ROLE OF FRESHWATER FISHES IN THE EPIDEMIOLOGY OF SOME ZOONOTIC TREMATODES IN ISMAILIA PROVINCE

A. M. Abou-Eisha; R. E. Saleh; Hanaa, M. Fadel; *Eman, M. Youssef and Yosra, A. Helmy

Department of Hygiene, Zoonoses and Animal Ethology and *Department of Parasitology, Faculty of Veterinary Medicine, Suez Canal University, Ismailia, Egypt

ABSTRACT

A total of 517 freshwater fishes; 119 Oreochromis niloticus (Bolti), 69 Clarias gariepinus (Karmou Lazeer), 96 Chrysichthys auratus (Abo-Riala feddy), 97 Bagrus bayad (Baiad), 64 Ctenopharyngodon idella (Mabrouk Hashaash) and 72 Barbus binny (Benni Asseel) were collected from Nile tributaries during the period from March 2007 to February 2008 in Ismailia province. Parasitological examination of these fishes revealed that the prevalence rates of the encysted metacercariae (EMC) among the examined freshwater fishes were 74.8%, 87%, 78.1%, 74.2%, 73.4% and 76.4% in the aforementioned fish species respectively. Clinostomum tilapia metacercariae (Yellow Grub) were found in only two of the examined Oreochromis niloticus fish (1.7%). Generally, the highest infection rate with EMC among the examined freshwater fish was recorded in Summer (94.7%) followed by Spring (84%) and Autumn (79.8%), and the lowest infection rate was in Winter (47.6%).

Experimental infections of white rats and chickens with encysted metacer-cariae, recovered the adult trematodes of Heterophyes heterophyes, Heterophyes heterophyes nocens, Haplorchis pumilio, Metagonimus yokogawi, Pygidiopsis genata, Stictodora tanayensis, Prohemistomum vivax, Mesostephanus appendiculatus, Mesostephanus burmanicus, Mesostephanus milvi, Cyanodiplostomum azimi, Neodiplostomum spathula and Echinochasmus liliputans. Grilling of infected Oreochromis niloticus fish with encysted metacercariae for 15-20 minutes at 60-80 °C or freezing of these fishes for 72-96 hours at -10 °C was sufficient to destroy the encysted metacercariae. Current research indicated that freshwater fishes act as an important intermediate host for maintenance of these trematodes and a possibly source for infections of human and other fish eating animals with these parasites.
INTRODUCTION

Parasitic diseases are considered a serious problem in warm water fishes (Axelrod and Szieszke, 1980).

Fishes act not only as final host but also as intermediate host for larval stages of many parasites like encysted metacercariae of different species of trematodes, which affect fish causing retardation of growth especially for young fish and increasing the possibility of the secondary infections by decreasing the fish immunity (Ilan and lengy, 1963 and Elamei, 2001).

Infection of fish by encysted metacercariae of digentic trematode is due to the contamination of water resources as rivers, lakes and sea water with human, animals and bird settlements which harboring eggs voided in their feces, reaching the water stream which contain the specific intermediate snail host completing their life cycle (Elamei, 2001).

From the public health point of view, the consumption of fishes infected with the larval stages of some parasites caught from local water, constitutes a human hazard. Freshwater fishes are considered as one of the important sources of parasitic infection to man and fish eating mammals particularly after the increased pollution of rivers and lakes in Egypt (Mohamed, 1996). The World Health Organization (1995) has estimated that the number of people currently infected with fish-borne trematodes exceeds 18 millions, but worldwide the number of people at risk, including those in developed countries, is more than half a billion. In view of human population in the region relies heavily on freshwater fish as a source of dietary protein and the importance of fish parasites as human health hazard. The present study was undertaken to elucidate the prevalence of encysted metacercariae in freshwater fishes and the role of these fishes as a source of some zoonotic trematodes in Ismailia Province.

MATERIAL & METHODS

(1) Sampling:-

- Fresh water fishes:-

  A total of 517 freshwater fishes were collected during the period from March 2007 to February 2008 from Nile tributaries in Ismailia province. The collected freshwater fishes were 119 Oreochromis niloticus (Bolti), 69 Clarias gariepinus (Karmout Lazeer), 96 Chrysichthys auratus (Abo-Riala feddy), 97 Bagrus bayad (Baiad), 64 Ctenopharyngodon idella (Mabrouk Hashaash) and 72 Barbus binny (Benni Asseel).

