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Field Studies on *Anguillicoliasis* in Cultured Freshwatewr (*Anguilla anguilla*) with Emphasis to Treatment Trails

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Abstract: The present study deal with 120 cultured *Anguilla anguilla* in Egypt to clarify the clinical pictures. Any divergence from normal to red colouration could be used as a diagnostic tool of *Anguillicoliasis*. Also, prevalence and intensity of *A. crassus* were recorded. Histopathological changes in swimbladder of infected *Anguilla anguilla* were done. *Anguillicola crassus* infection was more prevalent in small sized *Anguilla anguilla* than larger ones, while larger *Anguilla anguilla* harbored more parasites than smaller ones. In addition, trials for treatment of 150 cultured infected *A anguilla* with humates and Levamisol HCl. The survivability of the humates treated group at 70 and 135 g were 92 and 96 % respectively and that treated with levamisol HCl group at 70 and 135 g were 84 and 92 % respectively was 80%.While, the control group at 70 and 135 g were 60 and 80% respectively. The prevalence of *A. crassus* infection in humates treated group at 70 and 135 g were 30 and 23 % respectively and that treated with levamisol HCl group at 70 and 135 g were 30 %.While, the control group at 70 and 135 g were 70 %. In this study, *Anguillicoliasis* can be safely treated using humates than levamisol HCl.

Key words: *Anguillicoliasis* · *Anguilla anguilla* · Swim Bladder · Humates · Levamisol Hcl

INTRODUCTION

In Egypt, *Anguilla anguillla* is one of high commercial value in the cultured freshwater fish farms. It is characterized by tolerance of variation in temperature, salinity and high stocking densities which make it suitable species for culture [1]. On the other hand, it is sensitive to environmental stresses (parasitic, fungal and bacterial diseases) which are responsible for lowering survival and for higher costs in prevention and cure [2]. *Anguillicola crassus* infections may cause serious losses in *Anguilla anguilla* production [3,4]. It was suggested that infection with this parasite may soon be widespread in natural basins in Europe and North Africa [5,6]. In fact, this nematode has been reported in Egypt for the first time in 1989 via the live exported *Anguilla anguilla* fish from Egypt to Germany for the food trade [7,8]. Parasite-induced damage to the swim bladder inhibited vertical migrations and infected *A anguilla* tended to migrate in shallower waters, relatively close to the shore [9]. Infected *A anguilla* showed mild to severe degenerative, necrotic and inflmatory changes of swim bladder [10]. Two ways of controlling *anguillicoliasis* were proposed. First way, prophylactic methods were used, where by bodies of water is treated to eliminate the intermediate hosts (copepods) so that the life cycle of the parasites cannot be completed. Secondly, therapeutic treatment of infected *Anguilla anguilla* with anthelmintics was considered [2,11].

This study was performed for recording the prevalence and intensity of *Anguilla crassus* infection by *Anguilla anguilla* in relation to seasons and body size. Besides, study of clinical picture and histopathological changes in gas bladder and investigation of the efficacy of humates and levamisole HCl as trials for treatment of *Anguilla anguilla* infected with *A. crassus* reared and cultured in freshwater fish farms, Egypt.

MATERIALS AND METHODS

Fish for Examination: A total of 120 freshwater *Anguilla anguilla* were collected from different fish farms

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(earthen ponds) seasonally (2009-2010). Sixty *A anguilla* (30 in spring and 30 in summer seasons) with average body weight 70 g. Also, 60 *A anguilla* (30 in autumn and 30 in winter) with average body weight 135 g. The fish were examined for worm infection seasonally and size of eel fish.

Fish Used for Treatment: A total of 150 *A anguilla* were collected from the same fish farms (75 average body weight 70g in spring and 75 eels 135 g in winter seasons) used for trials of treatment by humates and Levamizole. 70 g *A anguilla* were divided into 3 groups, each of 25. Groups 1 and 2 were treated and group (3) kept as a control group. Also, 135 g *A anguilla* were divided into 3 groups, each of 25. Groups 1 and 2 were treated and group (3) remained as a control group. Finally, trials were examined to detect the percentage of survivability, then dissected and record infection.

Diet Preparation: The diet was formulated from fish meal, meat meal, soybean meal, corn flour, wheat bran, beside vitamins and mineral mixtures. Diet contained 42% crude protein and 3150 kcal/kg mtabolizable energy and all ingredients were finally ground to a size less than 1mm that could apprehended by fish easily, the ingredients were then weighted out according to the formulation, thoroughly mixed then water and oil were added to the dry ingredients mixture shortly before feeding to form a paste, then the feed used quickly after preparation. The diet was placed in a feeding basket that hanged close to the water surface at a fixed feeding place with two fixed times daily at a rate of 5% of body weight according to Dosoky [2].

