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AN EXPERIMENTAL TRIAL FOR INFECTING THE SCAVENGER CATFISH *CLARIAS LAZERA* WITH *TRICHINELLA SPIRALIS* LARVAE WITH SPECIAL REFERENCE TO CERTAIN FISH BIOCHEMICAL REACTIONS.

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ABSTRACT

Scavenger catfish *Clarias gariepius* were experimentally infected with viable infective *Trichinella spiralis* larvae (1000 larvae/fish). Examination of the intestinal contents revealed the presence of viable larvae (that infect albino rats, xenodiagnosis) up to 48 hours post infection. Adult worms were not detected in the intestines of any of the experimentally infected fish.

Trichinella spiralis larvae were not detected neither in the muscles of the infected catfish nor in the diaphragms of albino rats fed on muscles obtained from the experimentally infected fish by the 40th day post infection. Thus, *C. gariepius* might be considered as a paratenic host for *T. spiralis* infection and could play a role in the epidemiology of trichinosis.

Sequential serum samples from each fish were collected 7 and 40 days post infection. Small but statistically significant changes, in alanine aminotransferase and aspartate aminotransferase occurred after infection. Serum concentration of total protein remained constant, indicating little disturbance of liver function. Infection significantly lowered the relative mobility of serum protein fractions separated by polyacrylamide gel electrophoresis on the 40th day post infection. The relative intensities of the more mobile fraction (# 1) significantly rose, while fractions # 3, 5 and 11 decreased 40 days post infection.

It is concluded that the immune response of *C. gariepius* is probably held responsible for the failure of *Trichinella spiralis* larvae to establish in this abnormal host.

INTRODUCTION

Trichinosis is a cyclozoonotic disease affecting man and a wide variety of animals. Carnivorism is the key in understanding the parasite epidemiology. Noting that larvae of *Trichinella* spp. remain infective in the decaying carcasses, Madsen (1976) has stressed the probable importance of carnivore carcasses in maintaining the sylvatic cycle. As the vast majority of all carcasses are consumed by scavengers, infection is widespread within a given biome and throughout the world (Kim, 1983).

A list of naturally infected animals takes an astounding five pages to complete in Campbell's treatise (Campbell, 1983). Even sea mammals and herbivores have been found infected with *Trichinella* spp. (Campbell, 1983 and MacLean *et al.* 1989). It is not difficult to imagine how the infection occurs in the case of carnivores, but it is of interest to note that *Trichinella* spp. larvae have been found in the flesh of marine mammals. Rausch (1962) reported natural infestation rates of 1:0 % in walrus (*Odobenus rosmarus*), 0.8 % in bearded seal (*Erignatus barbatus*) and 0.06 % in ring seal (*Pusa hispida*). Żimmerman *et al.* (1958) stated that larvae passed in the feces of infected carnivores, rats and other hogs, at the period when the excysted mature larvae become established in the intestine of these animals, constitute an additional source of exposure.

Susceptibility of non-specific hosts to *Trichinella* spp. infection has been studied by many authors. Cram (1941) recorded experimental infection in guinea pigs, monkeys, sheep, cattle, horse young chickens, pigeons, magpies and rooks. In addition, experimental *Trichinella spiralis* infection was recorded in camels by Bommer *et al.* (1980), in horses by Khalina *et al.* (1988), in sheep by Smith and Snowdon (1989) and in rabbits by Yacoub *et al.* (1993). Recently, Asatrian *et al.* (2001) were able to establish *Trichinella* spp. infection in the reptiles (*Lacerta agilis* and *Agama caucasica*) under certain environmental conditions.

Clarias gariepius is a tropical, highly nutritive, popular carnivorous scavenger fish which lives in derelict, shallow, swampy water highly infested with pathogens and micro-organisms. Little is known about the immunological capabilities of this commercially important air-breathing catfish. The strong lytic activity of this fish serum might play an important role in natural resistance to diseases (Sinha and Chakravarty, 1997).

