STUDIES ON BLOOD FLUKE DISEASE (SANGUINICOLIASIS) AMONG SOME WILD AND CULTURED FISHES

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ABSTRACT

The present study was carried out to study sanguinicoliasis through recording the clinical signs and postmortem lesions in diseased wild *Clarias gariepinus* and cultured *Cyprinus carpio*. Isolation and identification of three sanguinicola sp. (*S clarias*, *S inermis* and *S chalmersi*). Monitoring the alterations in blood picture and serum components. Demonstration the histopathological alterations induced by such parasites. Infected fish showed lethargy, stop feeding, emaciation, sluggish movements, paleness, cyanosis in gills, gasping for air, swimming in spiral movement and loss of escape reflex. The internal examination revealed partially enlargement in kidney, partially yellowish coloration in liver, in some fish and distention of gall bladder. The total prevalence of sanguinicola sp. among all examined fishes was 45.31%. The prevalence of sanguinicola sp in wild and cultured fishes was 53.6 and 34.3% respectively. High prevalence was noticed in summer and spring while the lowest prevalence was in autumn and winter.

INTRODUCTION

Blood flukes infect the vascular system of freshwater and marine fishes comprised serious pathogens in cultured fishes lead to mass mortalities in ponds and cages in North America, Europe and Japan (*Stephen and Overstreet 2000*). Sanguinicoliasis is also a serious disease in cultured carp causing high mortality especially in early fry (*Sommerville and Iqbal 1991*).

*Sanguinicola inermis* parasitized carps throughout most parts of Europe, it infected wild and farmed fish causing extensive damage to fry and young fingerlings. Field studies showed that the adult flukes success fully in wild carp population, to release eggs in gills during the spring (*Ewens et al. , 1994*). Heart, branchial vessels were advantageous for egg deposition because eggs released there immediately carried to the gills; how-
ever, adults of fish blood flukes occurred in vessels involving the mesentery, intestine, kidney and brain (Smith, 1997). The hemoparasites of genus sanguinicola were found during summer in gill vessels of cultured eels (Anguilla anguilla) in fish farm located on Northern Italy (Prearo et al. 1995). Adult flukes were observed in the hearts and kidneys of infected fish where eggs were found in the gills (El-khatib and Elias 2003).

This work was aimed to investigate sanguicoliasis among some cultured and wild freshwater fishes with special regards to haematological and histopathological studies.

MATERIALS & METHODS

Fish:
A total number of (245) cultured and wild freshwater fishes 140 wild C. gariepinus were randomly collected from Manzallah fish farms and also from various water courses at Dakahlia governorate. The average body weights was (150±20g).

Clinical examination
The live fish specimens were clinically examined for detection of any macroscopic lesions and external abnormalities according to Conroy and Hermann (1981).

Postmortem examination
Internal organs were examined especially the liver, kidney, spleen and heart for any enlargement or abnormal colouration or paleness. (Noga 1996)

Parasitological examination:
The heart and its tributaries with portal vessels were extracted, placed into petri dish containing saline and opened longitudinally. The free flukes were recorded by dissecting microscope. Kidneys were placed into Petri dish containing saline. The gill filaments were compressed between two slides for miracidia. The obtained flukes were examined according to the technique described be Lucky (1977).

The parasites were collected in small vials containing 5% formalin solution and were washed with tap water to get rid of any traces of formalin solution. The specimens were stained with alum carmine stain. Dehydrated using ascending grades of ethyl alcohol, cleared using clove oil then: the specimens were mounted in Canada balsam, left to dry and covered with a cover slide, then identified microscopically.

Haematological examination:
Fresh blood samples were taken from all investigated groups by using plastic syring (Stoskopf, 1993) placed in Epindoof tubes containing EDTA as anti-coagulant. Erythrocytes and leukocytes count were performed by manual method according to Feldman et al. (2000) using improved Neubauer hemocytometer and Natt &
Herrick solution as diluting fluid (Natt and Herrick, 1952) - Erythrocytic count (Schaperclaus, 1992), Leukocytic count Differential leukocytic count, Haemoglobin (Hb) according to Dein (1984) Hematocrit value (PCV). Red cell indices, MCV (fL) MCH (pg) MCHC (%) were calculated from measured PCV%, Hb concentration and RBC count according to Feldman et al. (2000).

**Serum biochemical analysis:**
Prepared serum samples were used and analyzed for some serum components including alanine aminotransferase (ALT), asprate aminotransferase (AST) according to Reitman and Frankel (1957), glucose according to Werner et al., (1970), total protein according to Young (2001), Albumin was determined according to Dumas and Biggs (1972), urea according to Numann et al., (1957) and creatinine according to Henry (1974). A/G ratio was calculated by dividing albumin to globulin blood value (Kaneko et al. 1997).

