Cross Infection and Treatment Trials of Trypanosomiasis in Some Freshwater Fishes

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Abstract

A total number of 170 fishes were collected and represented as 30 *Trypanosoma mukasai* free fishes (10 *Clarias gariepinus*, 10 *Oreochromis niloticus* and 10 *Cyprinus carpio*) used in the cross experimental infection. In addition to 140 naturally infected *C. gariepinus* which used in the treatment trials.

The main clinical picture of the naturally infected *C. gariepinus* were skin abrasions, off food, emaciation, dullness and respiratory manifestations.

Blood was withdrawn from the caudal blood vessels of the naturally infected *C. gariepinus* using a heparinized syringe and injected I/P and I/M into *C. gariepinus*, *O. niloticus* and *C. carpio.* All injected fishes were examined for *Trypanosoma mukasai* infection for 2 weeks. Experimentally infected *C. gariepinus* and *O. niloticus* showed positive infection through both routes while, *C. carpio* showed positive infection through I/P route only.

The treatment trials revealed that the most effective treatment was *Artemisia annua* bath with a dose of 150 mg/l for 120 minutes followed by Trypano-Ject as I/M injection then Praziquental as a bath treatment.

Key words: Freshwater fish – trypanosomiasis – cross infection – *Clarias gariepinus* - *Oreochromis niloticus* – *Cyprinus carpio* - Trypano- Ject – *Artimisia annua* - praziquental

Introduction

Fish blood parasitic diseases were recorded in Egypt with high percentage due to the long periods of optimum warm water and consequent abundance of natural food as well as the availability of the intermediate hosts (Cyclops and mollusca and leeches) which affect fishes directly causing high morbidity, mortality or indirectly by lowering the body gain and results in high economical losses ***(Eissa et al., 2008 and Muhammad et al., 2017)***.

Parasites belonged to genus Trypanosoma (Kinetoplastida: Trypanosomatidae) are ubiquitous protozoans that infect a wide range of animals, including leeches, insects, fish, amphibians, reptiles, birds, and mammals. They are the

causative agents of some of the most neglected human and animal diseases ***(Fraga et al., 2016).*** Sleeping sickness disease caused by haemo-flagellates of genus Trypanosoma, was regarded as one of the most important economical internal diseases affecting freshwater fishes and transmitted by leeches as vectors, such disease makes affected fish anaemic, dull with slack appearance, thus, secondary infection as well as cannibalism may be occurred ***(Eissa et al.,1996).***

A multiplicative development of *Trypanosoma mukasai* occur in the gut of freshwater leech (*B. tricarinata)* before it infects *Clarias lazera* ***(Negm El-Din, 1997)***. The forms in the blood stream transformed into short flagellates and divided after 2 days of infection by binary fission to produce numerous epimastigotes which transformed to sphaeromastigotes, amastigotes, promastigotes and metacyclic trypomastigotes. ***Figueroa et al. (1999)*** stated that blood protozoa (Trypanosoma sp.) are not host specific to fish. While, ***Negm El- Din and Davies (1999)*** detected that, in Egypt *Trypanosoma mukasai, Babesiosoma mariae* and *Haemogregarina nili* were simultaneously and experimentally transmitted from *Tilapia nilotica* (*O. niloticus*) to *Clarias lazera* using leech vector.

Regarding control of trypanosomiasis in fish, ***Mishina et al. (2007)*** reported that Artemisinin compounds inhibit invitro growth of cultured *Trypanosoma cruzi* and *Trypanosoma bruceirhodesiense* at concentrations in the low micromolar range. Artemisinin also inhibits calcium-dependent ATPase activity in *T. cruzi* membranes, suggesting a mode of action via membrane pumps.

This study was aimed to make experimental trials for cross infection of *T. mukasai* into some freshwater species from naturally infected *C. gariepinius* and to determine the most effective treatment for trypanosomiasis in naturally infected *C. gariepinius*.