Biochemical reference ranges for *C. gariepius* have been reported (Rizkalla, 1982, 1988; Soliman *et al.*, 1991 and Rizkalla *et al.*, 1999, 2003), but there is little information available on serum biochemical profiles of fish with specific disease (Husien and Elias, 2000).

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the key enzymes in the protein to carbohydrate metabolism. They have a wide distribution in fish tissues (Gaudet *et al.*, 1975). These enzymes may leak into the plasma, following reservoir tissue damage or dysfunction. Hence, changes in the activity of enzymes have been studied as possible tools for aquatic pathological research (Kristofferson *et al.*, 1974).

The concentration of total proteia in the blood plasma is one of the basic parameters in the haematological examination of fish. The content of plasmatic protein and the relative proportion of individual fractions are, among others, affected by the type of nutrition, the technology of rearing, and the condition and state of health of fish (Vlasov, 1974 and R´nyai *et al.*, 1982). Ingram (1980) mentioned that the serum of teleost fish contains various proteins that may play a role in the non-specific and specific defense systems during infection. Changes in the pattern of these serum proteins during infection may reflect the mechanisms of pathogenesis. In Atlantic salmon, *Salmo salar*, serum protein changes have been observed during infection with *Renibacterium salmoninarum* (Bruno, 1986), as well as in fish with ulcerative dermal necrosis (Mulcahy, 1969). So, proteins of blood serum are a fairly labile biochemical system.

The purpose of the present study is to clarify the susceptibility of the scavenger catfish *Clarias gariepius* to *Trichinella spiralis* infection and to evaluate possible damage by this introduced parasite to free-swimming catfish populations of commercial importance for Egyptian fisheries. In addition, study the possible role of this non-specific host in the epidemiology of the disease.

MATERIAL AND METHODS

Trichinella spiralis Larvae:

Strain of *Trichinella spiralis* obtained from naturally infected pigs was propagated and maintained in the laboratory through frequent passage in albino rats. *T. spiralis* larvae used for

experimental infection were obtained from infected rats 30 – 40 days post infection.

Experimental Animals:

1. At the 15th of April, male catfish *Clarias gariepius* with body length ranged from 25.0 to 31.4 cm were obtained from the Nile River near El-Aiat (Giza Governorate). These fish were kept in 8-well aerated glass aquaria (6 fish/aquarium "187.5 Litre") and supplied with dechlorinated tape water. Water temperature was not controlled and recorded twice a day at 9 A.M. and 4 P.M. throughout the experiment. Water was partially changed every day and completely changed every 3 days. Fish fed once daily at 9 A.M. on cooked chips of poultry intestines. After 14 days of adaptation, fish were allocated into 2 groups:
   Group (I): Control group of 12 fish.
   Group (II): Thirty six fish given excysted *T. spiralis* larvae/fish.
2. Uninfected albino rats used for:
   - Maintenance and propagation of infected *T. spiralis* larvae.
   - Xenodiagnosis of experimental infection through detection of viable and infective *T. spiralis* larvae in the intestines of the experimentally infected fishes (first few days after infection) and in fish muscles at the end of experiment (40th day post infection).

Experimental Design:

1- Recovery and collection of the larvae from the muscles of infected pigs and rats were done through:
   - Trichinoscopic examination according to the technique described by Thornton and Gracey (1974).
     Separation and isolation of *Trichinella spiralis* larvae from trichinous pig, rat and fish muscles through a combined digestion and Baermann technique (Henriksen, 1973).

2- Preparation of infective larval dose:
   - The collected *T. spiralis* larvae were suspended in 20 % gelatin saline (37°C). After a thorough mixing, 0.1 ml of the suspension was spread onto a microscope slide and the whole larvae were counted to determine the appropriate infective dose (1000 larva/fish). A minimum of three counts were made to determine the mean count/ 0.1 ml of suspension (Mikhail and Tadros, 1973).
   - The infective dose was dispersed into firm gelatinous capsules and pushed into the stomach of the catfishes of group II.

Parasitological Examination:

Five fishes from the experimentally infected group (Group II) were

slaughtered on the 1st, 2nd, 3rd, 4th and 7th days' post infection. Intestinal contents were examined by direct smears for the presence of *T. spiralis* larvae or adult worms and the whole gut were fed to a group of three non infected albino rats which were then killed on the 40th day post feeding and examined for the presence of encysted *T. spiralis* larvae according to Beck (1953).

