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# Some Studies on *parasitic Anguillicoliosis* in wild Anguilla Anguilla off springs in Lake El Broullus, Egypt

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The present study deal with 240 *Anguilla Anguilla* offspring collected from Lake El Broullus in Egypt and it was conducted to clarify the clinical pictures. Anal divergence from normal to red colouration may used as a diagnostic tool of Anguillicoliasis. Also, prevalence, abundance and intensity of *Anguillicola crassus* was recorded. trials for treatment of 300 wild infected *A. anguilla* was with *Artemsa vulgarsim* and Levamisol HCl (ADWIA Company) . The survivability of the *Artemsa vulgarsim* treated group at 35 and 75 g size were 92 and 96% respectively and that treated with levamisol HCl group at 35 and 75 g size were 84 and 92% respectively. While, the control group at 35 and 75 g size were 60 and 80% respectively. The prevalence of A. crassus infection in Artemsa vulgarsim treated group at 35 and 75 g size were 30 and 23% respectively and that treated with levamisol HCl group at 35 and 75 g size were 30%. While, the control group at 35 and 75 g size were 70%. In this study, Anguillicoliosis can be safely treated using Artemsa vulgarsim without any side effects. In this study, Anguillicoliosis can be safely treaded using Artemsa vulgarsim than Levamisol HCl.

**Key words:** Anguillicoliasis, Anguilla anguilla, swim bladder, Artemsa vulgarsim, levamisol HCl.

## Introduction

*Anguilla anguillla* offsprings aremigrated from the area of reproduction to Egypt. They are characterized by their tolerance of variation in temperature and salinity (**Noor El Deen *et al.*, 2012**). On the other hand, they are sensitive to parasitic , fungal and bacterial diseases which are responsible for lower survival and for higher costs in prevention and cure (**Dosoky, 2007**). infections may cause serious losses in *A. anguilla* production ( **Pilecka andSobecka *Anguillicola crassus*, 2004**). It was suggested that infection with this parasite may soon be widespread in natural basins in Europe and North Africa (**Maillo et al., 2005; Abdallah and Maamouri, 2006**). In fact, this nematode has been reported in Egypt for the first time in 1989 via the live exported *A. anguilla* fish from Germany to Egypt for the food trade (**Koops and Hartman, 1989**). The number of helminths and the amount of exudates and debris present in the swim bladder lumen are also important factors for evaluating the severity of infection (**Lefebvre et al., 2002**). Parasitic infection levels were significantly a negative correlation with age and positive correlation with abundance and mean of intensity (**Heitlinger et al., 2009**). 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(**Sjoberg, 2009**).

In the first way, prophylactic methodswere used, where by bodies of water in fish culture is treated to eliminate the intermediate hosts (copepods) so that the life cycle of the parasites cannot be completed. Therefore, therapeutic treatment of infected *A. Anguilla* with anthelmintics was Levamisol Hcl (**Taraschewski et al., 1988**). This study was performed for recording the prevalence and intensity of A. crassus infection in A. anguilla in relation to seasons and body size, in addition to study the clinical picture and investigation of the efficacy of *Artemsa vulgarsim* as natural product and levamisole HCl as a chemical product as trials for treatment of *A. anguilla* infected with *A. crassus* reared and wild in freshwater fish farms, Egypt.

## Materials and Methods

### Fish Sampling For Clinical Examination

A total of 240 freshwater *A. anguilla* were collected from Lake Al Brollus seasonally (2013 to 2014), 120 *A. anguilla* (60 in spring season and 60 in summer season) with average body weight of 35 g and 120 *A. anguilla* (60 in autumn season and 60 in winter season) with average body weight of 75 g. The fish were examined according to Lucky,1977 for prevalence of worm infection according to season and size of eel fishes with detection of clinical syndrome.

### Experiment For Drug Applications

A total of 240 *A. anguilla* were collected from the same fish farms in plastic aquarium with penetrated cover 50*70*30 cm(120 *A. anguilla* with average body weight of 35 g in spring season and 120 *A. anguilla* average body weight of 75 g in winter season) used for trials of treatment of infected *A. anguilla* by *Artemsa vulgarsimsubstance* and Levamizole Hcl .All fishes were fasted before the trial treatment for 24 hours. *A. Anguilla* (35 g) were divided into 3 groups, each of 50 *A. anguilla*. Groups 1 and 2 were treated and group 3 remained as a control group. Also, *A. anguilla* (75 g) were divided into 3 groups, each of 50 *A. anguilla*. Groups 1 and 2 were treated and group 3 remained as a control group. The fish at the end of trial were counted to detect the percentage of survivability, then dissected and infection was recorded.

The experiment was done in the aquaria after adjustment the temperature ,PH, salinity and ammonia .The treatment was applied for two weeks.

### 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 3300 kcal/kg mtabolizable energy and all ingredients were finally ground to a size less than 1 mm that could be apprehended by fish easily, the ingredient 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 was used quickly after preparation. The diet was placed in a feeding basket that was 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 **Noor El Deen *et al*., 2012**.

