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SANGUINICOLOSIS SPECIFICITY FACTORS IN SOME FRESHWATER FISHES WITH SPECIAL REFERENCE TO FISH SPECIES HAEMOGRAM AND SERUM ELECTROPHORESIS.

(With 2 Tables and 1 Figure: a,b,c,d,e)

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العوامل المؤثرة في الإصابة بمرض السانجوينكولا في بعض أسماك المياه العذبة مع التأكيد على تحليل الدم و التحليل الكهربى للسيرم

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أصبحت الآن السانجوينكولا (ديدان الدم) تحتل الصدارة بين طفيليات الأسماك خاصا بعد التوسع في الاستزراع السمكي .في هذه الدراسة تم فحص نوعين من اسماك المياه العذبة وهما المبروك العادي و القراميط .تم فحص الخياشيم والقلب والكلى وتم عزل ديدان السانجوينكولا الناضجة الحية من القلب و الكلى مع وجود بيض الديدان في الخياشيم يحتوى على يرقات٠.بفحص اسماك المبروك اثبت اصابتها بنسبة ٢٠% بديدان السانجوينكولا انرمس اما اسماك القراميط كانت الإصابة بنسبة ٨٠% بديدان السانجوينكولا دينتاتا. والفحص المعملي للأسماك كشف عن وجود استسقاء مصحوب بهزال كعلامة مميزة. تم تقييم تأثير السانجوينكولا على الأسماك من خلال صورة الدم وتشمل نسبة الهيموجلوبين وعدد كلا من خلايا الدم الحمراء والبيضاء. هذا بالإضافة إلى التحليل الكهربائي والمنحنى الترددي ونسبة البروتين الكلى في سيرم الأسماك٠ أثبتت التحاليل تباينا ملحوظا بين نوعي الأسماك المختبرة حيث كان التأثير اقوى في اسماك القراميط اذ انخفضت نسبة الهيموجلوبين والبروتين الكلى انخفاضا ملحوظا بينما كان الانخفاض فياسماك المبروك غيرملحوظ وسجلت خلايا الدم البيضاء ارتفاعا ملحوظا في نوعى الاسماك نظرا لزيادة الاجسام المضادة. بينما كانت صورة التحليل الكهربائي أوضح في اسماك المبروك حيث انخفض الالبيومين فينوعى الأسماك المختبرة. بينما سجلت صورة الالفا١ والبيتا جلوبيولين انخفاضا ملحوظا وسجلت الجاما جلوبيولين ارتفاعا ملحوظا كخط دفاع للأسماك .وجاء اختبار المنحنى الترددي لبروتين سيرم الأسماك المصابة و الغير مصابة الذي أجرى لأول مرة تأكيدا لنتائج التحاليل.. اثمرت محاولة العلاج في اسماك القراميط بدواء درونسيت (بارازكونتال) عن خلو الأسماك تماما من الديدان والبيض. وقد تحسنت صورة الدم تحسنا طفيفا في زيادة نسبة الهيموجلوبين وعدد خلايا الدم الحمراء ونسبة البروتين الكلى ولكن ظل هناك ارتفاعا في عدد خلايا الدم البيضاء