2- Fish examination

- Parasitological examination

  Each fish sample was examined microscopically for the presence of
encysted parasitic larvae as described by Syme (1966).
Microscopical examination was carried out using compression technique as described by Morishita et al. (1965), for detection of EMC which were lodged in/or attached to different organs and tissues (gills, muscles, liver and gonads) and counting per gram of muscles. The recovered encysted metacercariae in the fish flesh were prepared for further study by applying the Tissue Digestion Method as described by Garcia (2001). Fixation, staining and mounting of the collected encysted metacercariae were done according to the technique described by Garcia (2001).

• Experimental infection
A total of 35 white rats (4 weeks old) and 35 chickens (15 days old) were used for experimental infection. These white rats and chickens were reared conventionally in separate groups in special cages where each cage contained a group of five animals. Their faeces were examined daily for seven successive days before experimental infection to ensure that they were parasite free. Of these white rats and chickens; one group of five white rats and other group of five chickens were left uninfected as negative control groups. The remaining groups of white rats and chickens were prepared for feeding on the infected fish flesh with EMC of the examined freshwater fishes as described by Mahmoud (1983).

The experimentally fed rats and chickens were put under observation for 15 days post-infection, and their feces was examined daily post-infection by direct examination and sedimentation techniques (Faust et al., 1976) for detection of the first appearance of parasitic eggs.

The experimentally fed birds and rats, which began to shed parasitic eggs, were sacrificed. The small intestine was opened, the content and scraped mucosa were collected in suitable jars containing normal saline. Washing with normal saline was carried out several times to remove the coarse particles of the intestinal contents and mucus that may be attached to the parasite. The sediments were examined using dissecting microscope. The trematodes were collected in normal saline then picked up in a small bottles containing 5% neutral buffered formaldehyde for preservation using Pasteur pipette. Fixation, staining and mounting of the collected adult trematodes were done according to the technique described by Garcia (2001).

3- Effect of grilling and freezing on the infectivity of the EMC
Twenty eight white rats were prepared as mentioned before to ensure that they were parasite free and were divided into 7 groups, each group was formed of four rats to test the effect of grilling, and freezing on the viability of EMC and one of them was control.
In the effect of grilling, the infected *O. niloticus* fishes ranged in size from 14-19 cm by 5-7 cm width were rolled with bran while be with scales and unevasciated then grilled at 60-80°C for different grilling periods 5, 10 and 15-20 minutes before feeding to experimental animals. While, in the effect of freezing, the same size of infected *O. niloticus* fishes were frozen to -10°C for 24, 48 and 72-96 hours then thawed before feeding to experimental animals (Raid, 1992 and Eissa et al., 2001).

All experimental rats of the previous groups were kept in wire cages and supplied with a source of clean water with wheat and bread. Feces of these rats were microscopically examined daily post-infection for detection of parasitic eggs. The rats were sacrificed in the period from 7th to 15th day post infection for detection of adult worms. The recovered adult worms were counted under the dissecting microscope. The fixation, staining and mounting of the adult worms was done as described by Garcia (2001). Statistical analysis was carried out using SAS (SAS, 1997).

**RESULT**

Parasitological examination of freshwater fish as shown in Table (1) and Figure (1) in this study, revealed that 398 (77%) out 517 examined fish were infected with encysted metacercariae with the highest infection rate was recorded in Summer (94.7%) followed by Spring (84%) and Autumn (79.8%) while the lowest infection rate was in Winter (47.6%). In *Oreochromis niloticus*, 89 (74.8%) out of 119 fish were infected, with the highest infection rate was in Summer (93.1%) followed by Spring (87.5%) and Autumn (71%), and the lowest infection rate was in Winter (44.4%). It was found that 60 (87%) out of 69 *Clarias gariepinus* were infected with EMC and the highest infection rates were recorded in Summer (100%), Autumn (93.3%) and Spring (90.9%) while the lowest one was in Winter (57.1%). In *Chrysichthys auratus*, 75 (78.1%) out of 96 fish were infected with the highest infections were recorded in Autumn (94.4%) and Summer (90.5%) followed by Spring (82.9%) and the lowest rate was in Winter (42.9%). In *Bagrus bayad*, 72 (74.2%) out of 97 fish were infected, with EMC with the highest infection rate was in Summer (95.7%) followed by Spring (76.9 %) and Autumn (76%) while the lowest rate was in Winter (47.8%). In *Ctenopharyngodon idella*, 47 (73.4%) out of 64 fish were infected with the highest infection rate was in Summer (94.7%) followed by spring (77.8%) and autumn (72.2%) and the lowest rate was in winter (50%). It was found that 55 (76.4%) out of 72 *Barbus binny* were infected.
with EMC, with the highest infection rate in summer (95.7%) followed by spring (85.7%) and autumn (81%) while the lowest infection rate was in winter (47.6%). Generally, there were no significant differences in the infection rates between the different fish species in the different seasons (P >0.05). However, there was highly significant difference in the totally infection rates with EMC among the examined freshwater fishes between the different seasons (P< 0.0001).

