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Trypanosoma Infection in Freshwater Fish and Its Corellation with Water Quality

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ABSTRACT

The Nile tilapia (*Oreochromis niloticus*) and the African sharptooth catfish (*Clarias gariepinus*) are the most widely cultivated fish species in Egypt. The production cycle runs from spring to autumn, during which water temperatures are conducive to fish growth and the propagation of leeches, which transmit fish hemoflagellates (*Trypanosoma* spp.). A total of 710 Nile tilapia and 385 catfish were randomly collected from three different sites—two private farms and Burullus Lake—throughout the year. Fish blood samples were examined for Trypanosoma infection. Regardless of fish species or collection site, the highest infection rate of *T. mukasai* was observed during summer. The catfish samples showed a higher infection rate than the Nile tilapia, and fish farms had higher infection rates compared to Burullus Lake. A positive correlation was found between Trypanosoma infection and water temperature, nitrogenous compounds (unionized ammonia, nitrite, and nitrate), total bacterial count (TBC), and total coliform count (TCC). Based on these findings, Trypanosoma infection can be expected during the production cycle (spring to autumn), and it is recommended to maintain optimal water parameters to prevent conditions favorable to infectious agents.

INTRODUCTION

In 2020, with 4.5 million tons, the Nile tilapia (*Oreochromis niloticus)* became one of the most highly cultured and profitable fish species worldwide **(FAO, 2020)**. This species is originated from Africa and belongs to the Cichlidae family. The Nile tilapia possess many characteristics that motivate fish farmers to cultivate them, such as fast growth, tolerance to a wide range of water temperatures from 16 to 38°C, and low maintenance costs **(Zhou *et al.*, 2019)**.

Although the African sharptooth catfish **(***Clarias gariepinus*) is an omnivore with high economic value in Africa, countless European countries claimed several ecological impacts after introducing it to the local environment **(Robins *et al.*, 1991)**. Fish originated from Africa with wide distribution in wild (tropical swamps, lakes, and rivers) and fish farms possess a wide range of feed kinds, such as higher plant debris, aquatic insects, and fish **(Holden & Reed, 1972; Bruton, 1979; Clay, 1979; Olufemi *et al.*, 1991)**.

Blood parasites are distributed in Africa especially *Trypanosoma* sp., which are kinetoplastid flagellates, polymorphic with deeply stained, lightly and finely granular cytoplasm, currently known to infect all classes of vertebrates, accounting for 500 different trypanosomes species **(Spodareva *et al.*, 2018)**. According to ecological and pathogenic studies, trypanosomes with a 200 species are isolated from both marine and freshwater fish **(Eiras *et al.*, 2012; Ferreira & Avenant-Oldewage, 2013)** in 54 African countries Botswana, Egypt, French West Africa, the Congo, the Sudan, Uganda, Mozambique, Namibia and South Africa **(McHugh *et al.*, 2016)**.

Trypanosoma muksai and *T. tilapiae* species are isolated from the Nile tilapia, *Tilapia zillii*, and the African butter catfish (*Schilbe mystus*) **(Imam *et al.*, 1985)**. Numerous *Trypanosoma* sp. were recorded as monomorphic *Trypanosome* in some fishes: *T. mansouri* from the *Chrysichthys auratus* and *Synodontis schall*, *T. tilapiae* from *Chrysichthys auratus* and *Tilapia zillii* fishes as diamorphic *Trypanosome* and *T. muksai* from *Clarias lazera* **(Negm El-Din, 1991)**. Additionally, **Overath *et al.* (1998)** found that *T. carassii* is a widely prevailed blood parasite of cyprinid and some non-cyprinid freshwater fish.

This study deals with the impact of changes in water physical parameters around the year and its impacts on fish, which became vulnerable to blood parasite infection. Water temperature affects fish metabolism and physiological processes (for example, enzyme functions), immunity and disease resistance, swimming activity and respiratory rate, and susceptibility to potentially toxic compounds such as ammonia and heavy metals **(Klontz, 1993)**. The presence of a pathogen along with deleterious water parameters resulted in the occurrence of fish diseases **(Sherif *et al.*, 2020, 2023a, 2024; Tawfeek *et al.*, 2024)**. Moreover, pollutants such as titanium and zinc nanoparticles affect fish immunity and tissues integrity **(Sherif *et al.*, 2019, 2021a, 2022, 2023b)**. Additionally, the fluctuation of water temperature is unavoidable in open-systems of culturing, which can trigger some physiological alterations in both the fish and pathogens **(Amal & Zamri-Saad, 2011; Sherif *et al.*, 2023a)**. Furthermore, it can reduce the immune response of fish becoming more vulnerable to various pathogens **(Karvonen *et al.*, 2010)**. Fish feed contaminated with aflatoxins could downregulate the immune status of fish, which is becoming more vulnerable to infections **(Sherif *et al.*, 2021b; Sherif & Zommara, 2023)**.

This study dealt with the prevalence of trypanosomes in fish farm and Burullus Lake impacting with the fluctuation of water parameters.