Material and Methods

Fishes:

A total number of 170 fishes collected from Fish Research Center, Suez Canal University. They were represented as 140 naturally infected *C. gariepinus* used to obtain blood with *T. mukasai* from five of them and then all were held to be used in the treatment trials. In addition to 30 *Trypanosoma mukasai* free fishes (10 *Clarias gariepinus*, 10 *Oreocromis niloticus* and 10 *Cyprinus carpio*) which were used in the cross experimental infection.

Aquaria:

Fully well prepared glass aquaria were used for holding fishes used for the treatment trials and cross experimental infection. All aquaria measured (120x50x48 cm), supplied with dechlorinated tap

water according to ***Innes (1991)*** and air pumps for continuous aeration as well as thermostatic heaters for maintaining water temperature to be 25±1ºC. Fishes were fed pellets of commercial ration containing 25-35 % crude protein obtained from Fish Research Center, Suez Canal University. All fishes were kept in the aquaria for 14 days as a period of acclimation before starting the experiment and the treatment trials.

Clinical examination: Fishes were clinically examined for detection of any gross sings and/or any external abnormalities according to ***Conroy and Hermann (1981).***

Sampling of blood: Blood samples were taken from caudal blood vessels of all fishes to make blood films to detect trypanosomes according to ***Lucky (1977).***

Experimental infection for other fish species (Cross infection):

From 5 naturally infected *C. gariepinus* with *Trypanosoma mukasai*, blood was withdrawn with a heparinzed syringe from the caudal blood vessels, pooled then injected by I/P and I/M routes. Five fishes from each experimental fish species (*C. gariepinus, O. niloticus and C. carpio)* were taken for each route of infection and each five were kept in a separate glass aquarium. All injected fishes were examined for trypanosoma infection for 2 weeks, 7 days intervals.

Treatment of naturally infected *Clarias gariepinus* with trypanosomiasis:

A total number of 140 naturally infected *Clarias gariepinus* with *Trypanosoma mukasai* were divided into 7 groups each contains 20 fish. The 1st, 2nd and 3rd groups were injected I/M with **trypano-Ject®** (Adwia pharmaceutical company) with a dose of 1, 2 and 3 ml/kg fish body weight respectively. While the 4th and 5th groups were subjected to bath treatment with *Artimisia annua* leaves ethanol extract (100 and 150 mg/l for 120 minutes, respectively) according to ***Ekanem and Brisibe (2010).*** The 6th group was subjected to bath treatment with praziquental **Biltricid®** (Alexandria pharmaceutical company) with a dose of 4 ppm for 60 min. according to ***Osman (2009).*** The 7th group was kept as a control group with no treatment. All groups were subjected for examination after 2 weeks and 7 days intervals for presence of trypanosome in blood films after treatment.

***Artimisia annua* leaves ethanol extract:**

Artimisia annua plant purchased from National Research Center, leaves were washed thoroughly in running tap water, dried by spreading under the sun for 3 days and finally in a hot air oven at 60°C for 8 hr. The dried leaves were crushed to powder in a mortar and pestle and subjected to Soxhlet extraction with 70% ethanol as extracting solvent. The solvent was exhausted from the extract with the help of a rotary evaporator. The

extract was stored in a refrigerator until required for use ***(Ekanem and Brisibe, 2010).***

Preparation of stock and working solutions of *A. annua*:

The stock solution was obtained by dissolving 1 g of the extract powder in 5 ml of dimethyl sulfoxide (DMSO) and made up to 100 ml with de-ionized water and up to 150 ml with de-ionized water ***(Ekanem and Brisibe, 2010).***

Results and Discussion

Clinical picture: The recorded clinical signs in the naturally infected *C. gariepinus* were emaciated bodies, pale gills and accessory respiratory organ **(Plate1, B)** with increased mucous secretion. Some fish suffered from abnormal coloration, and abrasions of skin **(Plate1, A)**. In advanced cases, fishes were laying on the bottom of aquaria, dull, off food with losing escape reflex**.** Meanwhile, paleness of liver, kidneys with enlargement and congestion of spleen in addition of watery blood. These findings were nearly in agreement with ***Eissa et al. (1996), Noga (1996), Mariam (2001) and Essam and El-Kateib (2004).***