SUMMARY

Sanguinicola species are becoming nowadays important fish parasites as the aquaculture industry continues to expand .In this study, two cultured freshwater fish species namely *Cyprinus carpio* and *Clarias gariepinus* were examined for blood flukes (Sanguinicola). Examination of gills, heart and kidneys revealed the presence of live adult flukes in heart and kidneys, and eggs containing different miracidia stages in gill vascular structures. Examination of *C. carpio* revealed the infestation of 20% with *Sanguinicola inermis* (Plehn, 1905). On the other hand, examination of *C. gariepinus* revealed the infestation of 80% with *Sanguinicola dentate* (Paperna, 1964). The clinical examination of infested fishes showed the presence of ascites as the most obvious sign of disease and/ or emaciated appearance. Effect of Sanguinicoliosis on the two fish species was evaluated through holding blood haemogram: haemoglobin concentration, erythrocytic count, total leucocytic count, and serum electrophoretic analysis. In addition, the UV Absorbance frequency curve of serum protein was held. Species variation proved more effectivness with *C. gariepinus* where the haemoglobin and total protein levels highly significant decreased while the same parameters decreased in *C. carpio* non significantly. The total leucocytic count registered highly significant increase in both species resulting in raising antibodies level. On the contrary, the use of serum electrophoretic analysis proved that infestation of *C. carpio* was more evident where albumin fraction decreased in both species .The picture for the alpha, beta and gamma globulins was highly significant drop for the first two and highly significant increase with the third being the fish defence line. The use of UV Absorbance protein frequency curve applied for the first time approved the obtained data. A treatment trial was held using Droncit 10 mg \L for 1 hour\ 3 days. Fish were completely free from flukes and eggs. Blood analysis revealed a slight increase in haemoglobin level and RBCs count, the TP and WBCs count remained high.

Key words:*Sanguinicolosis, Freshwater fishers, Electrophoresis.*

INTRODUCTION

Sanguinicola sp. are fish vascular parasites which occupy a tremendous position among the digenetic trematodes parasitizing fish. It is substantiated as the most important fish parasite among the expanding

aquaculture industry especially in tuna fish, (Colquitt *et al.* 2001). The natural intermediate hosts are Lymnaeid snails which have been found in Egypt. The definitive fish host acquires infection by the direct penetration of cercariae. The post penetration larvae migrate to the blood circulatory system of the fish and mature directly into adults releasing eggs into the circulation. The eggs are then carried by the blood flow to the host tissues. Eggs reaching the gills mature and liberate miracidia which emigrate to the water, searching for snail host to penetrate and reproduce finally large numbers of cercariae (Kirk and Lewis, 1992). Recently, there has been an increased interest in the immunological interaction between economically important fish species and their pathogens (Iwama and Nakanishi, 1996). However, information regarding immune responses of teleost fish against helminth parasites is limited, although it has been suggested that immunological processes may play a role in regulating parasites burden in fish (Thomas and Woo, 1995). The separation and characterization of the serum protein components of infected and non-infected fish can help us to evaluate the host-parasites relationship, (Woo, 1992).

The best preventative strategy for controlling blood fluke infections in farmed fish is the destruction of the intermediate host snail and elimination of adult flukes from infected fish (Paperna, 1995). Little is known about the means of eradicating sexually matured trematodes of fish and their control through infected fish removal, (Schaperclaus, 1992). Praziquantel is safe and effective against digeneans of animals and preliminary trials demonstrated its parasiticidal effect in rainbow trout, (Paperna, 1995).

The present study aimed to search for the presence of fish blood flukes, prevalence and clinical signs in two cultured freshwater fishes and to evaluate the haematological changes resulting of natural infection with Sanguinicola. The second main aim is the parasite close specification by the aid of sera electrophoretic analysis and UV Absorbance protein frequency curve. A trial to treat infected *Clarias gariepinus* (as one of the most popular fish) was evaluated.

MATERIAL and METHODS

Examined Fish:

Fifty fishes were collected alive from earthen fishponds of Central Laboratory for Aquaculture Research at Abbasa fish farm, Sharkia Governorate (25 *Clarias gariepinus* and 25 *Cyprins carpio*). Fish were examined for any clinical signs, and then parasitological

examinations were done for blood flukes. Heart and its vessels were flushed with PBS, the washings.

Collected and examined under a steroscopic microscope. Gills and kidneys were also dissected and separately examined. The obtained flukes were collected, preserved in formalin (5%) and stained with Carmine. The stained flukes were identified microscopically according to Schmidt (1993). Fresh blood samples were collected from both species, infected and non infected with or without EDTA.

Haemogram Picture:

Edetated blood used for haemoglobin concentration (Hb), erythrocytic count (RBCs) and total leucocytic count (WBCs) according to Stoskopf (1993).