By the end of experiment (40th day post infection), fishes were slaughtered and parasitologically examined for the presence of encysted muscular *T. spiralis* larvae through: trichinoscopy (Thornton and Gracey, 1974), combined digestion and Baermann technique (Henriksen, 1973) and feeding of fish muscles to non infected albino rats which were killed on the 40th day post feeding and examined for the presence of encysted *T. spiralis* larvae according to Beck (1953).

Biochemical Examination:

Blood samples were collected on the 7th and 40th day post infection from fishes of the groups I and II. Serum were separated and used to determine total proteins (King and Wootton, 1959); aspartate and alanine aminotransferases (Reitman and Frankel, 1957). The freshly separated serum was fractionated using 7.5 % polyacrylamide gel electrophoresis (Herzberg and Pasteur, 1975). Gels were stained by Amido black 10B and destained by 7 % acetic acid. The cleared fractions were scanned by computing photoelectric densitometer (Gelman DCD-16) at a wave length of 600 nm.

Statistical analysis:

t-test was analyzed from the obtained data using Microstate software version 2.01.

RESULTS

During the study, the mean water temperature was 18.6±3.5 °C.

Parasitological results:

Table (1) shows that the intestinal contents of the scavenger catfish *Clarias gariepius* given *Trichinella spiralis* larvae had viable larvae (that infect albino rats, xenodiagnosis) up to the 48 hours post infection. Adult worms were not detected in the intestines of any of the experimentally infected *C. gariepius* fish. By the 40th day post infection, *T. spiralis* larvae were not detected, neither in the muscles of the infected catfish, nor in the diaphragms of albino rats fed muscles of the experimentally infected catfishes.

Serum biochemical results:

The biochemical analyses are summarized in Tables 2, 3 and 4. Serum total protein of both control and experimentally infected *Clarias gariepius* with *Trichnilla spiralis* showed a significant decrease with time (from 7 to 40 days). No significant effect was detected in serum total protein due to *T. spiralis* infection (Table 2).

In experimentally infected fish, both ALT and AST showed a significant decrease with time (Table 2). Serum ALT concentration was significantly ($P<0.02$) lower in infected fish than in the control ones after 40 days, while serum AST concentration was significantly ($P<0.05$) higher in infected fish than in the control ones after 7 days (Table 2).

Polyacrylamide gel fractionation of serum protein demonstrated 12 fractions for both control and experimentally infected *Clarias gariepius*. The fractions were classified according to their relative mobility into three main groups (Table 3): distal fractions (# 2 & 3); mid fractions (# 4 - 8); proximal fractions (# 9 - 11) in addition to the fraction # 1 (fastest one) and fraction # 12 which is the closest one to the point of application. After 40 days, fraction # 1 was separated into 2 sub-fractions (1_a & 1_b) in both control and infected groups. 7 days after infection, fractions # 4, 5 & 12 showed a significantly higher relative mobility than that of the control. After 40 days, all fractions (except fractions # 2 & 4) showed a significantly lower relative mobility than that of the control (Table 3). The relative mobility of different fractions decreased with time. This observation was significant in fractions # 1_b, 8 & 11 in control fish and in all fractions in infected fish.

The relative area values of serum protein fractions shown in Table 4 demonstrate the concentration of protein in each fraction. After 7 days of infection, fraction # 3 showed a significantly ($P < 0.01$) lower value than that of the control, while fractions # 7 & 8 showed significantly higher values than those of the control. After 40 days of infection, fraction # 1_a showed a significantly higher value, while fractions # 3, 5 & 11 showed significantly lower values than those of the control. According to time, the relative areas of serum protein fractions fluctuated. In the control group, fractions # 1_a, 5 & 8 showed significantly increased values, while fractions # 6 & 7 showed significantly decreased values. In the infected group, fractions # 1_a & 5 showed significantly increased values, while fractions # 6, 7, 8, 9 & total proximal fractions showed significantly decreased values.