**Statistical analysis:**
Haematological values and serum biochemical parameters were analyzed by analysis of variance (ANOVA) according to State View (1993).

**Histopathological examination**
Specimens from the kidneys, heart and gills of infected fish were collected. They were fixed in 10% buffered formalin solution and dehydrated through different concentrations of ethyl alcohol (50,70 and 95%), treated with xylol then blocked in paraffin sections of 4-5 micron thickness were mounted on clean slides, stained by Haematoxylin and Eosin (Carleton, 1976).

**RESULTS**

**Clinical picture:**
Clinical signs noticed on *C. gariepinus* and *C. carpio* infected by blood fluke were lethargy, stop feeding, emaciation, sluggish movements, paleness, cyanosis in gills, gasping for air, swimming in spiral movement and loss of escape reflex. The internal examination of such fishes revealed congestion in heart, partially enlargement in kidney,, yellowish or pale of liver in some fish and distention of gallbladder.

**Parasitological examination:**
The parasitological examination revealed three sp of *Sanguinicolaa* body was elongated, flattened and tapered towards the anterior end and with rounded posterior end. Mouth opening is terminal and lead directly to a long oesophagus that ends in a short X or H-shaped. Four rounded lobed intestinal caeca at the level of 1/3 body length. The ovary appeared in form of double winged organ like a butterfly. Yolk glands fill the entire body. From the morphological
and parasitological examinations, this parasite was belonged to Phylum Platyhelminthes, Order Schistosomatida, Family Sanguinicolida, *Sanguinicola clarias*. (Fig.1) 
b- The body was elongated, delicate and tapered towards both ends with rounded extremities. The posterior end was not constricted off. The oesophagus was long in length terminating into an irregular shaped intestinal caecum with 4 or 5 lobes. The ovary was in the form of two symmetrical more or less a cuneiform wings. From the morphological and parasitological examination this parasite was belonged to *Sanguinicola chalmersi*, (Fig.2) 
c-The body was elongated lack anterior ventral suckers and pharynx. The intestinal caeca were short and x-shape. From the morphological and parasitological examination this parasite was belonged to *Sanguinicola inermis* (Fig.3)

**Prevalence:**
The total prevalence of *sanguinicola* sp. was 45.31% among *C. gariepinus* and *C. carpio*. The prevalence of in both fishes was 53.6 and 34.3% respectively. Also, high prevalence in summer season 68.6 & 48.6% while the lowest prevalence in winter and autumn 31.4 & 14.3% respectively (Table, 1).

**Hematological examination**

**Erythrogram**
The red blood cells (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV) were significantly decreased in *C. gariepinus* and *C. carpio* infected with *Sanguinicola* in comparison with control.

Mean while MCV (FL) and MCHC (%) were not significantly changed in *Sanguinicola* group in comparison with control in the two species. (Table, 2).

**Leukogram**
Concerning to the leukogram picture in our study, total leukocytes count (TLC) significantly decreased (leucopenia) in Catfish *C. gariepinus* and was not significantly changed in *C. carpio* in *Sanguinicola* infected groups in comparison to the control.

While lymphopenia was recorded in *Sanguinicola* group in both *C. gariepinus* and *C. carpio* in comparison with control group. Esinophilia and monocytosis were recorded in *Sanguinicola* group in both species. (Table, 3).

Serum alanine aminotransferase enzymes, (ALT) and Serum aspartate aminotransferase enzymes (AST) were significantly elevated in Catfish *Clarias gariepinus* and *C. carpio*, infected with *Sanguinicola* in comparison with control. Hypoproteinemia, hypoglobulinemia and hypoalbuminemia were recorded in *Sanguinicola* group in comparison with control in both species. While A/G ratio and glucose blood level were not significantly
changed in Sanguinicola group in comparison with control. Urea and creatinine blood level were not significant in comparison with control in both species. (Table, 4).

**Histopathological alterations**

The fish infected with Sanguinicola sp. showed in kidneys infected by sanguinicola sp., focal hemorrhage, tubular nephrosis, degeneration of epithelium of renal tubules and lymphocytic infiltration. The cross section of adult parasite was detected within kidney blood vessels (Figs. 4).

In heart, there were focal hemorrhage, intermuscular edema and hyaline degeneration with focal lymphocytic infiltration (Fig. 5). Gills showed oval body probably corresponding to egg of Sanguinicolid sp in gill filament was observed blocked in gill artery. The embryonated eggs were also surrounded by eosinophilic infiltration along with lymphocytes and edema (Fig. 6).

