Haematological Studies of Healthy and Helminthes Infected Rabbit fish (*Siganus rivulatus*) in Red Sea Coast, Sudan

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Abstract: The experiment was conducted in Abu Hashish Site in 2007. *Siganus rivulatus* fishes were caught for the present study and observed for external parasites and then examined for helminthes parasites and divided into four groups: Group A (control male), Group B (helminthes infected male), Group C (control female), and Group D (helminthes infected female). Blood samples were collected after fish catch, and then subjected to biochemical investigations which including haematological estimations, haemoglobin (Hb), packed cell volume (PCV), and erythrocyte count. The results revealed that there is decrease in packed cell volume (PCV), haemoglobin (Hb) and red blood cells count of helminthes infected groups as compared with control group of male and female.

Key words: *Siganus rivulatus*, Helminthes, Healthy and fish.

INTRODUCTION

There are about 250 species of fishes known from Sudanese Red sea coast out of 1000 encountered from Red Sea. Some biological aspects were studied in few species. The rabbit fishes (also known as marbled spine foot) were got their scientific name *siganidae* from the Arabic word "Sigan" used by fishermen for this type of fish. Member of *siganidae* are one of the fish family that migrated from Red Sea to the Mediterranean Sea. This is due to opening the Suez Canal in 19th centre.

A population of *Siganus rivulatus* was recorded from the Mediterranean Sea in 1927 [9]. *Siganus spp* are considered as one of the most important commercial fishes than in mammals are: First, a review of literature provides in consistencies concerning the nomenclature, cellular differential, maturation, and function of fish blood cells, Second, cell count must be determined manually, because the nucleated erythrocytes and the overlap in the leukocyte size prevent the use of automated methods and Third, the number of fish species and their diversity in morphological form and ecologic function makes generalization about fishes impossible. The generic term “fish” includes primitive jaw less vertebrates and vertebrate with cartilaginous skeletons as well as more advanced bony skeletons [4].

Abu Hashish Area is one of the landing and / or fishing grounds a long the Sudanese Coast. It's about 6 km long and extends to about 2 km north of the northern point of Port Sudan harbour entrance plate (2). This situation enhanced the execution of a number of studies in the area included: Impact of thermal pollution [11], the abundance of *Trochus spp.* [8] and Environmental impact assessment and diversity of Echinoderms [2]. Plate (1).

Nematodes that use fish as intermediate hosts are much more injurious than adults, mainly because they infected tissues. The host’s defensive response results deposition of fibrous capsule around the encysted larva. Extensive degenerative change and necrosis may occur around cyst, because they are coelozoic, adult nematodes rarely cause serious injury. At most they cause local lesions of no great significant to the host’s general health. Heavy nematode infection, especially in small and young, fish may more serious. However, as the intensity of infection usually increases with age; young fish are seldom heavy infected [22]. Infection is widespread in wild eels [25] and occasionally occurs in earth ponds, but it is unlikely to spread in hyperintensive systems where copepods, the intermediate hosts, cannot usually survive [12]. Pathological effects vary with growth conditions and eel species. Heavy infection causes haemorrhagic inflammation of the swim bladder, but it may not necessarily always disrupt fish growth. It may, however, decrease the eel’s tolerance to transport stresses [24]. In South Africa, infection by an endemic species was found in cultivated eels only. Nematode or round worms have along been known out to parasitize
marine fishes and are found throughout the seas of world. One recent systematic compilation\[32\] listed some 300 species representing more than 40 genera in 17 families, from marine fishes. Most adult nematodes in fishes, especially that living in digestive tract, likely have shorter life span, probably less than a years. Host specificity of acanthocephalans is variable and may be evaluated only where sufficient data are available, which is not the case for most African fish species. Acanthogyrus tilapiae is specific to Cichlidae, while other species have been found in Cyprinidae Paragorgorynchus albertianum is indiscriminate in its choice of hosts\[15\].

Epizootiological data are limited to natural infections: In the Sudan White and Blue Nile, 5–27 Tenuisentis niloticus occurred in 93% of Heterotis niloticus, 6–43 Neoechinorhynchus sp. in 26% of Citharinus citharus and 2–5 unidentified acanthocephala in 60% of Synodontis batensoda (Khalil, 1969). Similarly abundant infections were found in the same fish in L. Chad\[31\]. Acanthogyrus tilapiae is a fairly common parasite of cichlid fish in tropical Africa, in Lake Kariba, 63% of the Tilapia rendalli, and all four Oreochromis mortimeri examined harboured worms, of which, one specimen had over 100 worms\[5,6\].

