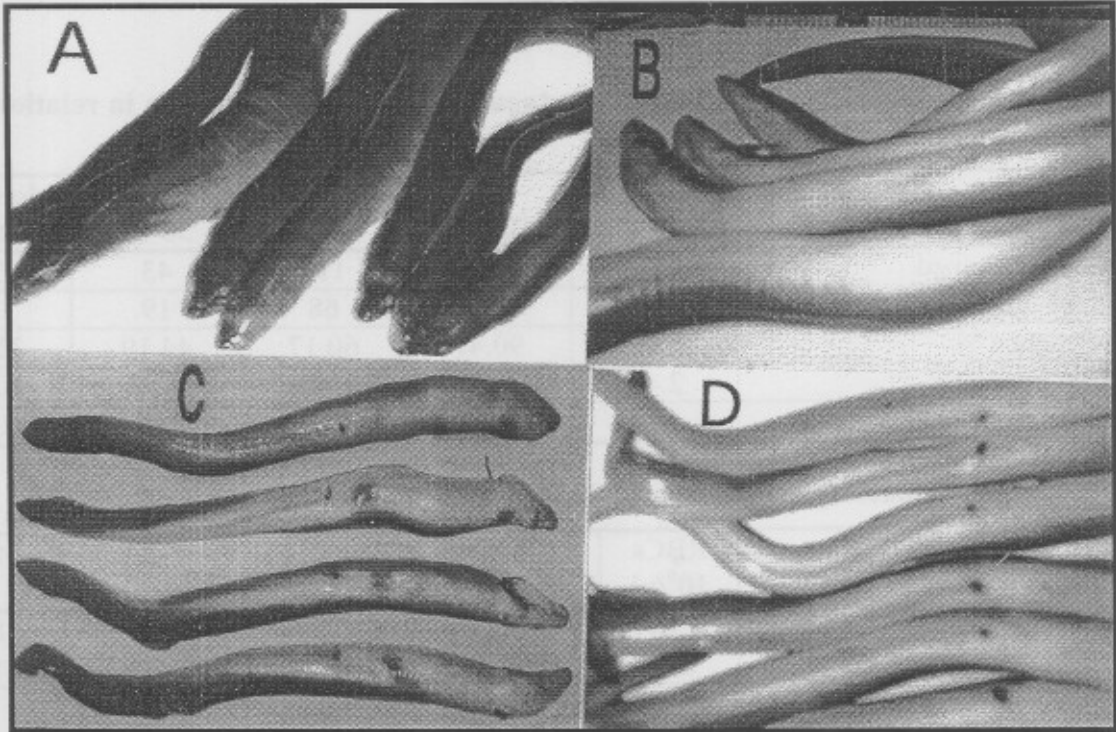


Fig. 1. Healthy eels (A and B) . Eels infected with *Anguillicola crassus* showed distended abdomen, yellow orange and pink hyperemic anus (C) . Eels infected with *Anguillicola crassus* showing pink and red hyperemic anus (D).