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**Fig. (1):** *Sanguinicola clarias* isolated from heart of *C. garepinus* (x400).

**Fig. (2):** *Sanguinicola chalmersi* isolated from kidney of *C. garepinus* (x400).
Fig. (3): *Sanguinicola inermis* isolated from heart of *C. carpio*. (x400).

Fig. (4): Kidney of *C. gariepinus* infected with *Sanguinicola clarias* showing focal hemorrhages, degeneration of the epithelium of renal tubules and lymphocytic infiltration cross section of the parasite (*sanguinicola* trematode). H&E. (X400).

Fig. (5): Heart of *C. garipinus* showed focal hemorrhage, intermuscular edema and hyaline degeneration along with focal lymphocytic infiltration.

Fig. (6): Gills of *C. gariepinus* showing oval body corresponding to egg of *Sanguinicolid* in gill filament and surrounded by eosinophilic infiltration and few lymphocytes. H&E. (X400).
Table. (1): Seasonal prevalence of blood parasites in *C. garepinus* and *C. carpio*.

| Season | *C. garepinus* | | | *C. carpio* | | |
|--------|----------------|----------------|----------------|----------------|----------------|
|        | No. | No. of infested | %  | No. of infested | %  | |
| Spring | 35  | 23             | 65.7 | 14              | 40  | |
| Summer | 35  | 24             | 68.6 | 17              | 48.6 | |
| Autumn | 35  | 17             | 48.6 | 5               | 14.3 | |
| Winter | 35  | 11             | 31.4 | 0               | 0   | |
| Total  | 75  | 53.6           |      | 36              | 34.3 | |

Table. (2): Erythrogram Picture (Mean ± S.E) in *C. garepinus* and *C. carpio* infected with sanguinicolosa sp.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Group</th>
<th>RBCs Mill/μL</th>
<th>Hb g/dl</th>
<th>PCV %</th>
<th>MCV FL</th>
<th>MCH Pg</th>
<th>MCHC %</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. garepinus</em></td>
<td>Control</td>
<td>2.42 ± 0.14</td>
<td>8.91 ± 0.41</td>
<td>29.10 ± 1.15</td>
<td>120.2 ± 3.82</td>
<td>36.8 ± 1.85</td>
<td>30.6 ± 1.05</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>1.72 ± 0.15*</td>
<td>6.31 ± 0.43*</td>
<td>21.05 ± 1.21*</td>
<td>122.4 ± 3.79</td>
<td>36.7 ± 1.92</td>
<td>29.9 ± 1.21</td>
</tr>
<tr>
<td><em>C. carpio</em></td>
<td>Control</td>
<td>1.91 ± 0.12</td>
<td>7.35 ± 0.31</td>
<td>28.9 ± 1.10</td>
<td>151 ± 4.95</td>
<td>38.5 ± 1.84</td>
<td>25.4 ± 1.35</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>1.34 ± 0.10*</td>
<td>5.35 ± 0.33*</td>
<td>20.05 ± 1.21*</td>
<td>150 ± 6.25</td>
<td>39.9 ± 2.85</td>
<td>26.7 ± 1.19</td>
</tr>
</tbody>
</table>
Table. (3): Leukogram Picture (Mean ± S.E) in *C. gariepinus* and *C. carpio* infected with *sanguinicola* sp.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Group</th>
<th>TLC Thou/µL</th>
<th>Neutro Thou/µL</th>
<th>Eosinoph Thou/µL</th>
<th>Basoph Thou/µL</th>
<th>Lymph Thou/µL</th>
<th>Monocy Thou/µL</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. gariepinus</em></td>
<td>Control</td>
<td>25.84 ± 2.15</td>
<td>8.51 ± 0.92</td>
<td>0.32 ± 0.03</td>
<td>0.0</td>
<td>15.61 ± 1.04</td>
<td>1.41 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>21.95 ± 1.82*</td>
<td>8.58 ± 0.95</td>
<td>0.78 ± 0.04*</td>
<td>0.010 ± 0.010</td>
<td>10.61 ± 1.16*</td>
<td>1.98 ± 0.12*</td>
</tr>
<tr>
<td><em>C. carpio</em></td>
<td>Control</td>
<td>29.98 ± 2.41</td>
<td>8.29 ± 0.84</td>
<td>0.63 ± 0.05</td>
<td>0.060 ± 0.060</td>
<td>19.48 ± 1.12</td>
<td>1.46 ± 0.17</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>27.46 ± 1.96</td>
<td>8.97 ± 0.92</td>
<td>1.12 ± 0.08*</td>
<td>0</td>
<td>15.64 ± 1.1*</td>
<td>1.91 ± 0.12*</td>
</tr>
</tbody>
</table>

Significant at P > 0.05

Table. (4): Serum biochemical parameters (Mean ± S.E) in *C. gariepinus* and *C. carpio* infected with *sanguinicola* sp.