Parasitological findings:

The morphological and parasitological examinations revealed a small, colorless, cylinder and thin flagellated protozoan with pointed end in one side. It had single, long flagellum originating from a small kinetosome and attached to the body by a well-developed and clear undulating membrane with a short free end. A large oval nuclus was situated in the center. The cytoplasm was neurophilic with fine granules so it is belonged to **Family:** Trypanosomatidae**, Species:** *Trypanosoma mukasai* (**plate 1, C**). This finding nearly similar to the description of ***Chong (2005)*** and ***Eissa et al. (2008).***

Results of cross experimental infection:

It was evident that transmission of *Trypanosoma mukasai* from infected *C. gariepinus* to free *C. gariepinus* was succsseded through both routes of infection. Also, *O. niloticus* showed positive infection through the both routes. While, I/P route only was succeeded to produce infection in *C. carpio* (**plate 2**) (**Table 1)**

Results of treatment of trypanosomiasis in naturally infected *C. gariepinus*

Results revealed that the most effective treatment was induced by *Artemisia annua* in the 5th group treatment as it succeeded by 100%, followed by the 2nd group treatment with 90% success followed by the 4th group treatment with 70% success, while the less effective treatment was recorded in the 3rd group treated with Trypano- Ject2 with a percentage of 10% and the 6th group treated with praziquental with a percentage of 20%. (**Table 2).** Artemisinin (the active principle of *A. annua*) thought to destroys the

cells of parasitic organisms through the generation of highly reactive oxygen-based free radicals or electrophilic intermediates, by alkylating and oxidizing proteins and lipids of parasite membranes as well as inactivation of channel proteins ***(Ridley and Hudson,1998).*** It has been demonstrated that the effect of artemisinin is equally mediated through disruption of membrane potential by interacting with the electron transport chain in the mitochondrial membrane, resulting in free radical damage and dysfunctional mitochondria ***(Li et al., 2005).***

Table 1: *Showing results of cross infection from naturally infected C. gariepinus to different fish species using T. mukasai*

Experimentally infected species	I/P injection	I/M injection
C. gariepinus	+ve	+ve
O. niloticus	+ve	+ve
C. carpio	+ve	-ve

+ve = positive -ve = negative

Table 2: *Showing treatment effeciency in naturally infected C. gariepinus with trypanosoma*

group	No. of naturally infected *C. gariepinus*	Drug	Dose	Treated	Infected	% of Treated
1st	20	Trypano-Ject®	1ml/kg b.wt	6	14	30
2nd	20	Trypano-Ject®	2ml/kg b.wt	18	2	90
3rd	20	Trypano-Ject®	3ml/kg b.wt	2	18	10
4th	20	*A. Annua*	100 mg/l for 120 min.	14	6	70
5th	20	*A. annua*	150 mg/l for 120 min.	20*	0*	100*
6th	20	Praziquental	4ppm for 60 min.	4	16	20
7th	20	No treatment	Control	0	20	0.0

Each value represents mean±S.E; n=20.
*Significant difference t-test at P ≤0.01.