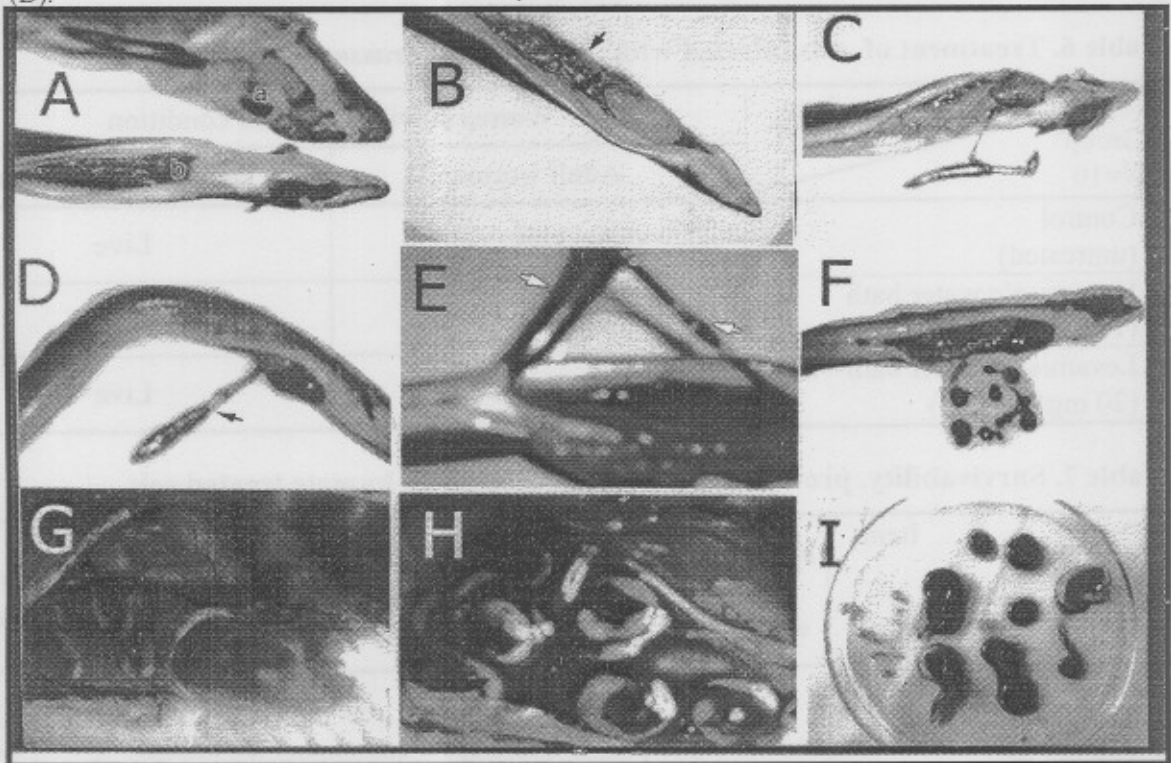


Fig.2. Eel infected with *Anguillicola crassus* show splenomegaly (Aa) in relation to normal spleen size in non infected eel (Ab). Ruptured swim bladder and swimming of the worms in the body cavity (B & C). Inflamed pneumatic duct and intact thickened wall swim bladder filled with worms (D). Swim bladder and pneumatic duct showed inflammation, hyperemia and filled with worms (E) . Worms of *Anguillicola crassus* after ruptured of swim bladder (F). Inflamed hemorrhagic swim bladder engorged with worms (G). Ruptured swim bladder with release of worms in body cavity (H) . *Anguillicola crassus* worms in Petri dish (I).