Drugs Used for Trial for Treatment: Humates substance: In the form of Biofarm dry (one kg), produced by Pharmavet Company, Turkey. It was used as1g/kg ration according to Dosoky [2].

Levamisol HCl: In the form of powder (one kg),produced by ADWIA Company, Egypt. It was used as 1g /10kg ration according to Koops and Hartman [7], who used of Levamisol HCl 10% for Anguilla *anguilla* treatment by the force-feeding experiment, the given dosage was based on the prescription for farm animals.

Clinical Examination: The collected fish were examined externally and postmortem using the methods described by Lucky [15] paying special attention to the, gas bladder, stomach, intestine and abdominal cavity.

Parasitogloical Examination

Isolation and Identification of Nematode: The nematode after being recovered were washed in saline solution and kept in refrigerator for killing and stretching. Worms were treated with 70% alcohol and 5% glycerol. After that for best clearing they were kept in lactophenol for 48 hours, mounted in polyvinyl alcohol, then microscopically examined for their morphological characteristics according to Lucky [15].

Histopathological Examination: Specimens from the infected *Anguilla anguilla* fish were collected including infected swim bladder of eels and fixed in 10% formol saline for twenty four hours.Washing was done with tap water then serial dilutions of alcohol (70% and absolute ethyl) were used for dehydration. Specimens were cleared in xylene embedded in paraffin at 56 degree in hot air oven for twenty four hours. Paraffin bees wax tissue blocks were prepared for sectioning at 4 microns thickness by slidge microtome. The obtained tissue sections were collected on glass slides, deparaffinized, stained by Hematoxylin and Eosin and examined microscopically [16].

Statistical Analysis: The results of infections were statistically analyzed using method of [17].

RESULTS

Clinical signs: The infected *Anguilla anguilla* showed loss of appetite and vitality, inverted swimming, retard in growth rate, absence of escape reflex, increase mortality rate, swollen and distended abdomen, the anal opening showed yellow-orange, pink or red coloration (characteristic sign) Fig. (1).

Postmortem Findings: As shown in Fig. (2). the worms filled the swim bladder giving a picture of a case engorged with worms (sausage like).The swim bladder of *A. carssus* infected *Anguilla anguilla* showed numerous different sizes (Fig. 3). The worms appeared from outside the intact swim bladder which looks like dissolved chocolate Fig. (4). The separated worms appeared in Petri dish with different size Fig. (5). The large worms appeared over 1cm (Fig. 6).

Prevalence Rate and Intensity in Relation to Seasonal Occurrence of Cultured *a Anguilla*: As shown in a Table (1), the total prevalence of *A. crassus* infection in cultured *A anguilla* was 54.1% and mean intensity was

Table 1: Seasonal occurrence of *Anguuillicola crassus* infection in cultured *Anguilla anguilla.*

Fish	Spring	Summer	Autumn	Winter	Allover the year
No. of examined	30	30	30	30	120
No. of infected	20	25	15	5	65
Prevalence %	66.6	83.3	50	16.6	54.1
Mean intensity	3±0.02	2±0.02	3±0.02	8±0.5	4.2±0.8
Chi^2			16.24*		

Means within the same row of different litters are significantly different at ($P < 0.05$). * = Significant at ($P < 0.05$)

Table 2: Prevalence and intensity of *Anguillicola crassus* infection in cultured *Anguilla anguilla*.

Anguilla anguilla	70 g	90 g	110 g	135 g
No. of examined *Anguilla anguilla*	30	30	30	30
No. of infected	20	25	15	5
Prevalence %	66.6	83.3	50	16.6
Mean intensity	3	2	3	8

Table 2: Prevalence, survivability and mean intensity in humates & Levamisole HCl treated groups in relation to control untreated.