Total Protein Concentration:

Sera collected from non-edetated blood used to calculate total protein (TP) by Biuret (Wotton and Freeman, 1982).

Electrophoretic Analysis:

Electrophoresis was performed as described by Laemmli (1970). The gels were stained with coomassie brilliant blue R-250 (Sigma), and destained with mixture of 45% methanol ,10% acetic acid and 45% distilled water. Polyacrylamide gels were subjected to densitometric analysis and peaks integration was accomplished using a Zeineh Video Ultrascan Densitometer.

Protein Frequency Curve:

Sera collected monitored through U V Absorbance spectrum at wave length 200- 550nm (Cecil 3000 – instrument) according to Mahmoud and Siam (1996).

Treatment Trial:

Twenty alive *C.gariepinus* which proved to be heavily infested with Sanguinicola by parasitological examination and free from other blood parasites. Fish were divided into two groups'(ten each) one control and the other treated. Fish kept in aquaria with dechlorinated water at 20°C ± 0.5. The drug used Droncit (Praziquantel – Bayer) as bath treatment in dose 10 mg / L for 1 hour, for 3 sequential days (Paperna, 1995). Blood was drawn from caudal artery before treatment and three weeks after treatment and used for haemogram picture and Protein UV Absorbance Frequency curve evaluation.

Statistical Analysis:

The obtained data were statistically analyzed using t-student test according to Petrie and Watson (1999).

RESULTS

Out of 25 *C. carpio* examined fish only 5 (20%) harbourd *Sanguinicola inermis* (Plehn, 1905) while on the other hand in *C. gariepinus* the percent increased up to 20 fish (80 %) were infested with *Sanguinicola dentata* (Paperna, 1964). The clinical examination of infested fish showed signs of ascites and / or anaemic appearance with pale colour of gills especially in *C. carpio*. Fig. (D & E). Examination of heart and kidney revealed the presence of live adult flukes and eggs in the gills.

Haemogram Picture: Hb, RBCs, and TP decreased non significantly with *C. carpio* and highly significantly with *C. gariepinus* The WBCs increased highly significant among both species as Table (1).

Table 1: Blood Haemogram in healthy and infected *C.carpio* and *C. gariepinus* with Sanguinicola sp. and after Treatment (Mean ± S.E.).

Item	*C. carpio*		*C. gariepinus*		
	Non Infected.	Infected	Non Infected	Infected	Treated
Hb (g/dl)	7.7 ± 0.21	7.3 ± 0.18	8.6±0.3	5.1 ± 0.15***	7.8 ± 0.16*
RBCs (X106/ml)	1.8 ±0.21	1.2 ±0.15	2.8±0.18	1.2± 0.21	2.5 ± 0.16
WBCs (X103/ml)	47.5± 3	66.3±3.2***	25.6±1.35	50.6 ± 1.5***	53.8 ±2.65***
TP (g/dl)	5.4± 0.29	4.7 ± 0.39	4.9 ±0.31	3.0 ± 0.12***	2.7 ± 0.26***

Electrophoretic Analysis: Within the two species, infected fish the albumin decreased by slightly significant in *C. carpio* and non significant in *C. gariepinus*. Among the two species the alpha and beta fractions highly significant decreased while the gamma globulins recorded a highly significant increase. Fig. (A) and Table (2).

Protein Frequency Curve: The UV Absorbance frequency curve of non infected *C. gariepinus* revealed 1-2 peaks at W. L 404 & 515 nm while in the infected fish 3 new peaks appeared at W. L 207, 206 & 209 nm in addition to the previous peaks. In non infected *C. carpio* 3 peaks were evident at W. L 404, 515 & 205 nm. These peaks

were substituted by 4 others at W. L 515, 540, 277 & 411 nm in infected cases.

Table 2: Electrophoretic analysis of healthy and infected *C.carpio* and *C.gariepinus* with Sanguinicola sp.