**Drugs used for trial of treatment Artemsa vulgarsim substance**

In the form of Biofarm dry (1 kg) produced by Pharmavet Company, Turkey. It was used as 1 g/kg ration according to **Noor El Deen *et al*., 2010**.

**Levamisol HCl**

In the form of powder (1 kg) produced by ADWIA Company, Egypt. It was used as 1 g/10 kg ration according to **Geets et al. (1992)** who used Levamisol HCl 10% for *A. anguilla* treatment by the force-feeding experiment, the given dosage was based on the prescription for farm animals.

**Clinical examination**

The 240 fishes collected were examined externally and postmortem using the methods described by **Lucky (1977)** paying special attention to the swim 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 a 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 h and then mounted in polyvinyl alcohol, then microscopically examined for their morphological characteristics according to **Lucky (1977)**.

**Statistical analysis**

The results of infections were statistically analyzed using method of**Maddison and Maddison (2008)**.

## Results and Discussion

In the present study, The infected *A. Anguilla* offspring showed loss of vitality, inverted swimming, absence of escape reflex , swollen and distended abdomen, ,abnormal behaviour by hanging near the surface, collected near water surfaces ,increase mortality rate percent tand the anal opening showed yellow-orange, pink or red coloration was its characteristic sign (Figure 1). The swim bladder of *A.carssus* infected *A. anguilla* showed numerous different sizes. The worms filled the swim bladder giving a picture of a case engorged with worms (sausage like). The worms appeared from outside the intact swim bladder and showing *A. crassus* containing contents which was mainly blood of infected *A. anguilla* which looks like dissolved chocolate (Figure1and 2).These results were inagreement with **Noor El Deen et al.,2012**. The postmortem lesion of *A. carssus* infected *A. anguilla,* it was observed that the swim bladder of *A. carssus* infected *A. anguilla* as sewage 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, the

swim bladder had become markedly enlarged and hyperemic, pneumonic 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 **Liewes and Hanen (1995)** and **Mohamed and Nouh (2004)**.

The total prevalence of *A. crassus* infection in wild *A. anguilla* was 54.1% and mean intensity was 4.2 parasites. Also, it was detected that the summer season showed highest prevalence rate (83.3%), followed by that in spring (66.6%) and autumn (50%) while the lowest was in winter season (16.6%) ,Table 1. This may be attributed to the times of low temperature when *A. 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 (**Wurtz et al. , 1998; Dosoky, 2007**).

Concerning *A. crassus* infection in relation to body size in wild *A. anguilla*, it was reported that the higher prevalence level observed at 35 g body weight was 83.3% while the lower prevalence level observed at 75 g body weight was 16.6%. In contrast, the mean intensity of infection increase as the *A. anguilla* body size increased which was the lowest 2 worms per infected *A. anguilla* in the 35 g size eels while it was highest (8 worms) in the 75 g sized *A. Anguilla* , Table 2.. This may be attributed to the crustaceans as intermediate host serve as a source of feeding and infection for smaller *A. anguilla* than larger ones. These results are in agreement with that observed by **Kirk (2003), Schabuss et al. (2005)** and **Abdallah and Maamouri (2006).** 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 swimbladder. These finding was nearly similar to that obtained by **Herzig (2005)** and **Abdallah and Maamouri(2006)**.

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Concerning the trials for treatment of *A. crassus* infected *A. anguilla* (70 g) at spring season was found that the prevalence 30, 30 and 70% for eels treated with

*Artemsa vulgarism*, Levamizol and control untreated group respectively. Also, survivability of the same fish was 92, 84 and 60%, respectively. While, *A. crassus*

Infected *A. anguilla* (75 g) at winter season, the prevalence were 23, 40 and 10% for eels treated with *Artemsa vulgarsim*, Levamizol and control untreated group, respectively. Also, survivability of the same fish was 90, 82 and 62%, respectively. These results indicated that the curative ability of the *Artemsa vulgarsim* substance is in treatment of *A. crassus* infection in *A. anguilla*. These result in agreement with **Meinelt et al. (2001)** whoreported the ability of *Artemsa vulgarsim* substance in treatment of parasitic infection of fish and safer on infected *A. anguilla* than chemical anthelmintic. This finding was by **Taraschewski et al. (1988)** and **Hartman (1989)** who recorded that Levamisol is less effective in curing the diseased *A. anguilla*. Chemical treatment is often associated with side effects that include parasite resistance undesired drug residues, host damage and pollution (**Geets et al., 1992**).

From the present investigation we can concluded that any divergence from normal to red colouration around the anus accompanied with off food, sluggish movement, loss weight and listless could be used as a diagnostic tool of *A. crassus* infection. Also, young or small sized *A. anguilla* was more susceptible than larger ones, while larger *A. anguilla* harbored more parasites than smaller ones. Anguillicoliasis can be safely treated using *Artemsa vulgarsim* without any side effects.