Fig. (1): Prevalence of encysted metacercariae among the examined fresh water fishes in Ismailia province according to season.
Fig. (2): Distribution of encysted metacercariae in different tissues of the examined fresh water fishes species in Ismailia Province.
Table (1): Prevalence of encysted metacercariae among the examined fresh water fishes in Ismailia province according to season.

<table>
<thead>
<tr>
<th>Fish types</th>
<th>Winter</th>
<th></th>
<th>Spring</th>
<th></th>
<th>Summer</th>
<th></th>
<th>Autumn</th>
<th></th>
<th>Total</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oreochromis niloticus</td>
<td>27</td>
<td>12 44.4</td>
<td>32</td>
<td>28 87.5</td>
<td>29</td>
<td>27 93.1</td>
<td>31</td>
<td>22 71</td>
<td>119</td>
<td></td>
</tr>
<tr>
<td>Clarias gariepinus</td>
<td>14</td>
<td>8 57.1</td>
<td>22</td>
<td>20 90.9</td>
<td>18</td>
<td>18 100</td>
<td>15</td>
<td>14 93.3</td>
<td>69</td>
<td></td>
</tr>
<tr>
<td>Chrysichthys auratus</td>
<td>21</td>
<td>9 42.9</td>
<td>35</td>
<td>29 82.9</td>
<td>21</td>
<td>19 90.5</td>
<td>19</td>
<td>18 94.7</td>
<td>96</td>
<td></td>
</tr>
<tr>
<td>Bagrus bayad</td>
<td>23</td>
<td>11 47.8</td>
<td>26</td>
<td>20 76.9</td>
<td>23</td>
<td>22 95.7</td>
<td>25</td>
<td>19 76</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>Ctenopharyngodon idella</td>
<td>18</td>
<td>9 50</td>
<td>9</td>
<td>7 77.8</td>
<td>19</td>
<td>18 94.7</td>
<td>18</td>
<td>13 72.2</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>Barbus binny</td>
<td>21</td>
<td>10 47.6</td>
<td>7</td>
<td>6 85.7</td>
<td>23</td>
<td>22 95.7</td>
<td>21</td>
<td>17 81</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>Grand Total</td>
<td>124</td>
<td>59 47.6</td>
<td>131</td>
<td>110 84</td>
<td>133</td>
<td>126 94.7</td>
<td>129</td>
<td>103 79.8</td>
<td>517</td>
<td></td>
</tr>
</tbody>
</table>

\[
\chi^2 = 0.850 \quad 2.346 \quad 1.99 \quad 6.727
\]

<table>
<thead>
<tr>
<th>P</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
<th>&gt;0.05</th>
</tr>
</thead>
</table>

Between the different seasons of totally infected fish species \( \chi^2 = 88.36 \) (P < 0.0001) (There is highly significant difference)
Table (2): Occurrence of encysted metacercariae in different tissues of the examined fresh water fishes species in Ismailia Province.