DISCUSSION

Table (1) shows that catfish (*Clarias gariepius*) might be considered as a paratenic host for *T.spiralis* infection and could play a role in the epidemiology of trichinosis. The results displayed in Table (1) are comparable with those recorded by Cram (1941) and Yacoub *et al.* (1993) in chickens, who mentioned that failure of detecting *T. spiralis* larvae in different tissues of non-specific hosts might be due to host specificity or dose and duration of infection. Presence of viable *T. spiralis* larvae in the intestinal contents of the scavenger catfish during the 48 hours post infection agreed with Yacoub *et al.* (1993) who stated that chickens (non-specific host) can act as a paratenic host during the first 48 hours of the experimental infection.

Presence of viable *T. spiralis* larvae within the intestinal contents of the catfish under study could explain and support the role played by fishes in the scavenger-marine mammal relationship explained by Thomas (1973) in which: 1- Carcasses of trichinous animals were consumed by a variety of scavengers (ranging from crabs to sea gulls). 2- The encysted larvae were transported and discharged with the scavenger feces over a wide area of sea and shore where both fish and crustaceans can pick up and transport trichina larvae to the marine mammals. These findings point to the importance of prohibiting contamination of water passages with offal, garbage or animal carcasses.

The activity of serum aminotransferases are good correlates of the health of reservoir organs (LaDue *et al.*, 1954). Since there exist kinetic equilibrium between serum and tissue levels of both alanine and aspartate aminotransferase and other enzymes, any increase in the serum levels of these enzymes may likely be due to imbalances in the physiology and/or anatomy of the reservoir tissues. In the control group of the present study (Table 2), the concentrations of ALT (19.333±6.356 and 14.667±1.862 IU/L after 7 and 40 days respectively) and AST (22.667±5.164 and 18.667±10.053 IU/L after 7 and 40 days respectively) are much higher than that recorded by Husien and Elias (2000) in the same fish species (5.3±2.3 and 14.5±4.1 IU/L/ml for ALT and AST respectively). Experimental infection with *Trichinella spiralis* significantly ($P < 0.05$) raised the concentration of AST to 36.143±12.034 IU/L after 7 days, whereas after 40 days the enzyme level decreased insignificantly. In case of ALT, the concentration was lowered after both time intervals, but it

showed a significant ($P < 0.02$) value (9.333±3.983 IU/L) after 40 days. Davis (1995) observed no significant differences for both ALT and AST concentrations on the 4th and 11th days post-exposure of farm-reared channel catfish *Ictalurus punctatus* with *Ichthyophthirius multifiliis* (ciliate) infection. Husien and Elias (2000) revealed an insignificant effect on serum AST and ALT levels by bacterial infection (*Pseudomonas fluorescence* and *Flavobacterium* sp.) in *C. gariepius* and a highly significant increase in *Oreochromis niloticus*. Thus, the observed increase in the AST activity in *C. gariepius* infected with *T. spiralis* may be due to tissue damage, particularly the liver and/or physiological perturbations following infection. The observed significant increase of serum AST activity after 7 days of infection could be a manifestation of the general adaptive response in animals. This postulation was supported by the significantly declining value with time after infection for both AST (36.143±12.034 and 10.000±7.720 IU/L on the 7th and 40th days respectively, $P < 0.001$) and ALT (16.143±5.581 and 9.333±3.983 IU/L on the 7th and 40th days respectively, $P < 0.05$) due to absence of the parasite in the muscles as shown in Table 1.

Average serum levels of total protein in control and infected *Clarias gariepius* are presented in Table 2. The control value after 7 days (6.533±1.426 g/dl) is higher than that recorded by Rizkalla (1982) "5.128±0.824 g/dl"; Soliman *et al.* (1991) "4.525±0.194 g/dl"; Moussa *et al.* (1994) "4.150±0.212 g/dl"; Husien and Elias (2000) "4.7±0.7 g/dl" and is similar to Rizkalla (1988) "6.699±0.642 g/dl" on the same fish species. The control value at 40 days (4.733±0.575) is similar to that recorded by Moussa *et al.* (1994) at 35 and 42 days (4.420±0.169 and 4.800±0.283 g/dl respectively).