Protein Fractions	*C. carpio*		*C. gariepinus*	
	Non Infected	Infected	Non Infected	Infected
Albumin (A)	30.6 ± 0.06	27.0 ± 1.5*	34.1 ± 0.3	24.4 ± 0.36***
T. Globulin(G)	73.0 ± 4.5	70 ± 6.66	76.2 ± 1.65	66.1 ± 2.11***
Alpha	30.2 ± 0.71	22.0 ± 0.39***	36.4 ± 0.28	26.5 ± 0.36***
Beta	25.1 ± 0.76	19.3 ± 0.38***	28.3 ± 0.17	22.4 ± 0.12***
Gamma	17.7± 0.74	28.6 ± 0.62***	11.5 ± 0.29	17.2 ± 0.41***
A / G Ratio	0.42 ± 0.038	0.39 ± 0.032	0.45 ± 0.035	0.37 ± 0.038

* Significant at P< 0.05 *** Significant at P < 0.001

Treatment Trial: Treating infected *C. gariepinus* succeeded as fish proved to be completely free from flukes and eggs. Blood haemogram reflected a slight increase in Hb content and RBCs but failed to reach normal levels. WBCs and TP remained highly significant increasing and decreasing respectively when compared to the non infected fish.

The UV Absorbance frequency curve of protein treated fish copied 3 peaks at W.L 224, 275 & 414. Fig. (B & C).

DISCUSSION

Blood flukes in cold – blooded vertebrates were first reported over a century ago but they have received relatively little attention especially in cultured freshwater fish.

The humoral immune response of infested fish against blood flukes infection was needed to be evaluated (Paperna, 1995).

In the present study, examined fish were naturally infested with different species of Sanguinicola. *C. gariepinus* infested with *S. dentata* (80 %) and *C. carpio* infested with *S. inermis* (20 %). All trematodes are host specific and presence of suitable vector snails in the habitat is a must for transmission (Paperna, 1996). The freshwater snail *(Lymnaea stagnalis)* is an intermediate host of Sanguinicola spp. (Smith, 1972) which is present in Egypt. The intensity, prevalence incidence of infection and fish losses depend especially on the density of snails in the pond. The clinical signs of infested fish coincided with Colquitt *et al.* (2001), who showed that chronic renal Sanguinicoliasis have signs of general dropsy and suggested that during escape of miracidia through the

gill epithelium causes loss of blood and lead to anaemia. Regarding the haemogram picture, Williams (1967) have stated that parasites may harm fish by introducing toxic metabolic by- products able to produce severe changes in blood. Both species registered a decrease in Hb level and RBCs count which was compatible with Naumova (1961) who found that infected *C.carpio* with *S. inermis* suffered of low Hb content and RBCs count. This was explained by Ronald *et al.* (1964) through the Sanguinicolids ability to affect fish blood capacity to carry and exchange gases by lowering the number of circulating blood cells.

Sanguinicola eggs subsequently become trapped in host tissues and elicit cellular immune responses: leucocytes (eosinophils, neutrophils and macrophages) which lead to encapsulation and destruction of eggs with the granulomatus lesions, Richards *et al.* (1994). The two examined species recorded highly significant increase in WBCs count which even remained after fish treatment. This could be referred to the leucocytes ability to adhere to the cercarial and adult stages causing their damage, Richards *et al.* (1996).

Martin (1960) found numerous Sanguinicola eggs in the liver, the organ of protein synthesis, resulting in its damage. Ezz- El Din & Mousa (1998) added that the presence of parasite in blood together with the involvement of the liver with parasite antigens resulted in lowering total protein content which appeared highly significant in *C. carpio* and *C. gariepinus* lasting even after treatment.

S. inermis being inhabitant of the vascular system , Richards *et al.* (1996) pointed out that it can produce extracts which break down host serum components . Sera analysis in both infected fish species pictured a decrease (more evidently with *C. carpio*) in both albumin and A/ G ratio which is referred to the hepatic dysfunction, Ezz- El Din & Mousa (1998). The data in hand registered a highly significant increase in gamma globulins (antibodies carrier) as a humoral defense mechanism performed by the infected fish against the parasite. This was confirmed by Ellis, (1988) who substantiated that the circulating antibodies usually migrated and were associated with the gamma globulins.