The main objective of these studies is to determine the effect of the alimentary tract parasites on the haematological parameters for helminthes infected rabbit fish Siganus rivulatus in Red Sea Coast, Sudan.

**MATERIALS AND METHODS**

1.0 Materials:
1.1 The Collection of Fish Sample: Siganus rivulatus plate (3) used for this study was collected from Abu Hashish area using a cast net 3× 3 cm mesh size. Collections were performed during early morning. The fishes were divided into 4 groups on the basis of sex and infection, fifteen fishes are confined each group as follow: control male and female, infected male and female. The study period was extended for four weeks continuously from February to March 2007. The specimens were rapidly taken to the Research Laboratory of the Faculty of Marine Science and Fisheries to perform biological studies enlisted below. Fish identification followed\[1,26\].

1.2 The Collection of Blood Samples: Blood was collected immediately after capture by severing the caudal peduncle\[18\]. EDTA container then was put below the tail to collect drawn blood. Blood from each fish was collected separately and put into two containers containing EDTA: one for haematological tests and the other for plasma separation.

1.3 The Collection of Helminthes Parasites: First of all the skin, fins, gills, and opercula were examined for parasites. A ventral incision was made from the anus to the pectoral fin. General cavity wall was cut at the level of the superior part of stomach and the level of the rectum, the digestive tract was cut and deposited into a Petri dish. The gut is carefully dissected throughout its length with scissors (direct the interior scissors tip on the interior superiors wall of the gut in the goal not to cut the parasites present in the intestinal tube). The worm was removed from the hosts intestine into a Petri dish in saline water, gently washed and placed into marked bottle with very small amount of hot 4% formalin. The worms were identified by using light microscope according to\[32\] plate (4) and (5).

2.0 Methods:
2.1 Haematological Methods: Haemoglobin, PCV, RBCs count were determined by the methods described by Lea and Febiger\[20\].

2.1.1 Mean Corpuscular Volume (MCV): The MCV in cubic micron was calculated from RBC count and PCV values as follows:

\[
MCV(\text{fl}) = \frac{\text{PCV}(\%) \times 10}{\text{RBC count in million/m}^3}
\]

Mean Corpuscular Haemoglobin (MCH): The MCH was calculated from Hb and RBC values as follows:

\[
MCH(\text{pg}) = \frac{\text{Hb}(\text{g/dl}) \times 100}{\text{PCV}(\%)}
\]

2.1.3 Mean Corpuscular Haemoglobin Concentration (MCHC): The MCHC was calculated from Hb and PCV as follows:

\[
MCHC(\text{g/dl}) = \frac{\text{Hb}(\text{g/dl})}{\text{PCV}(\%)} \times 100
\]

2.2 Statistical Analysis: Statistical Package for Social Science (SPSS) program was used to run the data analysis with each test being conducted at 0.05% level of probability by t-test.

Results: The mean value of haematological, standard error of the mean are presented in Tables for collection groups A (control male), B (infected male), C (control female) and D (infected female).

Packed Cell Volume (PCV): The results of PCV level of group A and B shown in Table (1) and Fig (1). The level of PCV in group B is extremely significant.
different (P<0.000) lower compared with control group A. In group D the level of PCV also is extremely significant (P<0.000) lower compared to control group C as shown in Table (1) and Fig (2). Control group A has high mean compared with control group C, there was extremely significant (P<0.000) between both. Group B showed high mean than group D, there was extremely significant (P<0.000) between both.