<table>
<thead>
<tr>
<th>Infected tissue</th>
<th>Fish species</th>
<th>EX. NO.</th>
<th>infected NO.</th>
<th>infected %</th>
<th>Muscle NO.</th>
<th>Muscle %</th>
<th>Liver NO.</th>
<th>Liver %</th>
<th>Gonads NO.</th>
<th>Gonads %</th>
<th>Gills NO.</th>
<th>Gills %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>Oreochromis niloticus</strong></td>
<td>119</td>
<td>89</td>
<td>74.8</td>
<td>89</td>
<td>74.8</td>
<td>33</td>
<td>27.7</td>
<td>18</td>
<td>15.1</td>
<td>43</td>
<td>36.1</td>
</tr>
<tr>
<td></td>
<td><strong>Clarias gariepinus</strong></td>
<td>69</td>
<td>60</td>
<td>87</td>
<td>60</td>
<td>87</td>
<td>51</td>
<td>73.9</td>
<td>23</td>
<td>33.3</td>
<td>Not done</td>
<td></td>
</tr>
<tr>
<td></td>
<td><strong>Chrysichthys auratus</strong></td>
<td>96</td>
<td>75</td>
<td>78.1</td>
<td>75</td>
<td>78.1</td>
<td>34</td>
<td>35.4</td>
<td>16</td>
<td>16.7</td>
<td>26</td>
<td>27.1</td>
</tr>
<tr>
<td></td>
<td><strong>Bagrus bayad</strong></td>
<td>97</td>
<td>72</td>
<td>74.2</td>
<td>72</td>
<td>74.2</td>
<td>34</td>
<td>35.1</td>
<td>15</td>
<td>15.5</td>
<td>25</td>
<td>25.8</td>
</tr>
<tr>
<td></td>
<td><strong>Ctenopharyngodon idella</strong></td>
<td>64</td>
<td>47</td>
<td>73.4</td>
<td>47</td>
<td>73.4</td>
<td>15</td>
<td>23.4</td>
<td>8</td>
<td>12.5</td>
<td>15</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td><strong>Barbus binny</strong></td>
<td>72</td>
<td>55</td>
<td>76.4</td>
<td>55</td>
<td>76.4</td>
<td>23</td>
<td>31.9</td>
<td>8</td>
<td>11.1</td>
<td>14</td>
<td>19.4</td>
</tr>
<tr>
<td></td>
<td><strong>Grand Total</strong></td>
<td>517</td>
<td>398</td>
<td>77</td>
<td>398</td>
<td>77</td>
<td>190</td>
<td>36.8</td>
<td>88</td>
<td>17</td>
<td>123</td>
<td>23.8</td>
</tr>
</tbody>
</table>

\[
\chi^2 = 5.2, \quad \text{P} > 5.2, \quad 50.94 < \chi^2 < 0.001, \quad 16.2 < \chi^2 < 0.001
\]

Between the different infected tissues of totally infected fish species in Ismailia, \( \chi^2 = 52.71 \) (P < 0.001)

Table (2) and Figure (2), show the tissue affinity of EMC in the examined fish species.
Most of recovered EMC, in the present study, were unidentified (Figures 3, 4, 5, 6, 7 and 8) with the exception of metacercariae of *Clinostomum tilapiae* (Yellow Grub) (Fig. 3J) and *Cyanodiplostomum azimi* (Fig. 4F, 5E and 8D) which were identified based on their morphological criteria. *Clinostomum tilapiae* metacercariae (Yellow Grub) were found in only two of the examined *Oreochromis niloticus* fish (1.7%).

In the present study, an experimental infection was carried out to identify the adult trematode species of the unidentified encysted metacercariae in the muscles and other tissues of the examined freshwater fishes by their developing and maturation in white rats and chickens as definitive hosts. The recovered trematodes from white rats and chickens experimentally fed on infected fish flesh with metacercariae were belonged to 4 families; *Heterophyidae* (*Heterophyes* heterophyes, *Heterophyes* nocens, *Stictodora* tanayensis, *Metagonimus* yokogawi, *Haplorchis* pumilio and *Pygidiopsis genata*) (Fig., 9), Family *Cyathocotylidae* (*Prohemitostomum vivax, Mesostephanus appendiculatus, Mesostephanus milvi* and *Mesostephanus burmanicus*) (Fig., 10), Family *Diplostomatidae* (*Cyanodiplostomum azimi* and *Neodiplostomum spathula*) and Family *Echinostomatidae* (*Echinochasmus liliputans*) (Fig.,11).

Fig. (3): Different types of recovered unidentified fresh encysted metacercariae (A, B, C, D, E, F, G, H and I) and (J) *Clinostomum* spp. metacercaria from *Oreochromis niloticus* fish.
Fig. (4): Different types of recovered unidentified fresh encysted metacercariae (A, B, C, D and E) and (F) Cyanodiplostomum azimi. metacercaria recovered from Clarias gariepinus fish.

Fig. (5): Different types of recovered unidentified fresh encysted metacercariae (A, B, C and D) from Chrysichthys auratus fish.
Fig. (6): Different types of recovered unidentified fresh encysted metacercariae (A, B, C and D) and (E) Cyanodiplostomum azimi. metacercaria recovered from Bagrus bayad fish.

Fig. (7): Different types of recovered unidentified fresh encysted metacercariae (A, B, C and D) from Ctenopharyngodon idella fish.
Fig. (8): Different types of recovered unidentified fresh encysted metacercariae (A, B and C) and (D) Cyanodiplostomum azimi metacercaria recovered from Barbus binny fish.