MATERIALS AND METHODS

1. Investigation sites

Three different sites were used to collect fish and water samples: Fish Farm 1 is located in Damro village, Fish Farm 2 is located in Tolompate 7 village, and Burullus Lake is located in northern Egypt. A total of 1,095 apparently healthy fish, including 710 Nile tilapia and 385 catfish, were randomly collected. Fish were sourced from Farm 1 (273 tilapia and 100 catfish), Farm 2 (257 tilapia and 165 catfish), and Burullus Lake (180 tilapia and 120 catfish). The body weight of the fish ranged from 110±50 grams for the Nile tilapia to 320± 205 grams for the catfish, while body length ranged from 12±6 cm for the Nile tilapia to 20± 8cm for the catfish. Water samples were collected alongside the fish samples. The live fish were then transported to the Animal Health Research Institute laboratory in Kafrelsheikh.

2. Parasite examination

Fish were examined for external signs on their eyes and skin, such as exophthalmia, erosion, ulcers, hemorrhages, and scale detachment, and then dissected to examine their internal organs **(Amlacher 1970)**. Fresh blood smears were prepared from all examined fish by taking a drop of blood sample directly from the caudal vein of alive fish on a clean slide and covered by a cover slip for rapid diagnosis of trypanosomes or any other organisms (**Lied *et al.*, 1975)**. Blood film from the caudal vein and impression smears were prepared according to **Carleton *et al.* (1967)**. The blood smears were air dried, fixed in absolute methyl alcohol for 5 minutes, then dried and stained with Giemsa stain (1ml of stain was added to 9ml of freshly prepared distilled water) for 10-20 minutes. The stained blood smears were examined microscopically for blood protozoa, according to **Lucky (1977)**. The identification of protozoan parasites was conducted according to **Hoffman (1970)**, **Levine *et al.* (1980)**, **Molnar and Baska (1986)**, and **Molnar and Fernc (1992)**, as well as other morphological descriptions given by previous authors who had studied the enteric protozoan parasites in Egypt and other countries.

3. Water parameters analyses

Temperature was measured at the site of sampling, using a thermometer (model YSI Environmental, EC 300) directly from ponds (farms) and sites (Burullus Lake), according to **APHA (1992)**. Total ammonia concentration was measured colorimetrically following the method reported by **APHA (1992)**. Unionized ammonia (NH_3), nitrate (NO_3), and nitrite (NO_2) were determined following the method recommended by **Boyd (1990)** using a spectrophotometer (Thermo, Electro Corporation, Nicollet evolution 100).

4. Determination of total bacterial count in water samples

Heterotrophic plate count (HPC) was determined using the pour plate method according to **APHA (1992)**. Nutrient agar was melted and kept at 44ºC in a water bath. A ten-fold dilution of the water sample was prepared. The plates were marked and arranged to prepare blank, duplicate positive control. The dilutions were shaken vigorously prior to pipetting onto the plates. One ml of sample was pipped onto plates. Twelve to 15ml liquefied culture medium was poured on previous inoculated plates and thoroughly mixed. The plates were incubated at 37°C for 48 hours. Plates having 30 – 300 colonies were selected for Colony Forming Unit (CFU) = Average number of colonies per plate x dilution rate.

2.5. 5. Determination of total coliform count (APHA, 1992)

A- Presumptive test:-

Serial dilutions were prepared from the original sample at concentrations of 0.1, 0.01, 0.001, 0.0001, and 0.00001. A series of culture tubes containing MacConkey broth with inverted Durham tubes were arranged. The sample and its dilutions were shaken vigorously, then incubated for 24 hours at 37°C. A positive result was indicated by gas production. Negative tubes (showing no gas or acid production) were reincubated for an additional 24 hours at 35°C. The cultures were verified by a confirmed test, and the result was calculated using the following formula for determining the most probable number (MPN) per 100ml:

MPN / 100 ml = MPN (from table) × (10 / Highest dilution).

B- Confirmed test:-

Each of the positive presumptive tubes was shacken carefully. Then, the growth was transferred to brilliant green lactose bile broth with a sterile loop and incubated at 37°C for 24h. The tubes were examined for gas production. Then, negative tubes were reincubated for 24 hours at 37°C.

C- Completion test:-

One or more EMB (Eosine-Methylene blue) agar plates were streaked from each positive tube. Then, the tube was incubated in an inverted position for 24 hours at 37ºC. The colonies developed were typical (pink to dark red with green metallic surface shine). From each plate, typical colonies were picked up. The Gram-stained preparation was microscopically examined. The presence of a Gram-negative rod from bacteria was a positive completed test.

6. Statistics

To determine the prevalence of blood parasites and their correlation with water parameters infection, the analysis of variance **(**ANOVA**)** test was performed with the calculation of Duncan's multiple range **(Duncan, 1955)**; a significance level was at $P \leq 0.05$. The SAS program ran all statistics on the computer **(SAS, 1998)**.