Table 2 shows that the serum protein levels were insignificantly decreased at both time periods in infected fish. Rizkalla (1982), working on the effect of helminthes infestation on *C. gariepius*, reported no remarkable changes observed in total serum protein and ascribed this to the fact that *Clarias* had initially a high total serum protein level that had not been markedly affected by infection. Also plasma proteins in *Oncorhynchus mykiss* infected with a tissue dwelling fish pathogenic fungus were not changed over a 6 week period (Rand and Cone, 1990). On the other hand, Mahoney and McNulty (1992) reported that most diseased winter flounder (*Pleuronectes americanus*) had a significantly lower level of plasma protein than healthy fish. Boon *et al.* (1990) concluded that infection of *Anguilla anguilla* with infective larvae of *Anguillicola crassus* may

decrease the plasma proteins. Comparisons between serum total protein concentrations on the 4th and 11th days post-exposure of farm-reared channel catfish *Ictalurus punctatus* with *Ichthyophthirius multifiliis* (ciliate) infection showed a small, but statistically significant drop (Davis, 1995). Also MØyner (1993) reported that the serum protein levels were significantly decreased in infected Atlantic salmon, *Salmo salar*, by *Aeromonas salmonicida*. Husien and Elias (2000) revealed a significant decrease in the serum total proteins in *C. gariepius* and *Oreochromis niloticus* infected by two bacterial strains (*Pseudomonas fluorescence* and *Flavobacterium* sp.) The comparison of the total serum protein levels of the two intervals of time in both control and infected groups in the present study demonstrated significantly decreased values after 40 days that may be attributed to impairment in protein biosynthesis or an increase in kidney excretion.

Polyacrylamide gel electrophoresis is the most effective method of investigation which provides an excellent opportunity to separate and calculate the ratio of protein fractions when influenced by any disease (Golovnev *et al.*, 1982). In this trial, emphasis has been focused on the mobility and percentage of the different protein fractions separated on the polyacrylamide gel (Tables 3 & 4). Twelve bands were identified in serum proteins of *C. gariepius* in both control and infected groups. Rizkalla (1988) mentioned that as many as 13 bands were discernible in some gels although 10 was the maximum number that appeared with consistency. Soliman *et al.* (1991) reported to 11 fractions while Rizkalla *et al.* (1999) recorded 10. These variations of electrophoretic studies might be a result of changes in the physiological and environmental conditions (Meisner and Hickman, 1962).

The relative mobility of the different protein fractions was significantly decreased in *C. gariepius* experimentally infected with *T. spiralis* larvae by the 40th day (Table 3). Previous studies revealed that the pathogenic infection strongly affected the mobility of serum protein fractions of *Salmo salar* (Mulcahy, 1969); *Clarias gariepius* (Rizkalla, 1982) and *Anguilla anguilla* (Höglund *et al.*, 1992).

Table 4 shows the relative intensity of each serum protein fraction separated by polyacrylamide gel electrophoresis. The intensity of the more mobile fraction (# 1) of infected fish on the 40th day is significantly ($P < 0.001$) higher (13.931±1.948 %) than that of the control fish (7.107±1.998 %). This fraction could be defined as the albumin fraction since its mobility on the disc gel is similar to that

of human serum albumin as was speculated by Komatsu *et al.* (1970) and proved by Nagano *et al.* (1975). However, its concentration is low which is harmonizes with the current hypothesis that albumin concentration is always very small, or even absent, in freshwater bony fish (Sulya *et al.*, 1961). Albumin has transport and carrier functions, participates in osmoregulation of blood and is a reserve protein of the organism (Vlasov, 1974). Low concentrations of this fraction were found in diseased and starved individuals; on the other hand, an intensive supply of food resulted in high levels of this fraction (Vlasov, 1974 and Rónyai *et al.*, 1982). Boon *et al.* (1987) suggested that the plasma protein fraction # 1 is an indicator for the health status of young *Clarias gariepinus*. Rizkalla *et al.* (1999) found that the intensities of the more mobile fractions were significantly lower in the +ve non-toxogenic *Clostridium perfringens* infected *C. gariepius* than in the –ve group. The decrease in serum albumin could be attributed to its disturbed synthesis because of hepatic functional impairment (Bano and Hasan, 1990). So the fraction of albumin, both in relative and absolute values or in relation to globulins (A/G), is considered to be an important criterion of the state of health and condition of fish (Paláčková *et al.*, 1992). On that basis, we can attribute the insignificantly lower value of fraction # 1 on the 7^{th} day post infection with *T. spiralis* then the subsequent significant rise on the 40^{th} day to the recovery after infection.