<table>
<thead>
<tr>
<th>Fish</th>
<th>Group</th>
<th>ALT U/ml</th>
<th>AST U/ml</th>
<th>Total. Protein (A) g/dl</th>
<th>Albumin (A) g/dl</th>
<th>Globulin (G) g/dl</th>
<th>A/G Ratio</th>
<th>Glucose g/dl</th>
<th>Urea gm/dl</th>
<th>Creatinine g/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. gariepinus</em></td>
<td>Control</td>
<td>35 ±2.15</td>
<td>115 ±5.8</td>
<td>3.41 ±0.42</td>
<td>1.42 ± 0.12</td>
<td>1.99 ± 0.19</td>
<td>0.71 ± 0.12</td>
<td>78.1 ± 5.4</td>
<td>12.1 ± 1.1</td>
<td>1.18 ± 0.11</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>85.1 ±6.84*</td>
<td>144 ±6.1*</td>
<td>2.38 ±0.34*</td>
<td>0.84 ± 0.08*</td>
<td>1.74 ± 0.17</td>
<td>0.48 ± 0.08</td>
<td>71.1 ± 6.9</td>
<td>14.2 ± 1.6</td>
<td>1.21 ± 0.10</td>
</tr>
<tr>
<td><em>C. carpio</em></td>
<td>Control</td>
<td>30.1 ±2.32</td>
<td>108 ±7.8</td>
<td>2.85 ±0.24</td>
<td>0.98 ± 0.10</td>
<td>1.87 ± 0.18</td>
<td>0.52 ± 0.10</td>
<td>91.4 ± 6.8</td>
<td>10.2 ± 0.91</td>
<td>0.71 ± 0.08</td>
</tr>
<tr>
<td></td>
<td>infected</td>
<td>61.1 ±5.84*</td>
<td>135 ±5.4*</td>
<td>2.12 ±0.25*</td>
<td>0.58 ± 0.06*</td>
<td>1.54 ± 0.13</td>
<td>0.38 ± 0.06</td>
<td>90.3 ± 7.6</td>
<td>11.8 ± 1.69</td>
<td>0.82 ± 0.09</td>
</tr>
</tbody>
</table>
DISCUSSION

The clinical signs of the fishes infected by sanguinicola sp. were nearly similar to *El-Khatib and Elias (2003)*, *Adawy and Deeb (2007)*.

Sanguinicola sp. produced renal dysfunction as a result to the presence of adult worms and eggs in the kidneys which were degenerated and the enclosure by eggs in thick capsules do not find their way into blood circulation. The wide spread of sanguinicola sp among wild and cultured fish species supported the result of *Sommerville and Iqbal (1991) Roberts and Hoole (1994) Stephen and Overstreet (2000) Guidelli et al. (2002)*. Regarding the seasonal prevalence of blood flukes in infected wild and cultured fishes, the high infection rate was noticed in summer season followed by spring, autumn then winter in the examined Cl. gariepinus while C. carpio, the infection rate with blood flukes infection was recorded mainly in summer, followed by spring and the lowest rate in autumn. The high infection rates in summer may be due to the high water temperature period that enhance the spread and propagations of intermediate hosts (infected snails and/or bivalve). Such results agree with *Roberts and Hoole (1994)*.

Concerning the prevalence of sanguinicola sp. among wild Clarias gariepinus was 53.6%. This result is nearly agree with *Negm el-Din (1991)*.

Concerning the prevalence of sanguinicola sp. among wild and cultured fishes, it was noticed that high prevalence among wild than cultured fishes and this may be due to the viability of water snails. The seasonal variations of sanguinicoliasis, it was noticed that the highest prevalence was in summer season followed by spring and the lowest prevalence was recorded in winter season. These results agree with *Ogawa et al. (1993), Adawy and Deeb (2007)*. The morphological identification of isolated Sanguinicola clarias and S. chalmersi was nearly similar to description of *Negm-El-Din (1991), Padros et al. (2001) and Eissa (2002)*.