Finally came the UV Absorbance frequency curve approving the previous obtained data. The obviously differences in the peaks appeared a copy of the changes in the serum protein configuration due to the synthesization of more gamma globulins in blood as well as decrease of albumin. All these data concerning electrophoresis and UV absorbance

frequency curve for Sanguinicola are held out for the first time inside and outside the Egyptian media.

Thus, it is concluded that the drastic changes of Sanguinicola sp. on fish blood besides originating a parasitic diagnostic specificity through the serum protein alternations.

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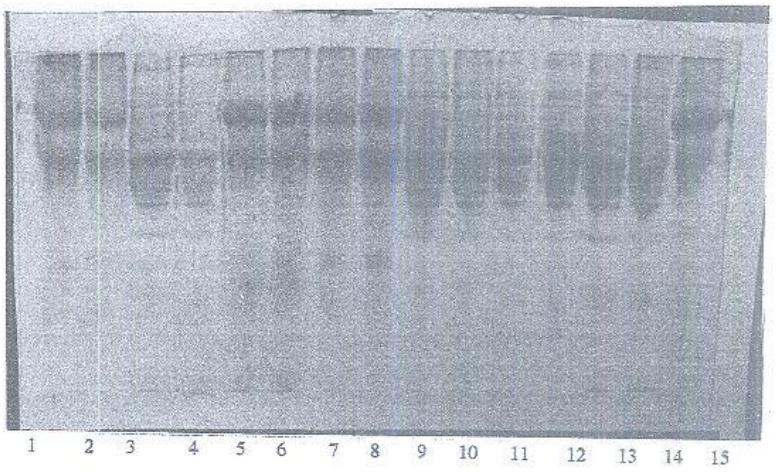

Fig A: Electrophoretic pattern of sera from infected and non-infected fishes with Sanguinicola.
Lanes 1, 2 and 15: Serum from infected C. gariepinus with Sanguinicola
Lanes 3, 4.13 and 14: Serum from non- infected C. carpio
Lanes 5-8: Serum from non- infected C. gariepinus
Lanes 9-12: Serum from infected C.carpio with Sanguinicola