Fig. (9): Adult Heterophyid trematodes; (A) Heterophyes heterophyes and (B) Heterophyes heterophyes nocens (isolated from rats fed on Oreochromis niloticus), (C) Stictodora tanayensis, (D) Metagonimus yokogawi, (e) Haplorchis pumilio and (F) Pygidiopsis generata (isolated from rats fed on Oreochromis niloticus and Chrysichthy auratus).
Fig. (10): Adult Cyathocotylidae trematodes; (A) Prohemistomum vivax and (B) Mesostephanus appendiculatus (isolated from rats and chicks fed on Oreochromis niloticus, Clarias gariepinus, Chrysichthys auratus, Bagrus bayad, Ctenopharyngodon idella and Barbus binny), (C) Mesostephanus burmanicus (isolated from rats and chicks fed on Oreochromis niloticus, Clarias gariepinus, Bagrus bayad and Barbus binny) and (D) Mesostephanus milvi (isolated from rats and chicks fed on Clarias gariepinus)
Fig. (11): Adult Diplostomatid trematodes; (A) Neodiplostomum spathula (isolated from rats and chicks fed on Ctenopharyngodon idella), (B) Cyanodiplostomum azimi (isolated from rats and chicks fed on Clarias gariepinus, Bagrus bayad and Barbus binny) and (C) Adult Echinostomatidae (Echinocirrus liliputans) (isolated from rats and chicks fed on Bagrus bayad)

Regarding the Effect of grilling and freezing on the infectivity of metacercariae, The results recorded from experimental feeding of rats on infected Oreochromis niloticus with EMC grilled at 60-80°C for (5, 10 and 15-20 minutes) and also on infected Oreochromis niloticus fish with EMC were frozen at -10°C for (24, 48 and 72-96 hours) were illustrated in Table (3) and Fig. (12). It was revealed that grilling for 5 and 10 minutes was not sufficient to destroy all encysted metacercariae in fish muscles. This finding was proved by recovering adult trematodes from small intestine of experimentally fed rats. The identified adult trematode species were Prohemistomum vivax and Mesostephanus appendiculatus. But grilling of fish for 15-20 minutes was sufficient to destroy all encysted metacercariae, where rats fed on these grilled fish were free of trematode.
On the other hand, it was found that freezing at -10°C for 24 and 48 hours was not sufficient to destroy all encysted metacercariae in fish muscles. This finding was proved by recovering adult flukes (*Prohemistomum vivax* and *Mesostephanus appendiculatus*) from small intestine of experimentally fed rats. While, freezing at –10°C for 72-96 hours was sufficient to destroy all encysted metacercariae where rats fed on these fish were free of parasites.

In the control group, adult trematodes; *Heterophyes heterophyes*, *Mesostephanus appendiculatus* and *Prohemistomum vivax* were recovered from from small intestine of rats that were fed on the infected fish without treatment.

Table (3): Effect of grilling and freezing on the infectivity of encysted metacercariae lodged in *Oreochromis niloticus*.

<table>
<thead>
<tr>
<th>Grilling &amp; freezing time</th>
<th>Infectivity</th>
<th>No. of rats fed on EMC/group</th>
<th>Infected rats No. %</th>
<th>Intensity of infection (No. of adult trematodes/infected rats)</th>
<th>Types of adult trematodes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Grilling time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 minutes</td>
<td></td>
<td>4</td>
<td>100</td>
<td>38</td>
<td><em>Mesostephanus appendiculatus</em> <em>Prohemistomum vivax</em></td>
</tr>
<tr>
<td>10 minutes</td>
<td></td>
<td>4</td>
<td>100</td>
<td>13</td>
<td><em>Mesostephanus appendiculatus</em> <em>Prohemistomum vivax</em></td>
</tr>
<tr>
<td>(15-20) minutes</td>
<td></td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>……………………</td>
</tr>
<tr>
<td><strong>Freezing time</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24 hours</td>
<td></td>
<td>4</td>
<td>100</td>
<td>45</td>
<td><em>Mesostephanus appendiculatus</em> <em>Prohemistomum vivax</em></td>
</tr>
<tr>
<td>48 hours</td>
<td></td>
<td>4</td>
<td>100</td>
<td>19</td>
<td><em>Mesostephanus appendiculatus</em> <em>Prohemistomum vivax</em></td>
</tr>
<tr>
<td>(72-96) hours</td>
<td></td>
<td>4</td>
<td>0.0</td>
<td>0.0</td>
<td>……………………</td>
</tr>
<tr>
<td>Control group</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(No treatment)</td>
<td></td>
<td>4</td>
<td>100</td>
<td>107</td>
<td><em>Heterophyes heterophyes</em> <em>Mesostephanus appendiculatus</em> <em>Prohemistomum vivax</em></td>
</tr>
</tbody>
</table>
Fig. (12): Effect of grilling and freezing on the infectivity of encysted metacercariae lodged in Oreochromis niloticus.