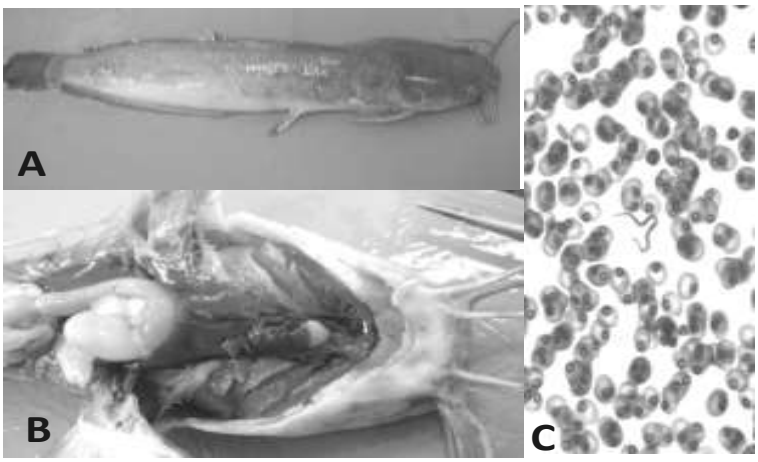

Plate (1): *Clarias gariepinus* **A**: infected fish showing skin abrasions and excessive mucous secretion on skin, **B:** pale gills and dendritic organs, **C:** blood film displaying magnified *Trypanosoma mukasai* (X 200).

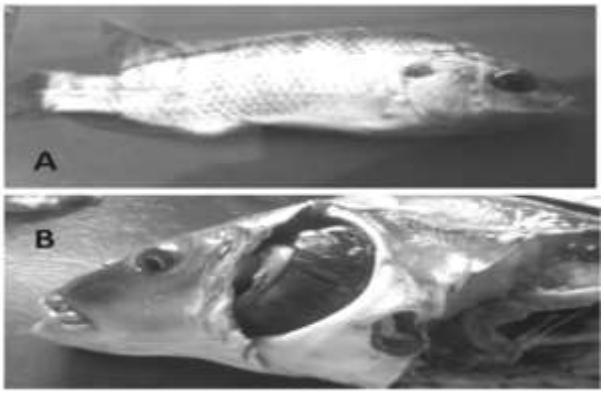

Plate (2): Infected A: *O. niloticus* with severe emaciation and eroded caudal fins. **B:** *C. carpio* showing severe branchitis

Conclusion

Trypanosoma mukasai is not host specific. Cross infection of *Trypanosoma mukasai* from naturally infected C. *gariepinus* to Nile tilapia *O. niloticus is* possible but it may failed in *Cyprinus carpio*. The treatment of choice for trypanosomiasis in *C. gariepinus* is the bath treatment using ethanol extract of *Artimsia annua* leaves (150ml/l for 120 minutes).

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العدوى التبادلية ومحاولات لعلاج مرض التريبانوسوما فى بعض أسماك المياه العذبة

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في هذه الدراسة، كان العدد الكلى 170 سمكة. كانت ممثلة على هيئة 10اسماك من القرموط الافريقى و 10اسماك من البلطى النيلي و 10اسماك من المبروك العادى و التى تم استخدامها فى العدوى التجريبية. بالإضافة إلى 140سمكة من القرموط الافريقى المصاب طبيعيا و التى تم استخدامها في التجارب العلاجية.

اهم العلامات المرضية للاسماك المصابة طبيعيا بالتريبانوسوما هى وجود سحجات على السطح الخارجى , فقدان الشهية, هزال عام مع كمون وعلامات تنفسية.

تم سحب عينات الدم بحقنة بها مضاد التجلط من الأوعية الدموية الذيلية لأسماك القرموط الافريقى المصاب بالتريبانوسوما موكاساي طبيعيا، وحقنها فى البروتون و العضلات فى كل من القرموط الافريقى و البلطى النيلي والمبروك العادى.

تم فحص جميع الأسماك المحقونة للكشف عن العدوى بالتريبانوسوما موكاسى لمدة اسبوعين.

أظهرت النتائج عدوى إيجابية فى أسماك القرموط الافريقى و البلطى النيلي باستخدام كلا الطريقتين في حين أن أسماك المبروك العادى أظهرت عدوى إيجابية من خلال الحقن البرتوني فقط.

أظهرت نتائج التجارب العلاجية أن العلاج الأكثر فعالية كان باستخدام أرتميسيا أنوا بجرعة 150 ملجم / لتر كحمام علاجى لمدة 120 دقيقة، يلية تريبانو-جيكت كحقن فى العضلات ثم بارازيكونتال كحمام علاجى.