The distal fractions (# 2 & 3) could be described as α_1 and α_2-globulins. Fraction # 3 showed significantly lower percentages in infected *C. gariepius* on the 7^{th} and 40^{th} days of infection (Table 4). An increase in the α-globulin fraction was recorded in diseased individuals (Rónyai *et al.*, 1982). The increase in α_2-globulin fraction may be attributed to an increased synthesis of acute phase proteins, which act as a buffer or as protective proteins against rapid a wide variety of inflammatory reactions and bacterial infections (Gewruz, 1982).

According to their mobility, the mid fractions (# 4 – 8) could be described as β-globulins (Table 4). Transferrin is the β-globulin protein in plasma which transports plasma iron and has antioxidant properties (Sheila and Brock, 1992). Fractions # 9, 10 and 11 (proximal fractions) which are the least mobile fractions could be assessed as the gamma globulins. *C. gariepius* experimentally infected with *T. spiralis* larvae had a significantly ($P < 0.05$) lower percentage of fraction # 11 on the 40^{th} day of infection than that of control group. In the same time, fraction # 9 and the total proximal

fractions showed significantly lower percentages in the infected fish on the 40th day than on the 7th day (Table 4). Golovnev *et al.* (1982) represented γ-globulins by one or two components in 70 % of the fish experimented. Soliman *et al.* (1991) observed that the concentration of γ_1- and γ_3-globulins significantly differs in male *C. gariepius* with time. These denote that time factor is functioning. The fraction of the γ-globulin includes immunoglobulins which are essential for the formation of antibodies. A higher percentage of the γ-globulin fraction was found in diseased fish (Rónyai *et al.*, 1982). Differences were also observed in the carp reared under different conditions and at different levels of nutrition (Vlasov, 1974 and Rónyai *et al.*, 1982). Höglund *et al.* (1992) said that the marked increase in the γ-fraction of serum proteins of the European eel, *Anguilla anguilla*, naturally infested by the sanguivorous nematode *Anguillicola crassus* is indicative of a humoral immune response. Highly significant positive correlations were also noted in this fraction with increasing numbers of parasites, and with an increased parasitization index (i.e. the weight of parasites per somatic weight of the host). Diseased salmon (*Salmo salar*) with acute furunculosis had a decreased intensity of serum protein of less mobile peaks as compared with the control pattern. This decreased intensity suggests that one or more proteins within this range had been consumed or degraded (Møyner, 1993). In the study of Rizkalla *et al.* (1999), the *C. gariepius* that had +ve non-toxogenic *Clostridium perfringens* in its intestine showed significant increases in the serum protein γ-globulin. Engle *et al.* (1958) proved that the cyclic metabolic pattern, proposed to be the cause of the changes in total serum protein, is in fact restricted to a cyclic pattern of serum globulin metabolism. Helmy *et al.* (1974) considered serum globulin fraction, more or less, a truthful mirror reflecting the cyclic nature of total serum protein.

From the previous discussion, we can conclude that the immune defense of *Clarias gariepius* is responsible for the failure of *Trichinella spiralis* larvae to establish in this abnormal host. Williams (1969) said that serum antibodies secreted in intestinal mucus are probably responsible for maintaining the host-specificity of the tetraphyllidean cestode *Acanthobothrium quadripartitum*, which occurs in *Raja naevus* in Scottish waters, but not in *R. radiate*, although both species occupy the same habitat and eat the same food. Physiological factors may influence the development of species of the pseudophyllidean *Bothriocephalus* in its teleost hosts. *B.*

acheilognathi, which does not have strict host-specificity, will infect almost all carp species, but its successful development depends on the physiological characteristics of the intestine of the host. It will establish in hybrids of its most common hosts, common and grass carp (common carp X silver carp, bighead X grass carp, crass carp X bighead) (Molnár *et al.*, 1984).