DISCUSSION

Fishes act as intermediate host for larval stages of many parasites like encysted metacercariae of different species of trematodes. In the present study, the overall prevalence rate of encysted metacercariae of trematodes among the examined fresh water fishes was 77% in Ismailia province. This result was nearly similar to the results obtained in Egypt by Ramadan (1994) in Ismailia province (73.32%) and Saba (2004) in Sharkia province (77.6%).

This result was nearly similar to the results reported in Egypt by Ramadan (1994) in Ismailia province (73.32%) and Saba (2004) in Sharkia province (77.6%).

In the different fish species, in this study, the prevalence rate of the encysted metacercariae in Oreochromis niloticus was 74.8%. This result was nearly similar to the results obtained in Egypt by Abo-Esa (1993) in Alexandria (73.43%). It was higher than that recorded in Egypt by Bazh (2003) in Tanta (50.19%). However, higher result was obtained by Ramadan (1994) in Ismailia province (92.5%), Egypt.

The highest prevalence rate of encysted metacercariae was recorded in Clarias gariepinus (87%). These results was nearly similar to the results obtained in Egypt by Elamei (2001) in Ismailia (83%) and Saba (2004) in Sharkia (84.1%). It was higher than those recorded by Bazh (2003) in Tanta (62.01%). While, sli-
ightly higher results obtained in Egypt by Raid (1992) (92%).

In Chrysichthys auratus, the prevalence rate of encysted metacercariae was 78.1%. This result was lower than that obtained in Egypt by Ibrahim (2000) in Cairo (96%). The prevalence rate of encysted metacercariae in Bagrus bayad was 74.2%. This result was slightly lower than those recorded in Egypt by Shalaby (1985) in Cairo (82.42%) and Ibrahim (2000) in Cairo (83.33%). While, it was higher than those reported in Egypt by Ramadan (1994) in Ismailia (53.5%) and Bazh (2003) in Tanta (35.16%).

In Ctenopharyngodon idella, the prevalence rate of encysted metacercariae was 73.4%. This result was lower than that obtained in Egypt by Abu El-Ezz et al. (2000) (90%) in Giza, Wadi Al-Rayan and Abbassa aquaculture.

While, the prevalence rate of encysted metacercariae in Barbus binny was 76.4%. This result was much higher than that reported by Shalaby (1988) in Giza (18.18%).

Such variations in the prevalence rates of EMC are expected and this may be attributed to various factors including the locality from which fish were caught and the degree of water pollution with human, animal and birds excreta.

In this study, the seasonal fluctuation of EMC among the examined fresh water fishes in Ismailia province showed that the highest infection rates were in summer followed by spring and autumn, and the lowest prevalence rates were in winter in Oreochromis niloticus, Clarias gariepinus, Bagrus bayad, Ctenopharyngodon idella and Barbus binny. While, in Chrysichthys auratus showed that the highest prevalence rate was in autumn followed by summer and spring and the lowest prevalence was in winter. This finding was in agreement with that reported in Egypt by Eissa et al. (2001) who found that the highest infection rate with EMC in Nile fishes was in summer followed by spring, autumn and winter in Ismailia province. Ramadan (1994) reported that the highest seasonal prevalence rate was in summer followed by autumn, spring and winter in Ismailia province furthermore, there were no significant differences in the prevalence rates of EMC between the different fish species (P > 0.05) in the different seasons. However, the difference in the overall infection rates with EMC among the examined freshwater fishes between the different seasons was highly significant (P < 0.0001). The high prevalence rates of EMC among the examined freshwater fishes in summer, spring and autumn may be attributed to the climatic conditions as temperature and humidity, which are suitable con-
ditions for growth and reproduction of snail (first intermediate host) (*El-Lea
ty, 1997*).