Anguilla anguilla fish	No. at start 70g	No. of infected Anguilla anguilla	Prevalence %	No. of fish at the end of exp.	Survivability %	No. of infected Anguilla anguilla at the end of exp. 135g	Prevalence %	No. of fish at the end of exp.	Survivability %
Control	25	17	70	15	60	25	70	20	80
Eels treated with humates	25	8	30	23	92	25	23	24	96
Eels treated with Levamisol HCl	25	8	30	21	84	25	30	23	92
	$Chi^2 = 6.55^*$					$Chi^2 = 6.78^*$			

* = Significant at ($P < 0.05$)

Fig. 1: Shwing red colouration in anal opening of diseased eel. X20

Fig. 2: Showing *Anguilla anguilla* engorged swim bladder sausage like.x20

Fig. 3: Showing worms of *A. crassus* after ruptured of swim bladder *Anguilla anguilla*. x20

Fig. 4 : Showing ruptured swim bladder and swimming of the worms in the body cavity, inflamed liver and congestion of intestine.x20

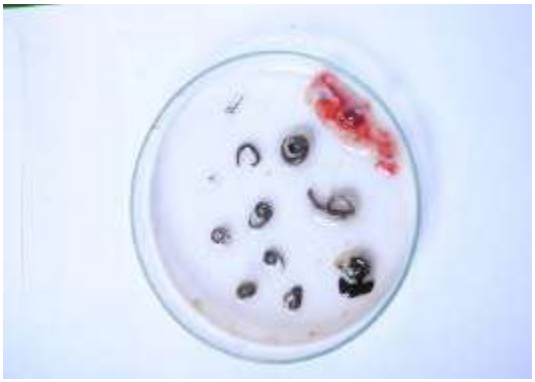

Fig. 5: Showing *Angiullicola crassus* worms in Petri dish.x20

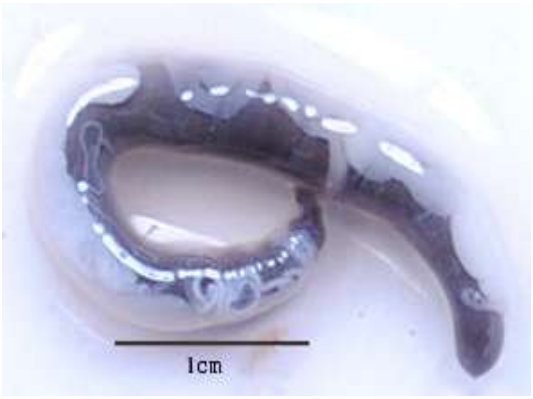

Fig. 6: Showing large *Angiullicola crassus* worms.x80

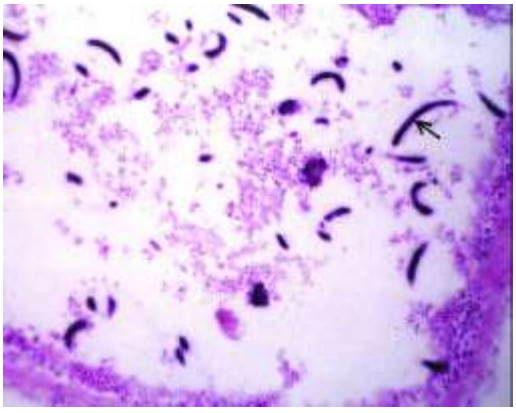

Fig. 7: Showing cross section of infected swim bladder with *A. crassus* surrounded with hyperplasitic epithelium of swim bladder. H&E x20.

4.2±0.8 parasites. Also, it was detected that the summer season showed highest prevalence rate (83.3%),followed by spring (66.6%) and autumn (50%) while the lowest in winter season (16.6%).

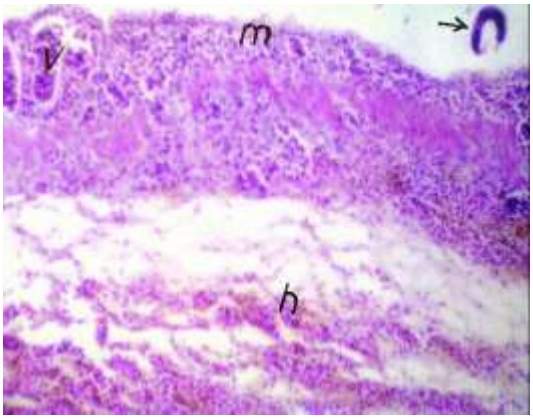

Fig. 8: Showing cross section in the *A. crassus* surrounded with edematous (m) and hemorrhagic layers of swim bladder (h). H&E x 20.

Prevalence and Intensity of Infection in Relation to Body Weight of Cultured *Anguilla Anguilla*: As shown in Table (2), the higher prevalence level observed at 90g body weight was 83.3 % while the lower level observed at 135g body weight was 16.6 %. In contrast the mean intensity of infection increase as the *Anguilla anguilla* body size increased which was the lowest 2 worms per infected in the 90g size eels while it was highest (8 worms) in the 135g *A anguilla*.