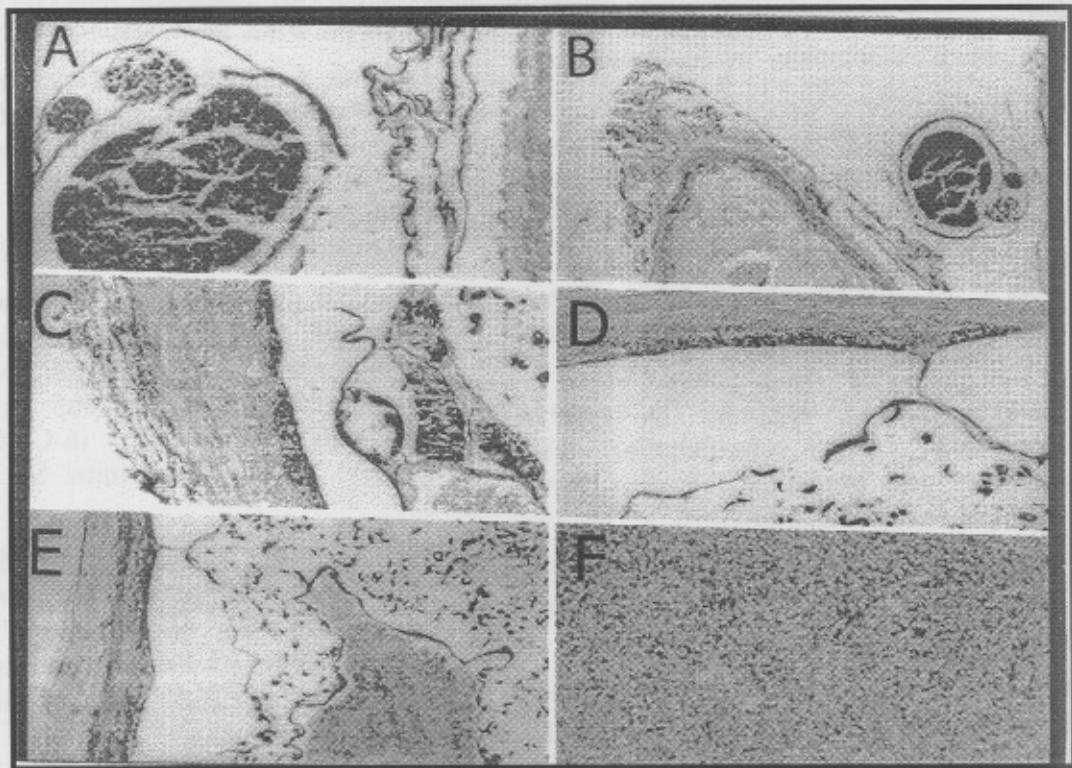


Fig. 3. Swim bladder containing cross sections of *Anguillicola crassus* surrounded with hyperplastic epithelium of swim bladder, H & E x 50 (A). Oblique section in the *Anguillicola crassus* within the cavity of swim bladder, H & E X 30 (B). Longitudinal section in the *Anguillicola crassus* surrounded with oedematous and hemorrhagic layers of swim bladder, H & E X 80 (C). *Anguillicola crassus* containing larvae surrounded by inflamed swim bladder wall, H & E X 50 (D). *Anguillicola crassus* containing numerous larvae surrounded by inflamed mucous membrane of swim bladder, H & E X 50 (E). Liver of infected eel showing degenerated hepatocytes, H & E X 80 (F).

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الملخص العربي

حدوث الأنجويليكولا كراسس (نيماتودا، دراكانكيوليدى) طفيل أسماك الثعابين الاوربية
(أنجويلا أنجويلا) فى مصر

جمال الدين صالح على ، جمال النوبى أحمد ، السيد عبده الدسوقى
قسم أمراض ورعاية الأسماك – كلية الطب البيطرى- جامعة الزقازيق

أستخدم عدد ٥٦٨ من أسماك الثعابين (أنجويلا أنجويلا) تم تجميعهم من أماكن مختلفة خلال فصول السنة المختلفة لدراسة انتشار طفيل الأنجويليكولا كراسس وتأثيره على صحة أسماك الثعابين. بالإضافة إلى ١٨٠ من أسماك الثعابين المصابة بطفيل الأنجويليكولا كراسس تم استخدامهم لمحاولات العلاج باستخدام الليفاميزول ومادة الهيومات.

أوضحت نتائج الدراسة مايلى:

- ١- أن نسبة انتشار الأنجويليكولا كراسس فى أسماك الثعابين المختبرة كان ٧٥,٢% مع متوسط كثافة ٦,١٥ دودة فى السمكة المصابة.
- ٢- وجد أن أعلى نسبة انتشار (٨٢,٨%) لوحظت فى قناة السلام والتي تحتوى على مياه عذبه.
- ٣- كما وجد أيضاً أن أعلى نسبة إنتشار (٩١,٠٦%) كانت خلال فصل الصيف.
- ٤- أن أسماك الثعابين الصغيرة (أقل من ٤٠سم) سجلت أعلى نسبة إنتشار (٩٠,٤٧%).
- ٥- أظهرت أسماك الثعابين المصابة سلوك غير طبيعى عن طريق تعليقها قرب سطح الماء ، تورم فى البطن، أحمرار فتحة الشرج ، تخانة جدار المثانة الهوائية مع تليفها وامتلائها بالديدان لتعطى شكل النقانق (السوسيج).
- ٦- وجد هناك تغييرات واضحة فى إختبارات الدم والأنسجة لاسماك الثعابين المصابة.
- ٧- وجد أن الليفاميزول (١٠-٢٠ ملجرام /لتر / لمدة ٢٤ ساعة) ومادة الهيومات (٢ جرام /كيلوجرام عليقة / لمدة ١٠ شهور) لهما تأثير فعال فى علاج أسماك الثعابين المصابة.