The recovered adult trematodes from white rats and chickens experimentally fed on infected fish flesh with metacercariae of the examined freshwater fishes, in this study, were thirteen species belonging to 4 Families; *Heterophyidae* (*Heterophyes heterophyes, Heterophyes heterophyes nocens, Stictodora tanayensis, Metagonimus yokogawai, Haplorchis pumilio and Pygidiopsis genata*), *Family* *Cya
thocotylidae* (*Prohemistomum vivax, Mesostephanus appendiculatus, Mesostephanus milvi and Mesostephanus burmanicus*), *Family* *Diplostomatidae* (*Cyanodiplostomum azimi and Neodi
plostomum spathula*) and *Family Echinostomatidae* (*Echinochasmus lilipu
tans*). These results were partially or completely in agreement with others (*Raef, 1994; Ibrahim, 2000; Elamei, 2001; Bazh, 2003; Saba, 2004 and El Gayar, 2007*)

The differences in the recovered adult trematodes from different laboratory animals as mentioned previously could be explained by the differences in physiological status and acidity of the stomach of all hosts, as well as other anatomical and physiological factors and also differences between final hosts, helping the establishment of these parasites (*Shalaby et al., 1989*).

The previous recovered species of adult trematodes of the EMC in the examined freshwater fishes in the present study, have zoonotic importance as recorded by *Hong et al. (1996)* and *Ryang et al. (1999)*. In Egypt, human infections with *H. heterophyes* are prevalent among the inhabitants of the northern part of the Nile Delta. A large number of *Heterophyes* species have been reported from humans, among which *Metagonimus yokogawai* and *H. heterophyes* are generally considered the most important species (*Yu and Mott, 1994*). However, because an extraordinary number of heterophyid species are zoonotic (about 35 species) and have very similar transmission patterns, this group is in the aggregate a very significant food safety and quality problem. The importance of these flukes is being increasingly recognized through recent studies from the Philippines (*Belizario et al., 2001*), from Thailand on *Haplo
crich taichui* (*Waikagul, 1991* and *Su
tontason et al., 2001*) and from Korea on several species including *Hetero
ychyes nocens* and *Metagonimus* spp. (*Chai and Lee, 2002*). Although generally not considered of significant clinical importance relative to the liver flukes, several heterophyid species, including *Haplorchis* spp. can cause significant pathology, often fatal, in the heart, brain, and spinal cord of humans (World Health Organi-
Most human Echinostome infections have been reported from Asia and the Western Pacific, but infections probably occur also in Africa (Yu and Mott, 1994).

In this study, Clinostomum Tilapiae metacercariae were detected in only two of the examined O. niloticus fish (1.7%) in Ismailia province. This result was very low in comparing with that reported by Laya (1994) who found that O.niloticus fish of Nile tributaries at Sharkia governorate and cultured fish at Abbasia were infected with C. Tilapiae in prevalence rate of 30% and 45.33% respectively. Clinostomum spp. metacercariae can be transmitted to human as a result of ingesting raw or improperly cooked freshwater fish, causing Halazoun-like disease leading to laryngo-pharyngitis (Chung et al., 1995). The infection of fish with C. Tilapiae metacercariae may be attributed to the richness of water with the intermediate host (aquatic snails) and the presence of final host (aquatic birds).

Regarding the Effect of grilling on the infectivity of metacercariae, in this study, it was revealed that grilling for 5 and 10 minutes was not sufficient to destroy all encysted metacercariae in fish muscles. This finding was proved by recovering adult trematodes from small intestine of experimentally fed rats. The identified adult trematode species were Prohemistomum vivax and Mesostephanus appendiculatus. While, grilling of fish for 15-20 minutes was sufficient to destroy all encysted metacercariae, where rats fed on these grilled fish were free of parasites. These results agreed with those reported by Mikhail, 1993 and Eissa et al., 2001. On the other hand, it was disagreed with that obtained by Yousef et al. (1981) who reported that grilling of Tilapia spp. for 15-20 minutes resulted in the recovery of 3-5 adult Pygidiopsis genata worms from each mouse fed with one gram of grilled fish meat. He also stated that the inner temperature of grilled fish meat for 15-20 minutes ranged from 51 -56°C and such preparation does not make the fish free of metacercariae. Mahmoud (1983) stated that heating of fish to temperature 45°C for 30 minutes may be safe guard against infection with H. heterophyes metacercariae. El-Leathy (1997) found that grilling of Tilapia spp. for 5 minutes was not sufficient to destroy all EMC, while good grilling for 10 minutes and frying for 5 minutes were sufficient to destroy all EMC.