With regard to development and reproduction of a parasite in a host, an extensive literature has shown that they are directly related to temperature (Kennedy, 1971; Skörping, 1981 and Gelnar, 1987). A correlation between body temperature of host and metabolic rate of parasite was demonstrated by Vernberg and Hunter (1961). Watson *et al.* (1998) found that the effect of water temperature fluctuations on fish survival partly reflects the combined influence of temperature on pathogen development and the host immune response. Such studies indicated that humoral and cell-mediated defense mechanisms can be suppressed by temperatures averaging 10°C below the host's optimal growth temperature (Stolen *et al.*, 1984). In this respect, Asatrian *et al.* (2001) were able to obtain complete development of *Trichinella* sp. muscle larvae in the reptiles *Lacerta agilis* and *Agama caucasica* held at 37-40 °C environmental temperature. Such a result arouses an inquiry about the exact rule of environmental temperature in the completion of *Trichinella* sp. cycle in scavenger fishes (the present water temperature is 18.6±3.5 °C) that needs further investigation.

Conclusion and recommendations:

As trichinosis constitutes a serious public health hazard, it should be kept under continuous surveillance. It may not be possible to eradicate *Trichinella spiralis* but measures should be implemented to prevent it from entering human food chain and limit its spread. Dogs and cats fed fish offal that might contain trichina larvae could play a role in perpetuating trichinosis infection to various animal species and consequently man. Legislation should be enforced up to criminality for contaminating water passages with offal, garbage or carcasses. All possible measures should be taken for sanitary disposal of offal, garbage and carcasses and to properly eliminate rats, stray dogs and cats.

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Table (1): Results of xenodiagnosis examination of the intestine and muscles of the *Clarias gariepius* given *Trichinella spiralis* larvae (1000 larvae / fish).

Time of examination	Fish intestines N = 6		Fish muscles n = 6
	No. of + ve*	% of + ve	No. of + ve*
24 hours P.I.	6	100 %	---
2nd day P.I.	4	66.67 %	---
3rd day P.I.	None	0 %	---
4th day P.I.	None	0 %	---
7th day P.I.	None	0 %	---
40th day P.I.	---	---	None

* Samples are considered positive by finding encysted muscle larvae in infected rats.

Table (2): Some biochemical parameters in the serum of control and experimentally infected *Clarias gariepius* with excysted *Trichnilla spiralis* larvae after 7 and 40 days of infection.

Parameter	Control				*Trichnilla spiralis* larvae infection			
	7 days (6)		40 days (6)		7 days (7)		40 days (6)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
Total proteins (g/dl)	6.533	1.426	4.733	0.575[b]	5.400	0.614	4.533	0.547[a]
ALT (IU/L)	19.333	6.346	14.667	1.862	16.143	5.581	9.333	3.983[**a]
AST (IU/L)	22.667	5.164	18.667	10.053	36.143	12.034[*]	10.000	7.720[d]

( ): Number of samples. S.D.: Standard deviation.

Significant in comparison with the control: * $P < 0.05$ and ** $P < 0.02$.

Significant in comparison between 7 and 40 days; a: $P < 0.05$, b: $P < 0.02$ and d: $P < 0.001$.

Table (3): Relative mobility values of polyacrylamide gel serum protein fractions in the control and experimentally infected *Clarias gariepius* with excysted *Trichnilla spiralis* larvae after 7 and 40 days of infection.