**Haemoglobin (Hb):** The results of Hb level of group A and B shown in Table (1) and Fig (1). The level of Hb in group B is extremely significant different (P<0.000) lower compared with control group A. In group D the level of Hb also is extremely significant (P<0.000) lower compared to control group C as shown in Table (1) and Fig (2). Group A and group C showed extremely significant, group A mean is high
Table 1: Means of blood parameters of healthy and helminthes infected male and female *Siganus rivulatus*

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>PCV (%)</th>
<th>Hb (g/ dL)</th>
<th>MCV (fl)</th>
<th>MCH (pg)</th>
<th>MCHC(%)</th>
<th>RBCs(10⁶/µl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>26.66±0.66&lt;sup&gt;a&lt;/sup&gt;</td>
<td>8.40±0.34&lt;sup&gt;a&lt;/sup&gt;</td>
<td>86.09±2.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>27.33±0.80&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.34±0.74&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.16±0.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>13.94±0.53&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.67±0.18&lt;sup&gt;b&lt;/sup&gt;</td>
<td>86.09±2.69&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.76±0.80&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.48±0.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.46±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>C</td>
<td>19.28±0.58&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.49±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.34±2.74&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.54±1.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>33.45±0.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.21±0.10&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>D</td>
<td>9.46±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.17±0.11&lt;sup&gt;a&lt;/sup&gt;</td>
<td>87.82±2.26&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.39±0.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.69±0.79&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.09±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means ± (SE) within same column followed by different superscript small letters are significantly different at (P<0.05) based on t-test. * Group A = Control male, * Group B = Infected male, * Group C = Control female and * Group D = Infected female.

than of group C. Group B showed extremely significant in Hb as compared with group D hemoglobin mean is lower than of group B.

**Mean Corpuscular Volume (MCV):** Control group A and group B showed same MCV are shown in Table (1) and Fig (1). There was no significant differences (P>0.05) in group D compared with control group C as showed in Table (1) and Fig (2). There was significant (P<0.05) between group B and group D as same between group A and group C.

**4. Mean Corpuscular Haemoglobin (MCH):** The results of MCH level of group A and B shown in Table (1) and Fig (2). The level of MCH in group B is extremely significant different (P<0.000) lower compared with control group A, but there was no significant (P>0.05) between infected group D compared with control group C as shown in Table (1) and Fig (2). *Siganus rivulatus* MCH in group B (infected male) is significantly (p<0.007) higher than of group D (infected female). In contrast there was no significant between group A and C.
Fig. 1: Means of blood parameters of control and helminthes infected male *Siganus rivulatus*.

Fig. 2: Means of blood parameters of control and helminthes infected female *Siganus rivulatus*. 
Mean Corpuscular Haemoglobin Concentration (MCHC): The results of MCHC level of group A and B shown in Table (1) and Fig (1). The level of MCHC in group B is extremely significant different (P<0.000) lower compared with control group A. In group D, the level of MCHCs also is extremely significant (P<0.000) lower compared to control group C as shown in Table (1) and Fig (2). *Siganus rivulatus* MCHC in group B (infected male) is significantly (P<0.05) higher than of group D (infected female). In contrast there was no significant between group A and C.

Red Blood Cells (RBCs): The results of RBCs level of group A and B shown in Table (1) and Fig (1). The level of RBCs in group B is extremely significant different (P<0.000) lower compared with group A. In group D, the level of RBCs also is extremely significant (P<0.000) lower compared to control group C as shown in Table (1) and Fig (2). In group A the level of RBCs is significantly (P<0.000) high as compared to group C. As same RBCs level of group B is significantly (P<0.000) high as compared with group D.

**Discussion:**

1. **Packed Cell Volume (PCV):** Korzhuez stated that fish hematocrit values usually vary from 20 – 35%, but only attain values greater than 50%. The mean PCV value reported here for group A and C fall within this range but group B and D is lower than the normal value. This change in PCV may due to effect of helminthes parasites or due to seasonal variation these results is also reported by Mahoney[23]. Group A PCV mean was higher than group C, this attributed to sex differences. Also Sand[27] and Mulcahy[23] reported that sex differences in values were noticed, male fish has wider range as well as higher mean value. Differences in PCV values have been reported for sexually mature brown trout, *Salmo trutta*, rain bow trout, *Salmo gairdneri* and pike, *Esox lucius*[29]. PCV values and haemoglobin concentration were positively correlated in four groups.