Our result showed normocytic normochromic anemia in Sanguinicola group in C gariepinus, in comparison with control. Also, anaemia was observed in C. carpio parasitized by Sanguinicola sp. *El-Khatib and Elias (2003)* observed anaemia in common carp infested with sanguinicola sp. Our result was in accordance with *Adawy and Deeb (2007)* who demonstrate anaemia as well as decrease erythrocytic count, hemoglobin and PCV% in C gariepinus infected with Sanguinicola clarias.
Regarding leukogram, it was shown that leucopenia was observed in C gariepinus infected with Sanguinicolasp. Leucopenia could be attributed to lymphopenia as result of stress effect of parasitism on fishes (Barton and Iwama, 1991). Esinophilia was recorded in C gariepinus and Common carp infected with Sanguinicolasp. Anisworth, (1992) concluded that an absolute increase esinophils number in fish is common in response to parasite infection. The cytosolic enzyme alanine aminotransferase (ALT) is nearly specific for hepatocellular injury. The increased serum levels parallel the magnitude of hepatocellular damage. Aspartate aminotransferase (AST) occurs in most cells however it is useful in evaluating hepatocellular and muscular injury because of its high activity in these tissues (Stoskopf 1993). Liver transaminase enzymes, (ALT & AST) in our study were elevated in all investigated groups in compare with control. Liver damage was documented by severe hemorrhage and leukocytic infiltration in C. garipinus and C. carpio infected by sanguinicolasp. In the same aspect Adawy and Deeb (2007) recorded elevation of AST and ALT in catfish Clarias garipinus infested by Sanguinicolasp. This could be attributed to the renal glomerular damage induced by Sanguinicolasp. was not severe enough to elevate blood creatinine value. This conclusion confirmed histopathologically by focal hemorrhage between the renal tissues.

Histopathologically, the fish species infested by sanguinicolasp. showed gill sloughing of secondary lamellae with congestion of blood vessels, edema & leukocytic infiltration in gill arch. Eggs of sanguinicolasp. in gill filament were observed blocked in gill artery. Kidney revealed focal hemorrhage, degeneration of epithelium of
renal tubules and lymphocytic infiltration. Such results were nearly similar to that produced by Kirk and Lewis (1998), Stephen and Overstreet (2000).

REFERENCES


الملخص العربي

دراسة على مرض ديدان الدم (السانجوينيكولا) في بعض الأسماك الحرة والمستزرعة

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معهد بحوث صحة الحيوان بالمنصورة

تعتبر السانجوينيكولا من أهم طفيليات الدم التي تصيب الأسماك الحرة والمستزرعة، وتعتبر أضراراً اقتصادية كبيرة. في هذه الدراسة تم فحص نوعين من الأسماك وهما القرموط الأفريقي الحر والمبروك العادى المستزرع، وقد وجد أن هذا الأسماك هزيله وتسحب بطريقة أهتزازية بطيئة بالإضافة إلى اختفاء ظاهرة الهروب والفحص الداخلي، وجد أن الخياشيئم لونها باهت وتميل للزرقة وإحتقان في القلب وزيادة طفيفة في حجم الكلى وإصفرار في الكبد دوامتملاء الحويصلة المرارية. وتم عزل ديدان السانجوينيكولا الناضجة الحية من القرموط والكلى، ووجد أن نسبة الاصابة كانت 3% ومثله في السانجوينيكولا كلاريز وكلاميرس، أما في المبروك العادى كانت نسبة الاصابة 43% ومثله في السانجوينيكولا انرمست. تم تقييم تأثير السانجوينيكولا على كلا النوعين من خلال صورة الدم والتحليل البيوكيميائي للسيرم ووجد نقص معنوي في عدد خلايا الدم الحمراء والهيموجلوبين بنسبة الخلايا المضغوطة ونقص في العدد الكلي لخلايا الدم البيضاء في كلا النوعين نتيجة لضغط العدوى.
الطفلية وقلة الخلايا الليمفاوية كما وجد زيادة في الخلايا الحمضية والخلايا الاكولية في كل من الأسماك المصابة مقارنة بغيرها من الأسماك الغير المصابة. كما وجد زيادة ملحوظة في وظائف الكبد ونقص في البروتين الكلي والأليافين أما الجلوبولين والبيوريا والكرياتينين فلم يحدث فيهم أي تغيير ملحوظ في الأسماك المصابة مقارنة بغيرها من الأسماك الغير المصابة. كما وجدت تغيرات باثولوجية واضحة على الكلى والخياشي. كما أن النسج الكلى بها بقع نزيفية المكونة للكلى أيضا وجد قطاع في السانجونيكونولا في الأوعية الدموية للكلية ووجود تنكر في خلايا نسيج الخياشي واحتفان في أوعيتها الدموية مع تجمع كبير لخلايا الدم البيضاء ووجود جسم بيضاوي لبيضة ديدان السانجونيكونولا محاطة بخلايا الأزيزونبيل والخلايا الليمفاوية.