Fraction	Control				*Trichnilla spiralis* larvae infection			
	7 days (6)		40 days (6)		7 days (7)		40 days (6)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
l_a	-	-	100	0	-	-	100	0
l_b	100	0	90.647	1.611^{d}	100	0	86.085	0.563^{****d}
Distal fractions:								
2	83.155	0.831	80.819	5.724	84.308	1.154	78.381	1.069^{d}
3	77.285	0.997	76.834	5.037	76.709	1.015	71.240	1.901^{*d}
Mid fractions:								
4	63.614	1.857	65.894	5.057	66.751	0.979^{***}	60.943	3.223^{d}
5	55.985	0.204	56.399	2.611	58.261	2.230^{*}	51.042	1.215^{***d}
6	47.678	0.627	46.328	1.907	49.525	2.530	41.214	1.841^{****d}
7	38.179	1.472	37.986	3.856	40.746	2.566	34.162	1.237^{*d}
8	24.511	1.740	22.446	1.379^{a}	27.733	4.095	19.271	1.189^{***d}
Proximal fractions:								
9	21.408	2.229	20.142	0.931	23.551	4.535	16.308	1.843^{***c}
10	10.465	1.695	11.364	1.439	12.535	4.367	7.553	1.710^{***a}
11	8.731	1.606	6.763	0.231^{b}	10.428	4.340	4.651	1.565^{***b}
12	2.084	0.109	2.014	0.443	2.831	0.404^{***}	1.098	0.321^{***d}

( ): Number of samples. S.D.: Standard deviation.

- : Fraction not detected.

Significant in comparison with the control:

* $P < 0.05$, ** $P < 0.02$,

*** $P < 0.01$ and **** $P < 0.001$.

Significant in comparison between 7 and 40 days;

a: $P < 0.05$, b: $P < 0.02$,

c: $P < 0.01$ and d: $P < 0.001$.

Table (4): Relative area values of polyacrylamide gel serum protein fractions in the control and experimentally infected *Clarias gariepius* with excysted *Trichnilla spiralis* larvae after 7 and 40 days of infection.

Fraction	Control				*Trichnilla spiralis* larvae infection			
	7 days (6)		40 days (6)		7 days (7)		40 days (6)	
	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.	Mean	± S.D.
I_a	-	-	2.732	0.742^{d}	-	-	11.133	1.925^{****d}
I_b	4.411	1.437	4.375	2.507	3.927	1.073	2.798	0.784
I_{Total}	4.411	1.437	7.107	1.998^{a}	3.927	1.073	13.931	1.948^{****d}
Distal fractions:								
2	4.102	1.679	4.615	0.434	7.010	3.288	4.189	4.846
3	20.848	1.318	23.040	3.660	16.179	3.217^{***}	15.302	6.492^{*}
Total	24.950	2.945	26.117	5.299	23.189	5.193	19.491	5.627
Mid fractions:								
4	12.387	3.859	12.961	4.555	9.490	2.910	13.197	5.046
5	15.662	3.274	25.997	3.900^{d}	14.238	3.247	20.259	2.575^{**c}
6	10.673	3.195	5.606	1.843^{c}	10.050	2.512	6.644	1.764^{b}
7	11.279	0.164	5.920	4.027^{c}	13.301	1.828^{*}	8.775	0.918^{d}
8	1.580	0.273	2.222	0.110^{c}	3.671	0.383^{****}	1.544	0.650^{d}
Total	51.582	2.754	51.966	2.200	50.750	6.122	50.419	3.179
Proximal fractions:								
9	9.137	3.089	7.620	1.247	11.492	1.155	8.991	2.590^{a}
10	1.283	0.412	1.111	0.097	0.868	0.281	1.064	0.198
11	7.485	1.983	7.620	1.247	7.658	2.711	5.664	1.452^{*}
Total	17.477	5.338	15.981	2.638	20.018	2.252	14.598	2.910^{c}
12	1.580	0.273	1.478	0.305	2.116	0.612	1.873	0.865

( ): Number of samples. S.D.: Standard deviation.

- : Fraction not detected.

Significant in comparison with the control:

* $P < 0.05$, ** $P < 0.02$,

*** $P < 0.01$ and **** $P < 0.001$.

Significant in comparison between 7 and 40 days;

a: $P < 0.05$, b: $P < 0.02$,

c: $P < 0.01$ and d: $P < 0.001$.