**Haemoglobin:** Bernardf et al.[4] presented mean haemoglobin values for several species of fish ranging from 5 – 10g/dl; our study revealed haemoglobin means 5.68 g/dl for 60 *Siganus rivulatus*. These data for group A (control male) and group C (control female) within range of values found by Bernardf and other workers. The results further suggest positive correlations between haemoglobin concentration and RBCs count and PCV value as already mentioned. Haemoglobin mean of control male (group A) higher than of control female (group C), this appear to be related to the sex of the fish but many investigators have attempted to correlate relative haemoglobin concentration with fish activity i.e. higher fish activity means the higher haemoglobin concentration (Eisler, 1965). Haemoglobin values are one of the most thoroughly documented hematological indices for evaluating fish health; anemia has been diagnosed in fish[14,28].

There was reduction in haemoglobin concentrations below references values, this is may be due to the effect of helminthes parasites which was observed previously by Klinger et al.[17] who found that the signs of nematodiases include anemia, emaciation, unthriftness and reduced vitality.

**Red Blood Cells (RBCs):** Erythrocyte counts have proven to be highly variable blood parameters among fishes. Bernardf et al.[4] found RBCs range from 1 to 5×10^6/µL. Smith et al.[28] also found RBCs to range from a low mean of 1.24 million mm⁻³ in gold fish *Carassius auratus* to high mean of 1.88 million mm⁻³ in the warmouth *Leptomis gulosus* when comparing for ten species. Significant differences were found for RBCs count between group A, B and group C, D.

The combined data for 60 samples yield a mean value of 1.98×10⁶/µL. Denyes and Joseph reported a range of 1.72 to 3.48 million mm⁻³ for a large mouth basis, as well as a positive linear relation ship between blood cell count and haemoglobin concentration. Our data also support such relationship, but in addition we found a linear relationship between RBCs count and PCV. In the present work, group B and group D exhibited a decline in RBCs during study period. This reduction may be due to the effect of helminthes parasites. Iwama et al.[13] and Mahoney and McNulty[21] showed that erythrocytes counts may decrease with disease but it's unclear whether the decrease is due to the change in red cell number or simply a change in the hydration status of the fish. The results revealed that sex differences between all groups’ male fish showed a wide range than female fish. This also have been reported by Knoll[19] who found sex differences male pike having a wider normal range than female pike and also Ezzat et al.[10] found a high erythrocyte count in males than females of *tilapia zillii*. These appear to be related to actives of sexes, male being more active and also appears to be associated to ganado activity. Infected group (B & D) showed decrease in RBCs count as indicator of anemia. Kawatsu[14] found a decreased in the mean erythrocyte value to be 800,000/ mm³ using a brook trout *Salvelinus fontinalis* (Mitchill) control in an investigation of macrocytic anemia the count were 1092000 to 1555000/ mm³.
Mean Corpuscular Volume (MCV): Control male and helminthes infected male had statistically same mean corpuscular volume but there were no significant differences that were found for MCV between group C and D. Bernardf et al.\textsuperscript{[4]} proposed that the MCV average range from 150 to 350fl. In the fishes under study revealed mean 89.27fl for 60 samples. These variances may due to spawning season. Blaxhal and Daisley reported that at spawning season when the rate of erythropoiesis is greatest, MCV decline sharply since young red cells appear to be smaller than old red cell.

Mean Corpuscular Haemoglobin (MCH): Bernardf et al.\textsuperscript{[4]} presented MCH values for several species of fish runs between 30 and 100pg, the results were ranged 18.50pg - 29.26 pg for 60 samples, group A had lower MCH than group B but there is no statistically between group C and group D in contrast, there is higher significant difference between group B and group D which may be due to effect of sex hormones. This difference is unclear.

Mean Corpuscular Haemoglobin Concentration (MCHC): As with MCV and MCHC, group A showed lower values for MCHC than group B (P<0.05) and no significant between group C and D but there is significant different between group A and C which may be may be due to effect of sex differences. The MCHC range from 18 to 30 g/dl and is lower in fishes than in mammals due to the space occupying erythrocyte nucleus Bernardf et al.\textsuperscript{[4]}. The result revealed high value than these range.

Conclusions and Recommendations: From the finding of this study on haematological and biochemical of healthy and helminthes infected Siganus rivulatus from Abu Hashish area, the following are concluded: Siganus rivulatus healthy males had higher packed cells volume, haemoglobin and red blood cells counts as compared with healthy female. Helminthes infected male and female showed decrease in packed cell volume, haemoglobin and red blood cells count

REFERENCES