**Table 1.** *seasonal occurrence of Anguuillicola crassus infection in wild Anguilla*

| **Fish** | **Spring** | **Summer** | **Autumn** | **Winter** | **All over the** |
|---|---|---|---|---|---|
| No. of examined | 60 | 60 | 60 | 60 | 240 |
| No. of infected | 40 | 50 | 30 | 10 | 130 |
| 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 $P < 0.05$.

| ***Anguilla anguilla*** | **35 g** | **35 g** | **75 g** | **75 g** |
|---|---|---|---|---|
| No. of examined *Anguilla anguilla* | 60 | 60 | 60 | 60 |
| No. of infected | 40 | 50 | 30 | 10 |
| Prevalence (%) | 66.6 | 83.3 | 50 | 16.6 |
| Mean intensity | 3 | 2 | 3 | 8 |

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

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**Table 3.** Prevalence, survivability and mean intensity in Artemsa vulgarism and Levamisol HCl treated groups for 10 days compared to control untreated group.

| **Anguilla Anguilla Start 70** | **No. of Fish 35 g** | **No. of infected *Anguilla anguilla*** | **Prevalence N** | **No. of fish at the end of exp.** | **Survivability N** | **No. of infected *Anguilla anguilla* at the end of exp. 75 g** | **No. of infected *Anguilla anguilla*** | **Prevalence N** | **No. of fish at the end of exp** | **Survivability N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **control** | **50** | **34** | **70** | **15** | **60** | **50** | **10** | **10** | **7** | **62** |
| **Artemsa vulgarism** | **50** | **16** | **30** | **23** | **92** | **50** | **23** | **23** | **30** | **90** |
| **Levamisol HCl** | **50** | **16** | **30** | **21** | **84** | **50** | **25** | **40** | **35** | **82** |

**Figure1**, Showing red colouration in anal opening of diseased eel and engorged swim bladder sausage like.x20

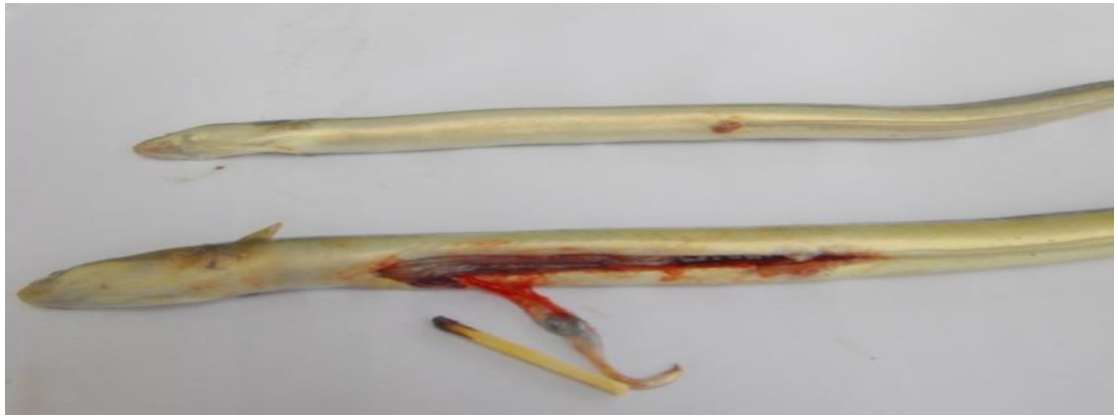
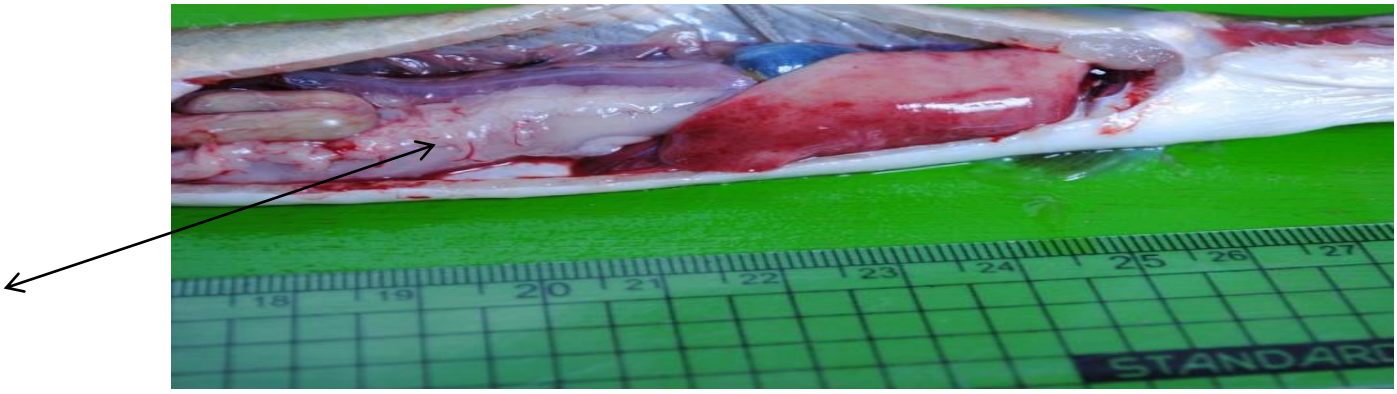


**Figure2,**Showing the worms in the body cavity, inflamed liver and congestion of intestine.x20



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