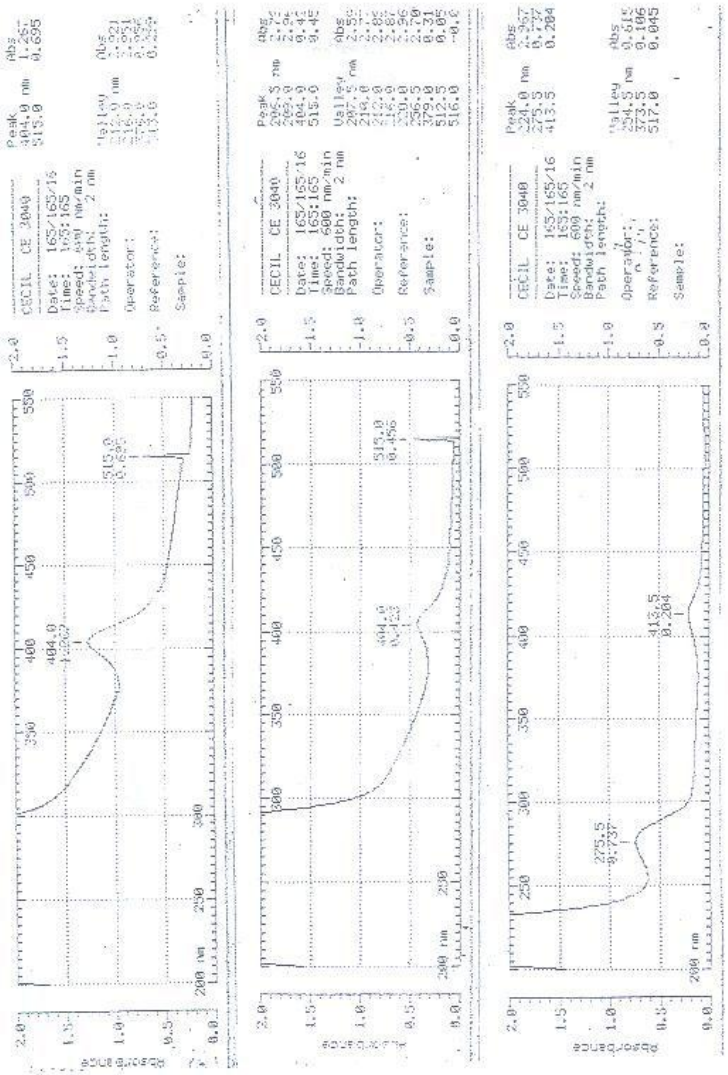

Fig. B: UV absorbance protein frequency curves of non infected, infected, & treated *C. gariepinus*.

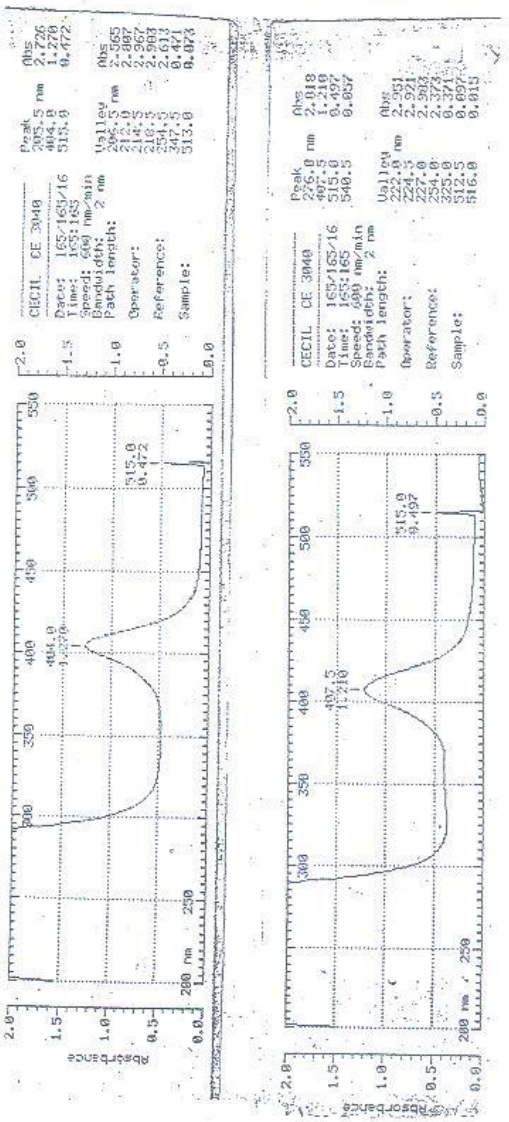

Fig. C: UV absorbance protein frequency curve of non infected. and infected *C. carpio*.

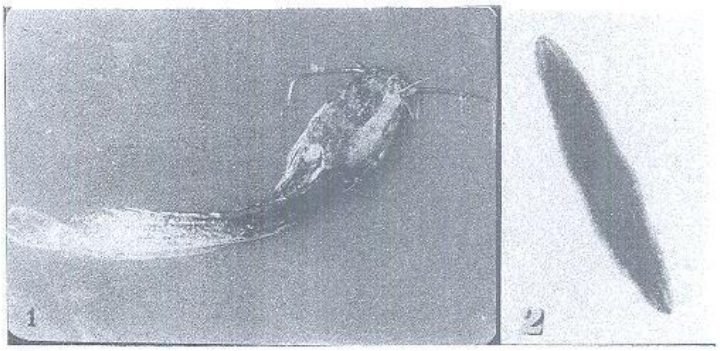

Fig. D: 1- *Clarias gariepinus* showing signs of anaemic appearance.
2- *Sanguinicola dentata.*

Fig. E: *Cyprinus carpio* showing signs of general dropsy.