Histopathological Findings: As shown in Fig (7); the swim bladder of infected *Anguilla anguilla* containing numerous of parasitic worms with desquamated cells.Moreover, severe congestion in the mucosal layer and hemorrhagic layers of swim bladder Fig (8).

Treatment Trials: As shown in Table 2 the survivability of humates& Levamisol treated groups allover the breading period was 96 and 90 % respectively. While that of untreated group was 80 %.

DISCUSSION

In the present study, the infected *Anguilla anguilla* showed loss of appetite, abnormal behaviour by hanging near the surface, collected near water inlet, retard of growth, increase mortality rate and the anal redness as a characteristic diagnostic feature and an indicator of infection by *A. crassus*. These results were in agreement with Dosoky [2]. The postmortem lesions of *A. carssus* infected *A anguilla*, revealed that the swim bladder of *A. carssus* infected eels as sausage like appearance from outside the intact swim bladder and filled with worms of

A. carssus. The swim bladder wall in some infected *A anguilla* showed thickening and extreme inflammation, it had become markedly enlarged and hyperemic, pneumatic duct showed inflammation. In severe cases, the bladder was dilated and its wall became thickened, opaque and showed signs of inflammation. These results were in agreement with Mohamed and Nouh and Liewes and Haenen [18,19].

The prevalence rate of infection in cultured *Anguilla anguilla* during summer season was 88.3 % while in winter season 16. 6%, it may attributed to the times of low temperature when *Anguilla anguilla* are in quiescent period and off food, the number of larvae could decrease as no new infections should take place and increased during summer [2,20].

Concerning *A. crassus* infection in relation to body size in cultured *Anguilla anguilla,* it was reported that the high prevalence of *A. crassus* infection in *A anguilla* of 70 and 90g size were 66.6 and 83.3 % respectively. While the prevalence in the at 110 and 135 g were 50 and 16.6% respectively, it may attributed to the crustaceans as intermediate host serve as a source of feeding and infection for smaller *Anguilla anguilla* than larger one. These results nearly agree with that observed by Abdallah and Maamouri and Kirk and Schabuss *et al.* [6, 21, 22]. Also, mean of intensity decrease in small eels and increase in large eels. These results may be attributed to maturation of non detected worms in swimdladder. These findings were nearly similar to that obtained by Abdallah and Maamouri [6].

Regarding the histopathological changes in the swimbladder in infested eels due to *A. crassus*, containing numerous worms having their guts filled with blood, inside the lumen and surrounded by thickened swimbladder wall. These results may be attributed to regular blood sucking and adverse reaction occurred by adult worms and larvae of these worms. These findings were nearly similar to that obtained [23,24,25]. The swimbladder showed both hyperplasia and proliferative changes of the mucosa inside its lumen, oedema, hemorrhage and congested blood vessels, beside few leukocytes could be seen in the remaining layers of swimbladder. These results may be attributed to continuous irritation of swimbladder mucosa by adult worms. These findings were nearly similar to that obtained by Dosoky and Mohamed and Nouh and Sokolowski and Dove [2,19,26].

Concerning to the trials for treatment of *Anguillicola crassus* infected *Anguilla anguilla* (70g) at spring season was found that the prevalence30,30 and 70% for eels treated with humates, Levamizol and control untreated group respectively. Also, survivability of the same fish was 92,84and 60% respectively. While, *Anguillicola crassus* infected *Anguilla anguilla* (130g) at winter season t the prevalence were 23,30 and 70 % for eels treated with humates, Levamizol and control untreated group respectively. Also, survivability of the same fish was 96, 92and 80% respectively. These results indicated that the curative ability of the humates substance in treatment of *Anguillicola crassus* infection in *Anguilla anguilla*. These result in agreement with Meinelt *et al.* [27] who reported the ability of humates substance in treatment of parasitic infection of fish and safer on infected *Anguilla anguilla* than chemical anthelmintic. Chemical treatment is often associated with side effects that include parasite resistance undesired drug residues, host damage and pollution [14].

From the present investigation it was concluded that any divergence from normal to red colouration could be used as a diagnostic tool of *Anguillicola. crassus* infection. Also, young or small sized *Anguilla anguilla* was more susceptible than larger ones, while larger *A anguilla* harbored more parasites than smaller ones. Also, *Anguillicoliasis* can be safely treated using humates than Levamesol.

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