It was concluded that the proper timing and temperature of grilling should be emphasized because superficial grilling doesn't affect deep muscles which of course contain encysted metacercariae are not affected by the fire (El-Sherbiny, 1988).

On the other hand, the Effect of freezing on the infectivity of meta-
cercariae revealed that freezing at -10°C for 24 and 48 hours was not sufficient to destroy all encysted metacercariae in fish muscles. This finding was proved by recovering adult worms (*Prohemistomum vivax* and *Mesostephanus appendiculatus*) from small intestine of experimentally fed rats. While freezing at -10°C for 72-96 hours was sufficient to destroy all encysted metacercariae where rats fed on these fish were free of parasites.

These results agreed with the results reported by *Eissa et al.* (2001). *Yousef et al.* (1981) found that freezing of *Tilapia* spp. at -4°C for 10 and 12 days failed to produce infection to experimental animals.

*Mahmoud* (1983) found that freezing of *Tilapia* spp. fish muscles at -2°C for a period not less than 9 days may be considered enough to kill the contained metacercariae. It was concluded that deep freezing of fish lead to prevent the danger of fish parasites which have a public health importance (*Raid, 1992*).

In conclusion, control the hazards associated with fish consumption can be done by efficient heat processing (80°C), of the fish before consumption for period not less than 15-20 minutes or simple freezing of the fish before processing for consumption for period not less than 72-96 hours.

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الملخص العربي

دور أسماك المياه العذبة في وبائية بعض الديدان المثقوبة المشتركة في محافظة الإسماعيلية

عبدالكريم محمود أبو عيشه، ربيع السيد صالح، هناء محمد فاضل، أيمان محمد يوسف، يسرا أحميد حلمي

كلية الطب البيطرى، جامعة قناة السويس، مصر

فى دراسة على وبائية بعض الديدان المثقوبة المشتركة في أسماك المياه العذبة، تم جمع عدد 517 سمكة من أسماك المياه العذبة (119 من سمك البلطي النيلى، 29 من سمك القراميط، 96 من سمك أبورياله)
القضي، 97 من سمك البلاط، 64 من سمك المبروك، 72 من سمك البنى الأصيل) من فروع النيل أثناء الفترة من مارس 2007 حتى نوفمبر 2008 بمحافظة الإسماعيلية. وتبين من الفحص الطفيلي لهذه الأسماك أن معدلات انتشار الذنبات المتحوصلة للديدان المثقوبة 70% في أسماك البلاط النيلي، 87% في أسماك القراميط، 96% في أسماك أبيض البلاط، 84% في أسماك أبيض البلاط. وجدت حويصلات كليوستوم تيليبي (البرقات الصفراء) والتي تم التعرف عليها من فحص الشكل الظاهر والطفيلى لها في عدد 3 سمكة من أسماك البلاط النيلي (71%). وعموما كان أعلى معدل إصابة بالمذنبات المتحوصلة في الأسماك التي تم فحصها في موسم الصيف (75%), ثم الربيع (74%) والخريف (78%), وكان أقل معدل للإصابة في أشهر الشتاء (75%). والتجربة التجريبي للفئران والكتاكيت على الأسماك المصابة بالمذنبات المتحوصلة تم الحصول على الديدان المثقوبة: هيتروفيس هيتروفيس، هيتروفيس نوسينس، هالبوركس باميليو ميتاوناموس يوكوكاوي، بايديديوس جيناتا، وستيكتودرا تانابيس، وبروهيمستوم فايفيس، ميزوستيفانس إبديكيلايس، ميزوستيفانس بيرمانيس، ميزوستيفانس ميلفى، سيانيديسطوم عميمي، نيديليوستوم سباسيولا واكينكاسينس فالبيةتانيسي. وللقضاء على المذنبات المتحوصلة في الأسماك المصابة باستخدام المعالجة الحرارية (الشوى) والتجميز لأسماك البلطي النيلي المصاببة بالمذنبات المتحوصلة للديدان المثقوبة، تبين أن شوي أسماك البلطي النيلي المصابة عند درجة حرارة من 80-90 درجة مئوية لمدة تتراوح من 15-20 دقيقة أو تجميد هذه الأسماك عند -20 درجة مئوية لمدة تتراوح من 22-26 ساعة كانت كافية للقضاء على المذنبات المتحوصلة. وأظهر البحث الحالي أن أسماك المياه العذبة تعتبر كعارل وسيط مهم لتواجد هذه الديدان المثقوبة ومصدر للإصابة الإنسانية والحيوانات الأكلة للأسماك بهذه الطفيليات.