RESULTS

1. Prevalence of *Trypanosoma* sp. in the Nile tilapia and *Clarias gareipinus*

On Farm 1, the catfish had a higher infection rate of *Trypanosoma mukasai* (Fig. 1) than the Nile tilapia during the summer and autumn seasons, with rates of 30% and 28% for catfish, compared to 24.3% and 16.2% for the Nile tilapia, respectively. In contrast, during the winter season, both the Nile tilapia and catfish showed no infections (Table 1). For Farm 2, *Trypanosoma* infection in the catfish was notably high during the spring season, followed by autumn, with rates of 32% and 24%, respectively. Conversely, the Nile tilapia exhibited a higher infection rate during summer, followed by spring (Table 1). In Burullus Lake, the pattern of *Trypanosoma* infection in the catfish mirrored that of Farm 1, while the infection rate in the Nile tilapia was consistently 6% during both the summer and autumn seasons (Table 1).

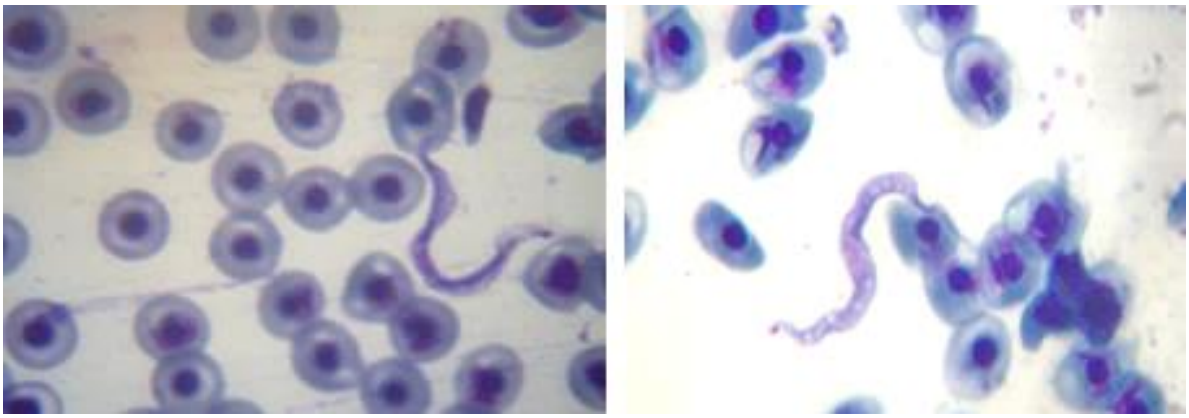

Fig. 1. *Trypansoma* sp. in blood samples of (A) the Nile tilapia and (B) catfish stained with Giemsa (x1000)

Table 1. Prevalence of *Trypansoma* sp. in fish farms and Burullus Lake

Items		**Nile tilapia**				**Catfish**			
		Winter	**Spring**	**Summer**	**Autumn**	**Winter**	**Spring**	**Summer**	**Autumn**
Farm1	Fish examined	70	53	70	80	25	20	30	25
	Fish infected	0	7	17	13	0	1	9	7
	%	0	13.2	24.3	16.2	0	5	30	28
Farm2	Fish examined	53	54	80	70	50	50	25	40
	Fish infected	0	4	11	3	2	16	6	6
	%	0	7.4	13.7	4.3	4	32	24	15
Burullus Lake	Fish examined	40	40	50	50	20	50	10	40
	Fish infected	0	0	3	3	0	3	2	7
	%	0	0	6	6	0	6	20	17.5

Note: *; /, ** %.

2. Correlation between infection rate of *Trypansoma* sp. and water parameters

The water temperature insignificantly differed between fish of farm 1, farm 2, and Burullus Lake during the same season. The highest water temperature was recorded during the summer season, 32.2± 2.01 ºC, while the lowest was during the winter season, 18.6± 0.34ºC. Water temperature and infection rate of *Trypanosoma* sp. were positively correlated regardless of fish kind (Table 2). In Table (2), it is clear that there is no significant difference between the fish farms during the breeding season (spring till autumn) in unionized ammonia levels in the water; the highest values were recorded during the summer season, and the lowest was recorded during the winter season ranging between 3.25 and 0.5mg/ L, respectively, moreover nitrite and nitrate took the same pattern of unionized ammonia. The infection rate was positively correlated with the level of the nitrogenous compound.

The total bacterial count (TBC) reached the the highest value during summer and the lowest during winter, with Farm 1 and Farm 2 recording 3.1×10^6 CFU and 2.1×10^3

CFU, and 2.5 × 10^6 CFU and 1.2 × 10^3 CFU, respectively. In Burullus Lake, the TBC recorded the lowest value in winter, at 3.6 × $10^{\wedge 2}$ CFU, with no significant differences recorded during spring, summer, and autumn, with values of 1.9 × 10^4, 4.1 × 10^5, and 1.9 × 10^4 CFU, respectively (Table 2)

In Table (2), the TCC followed the same pattern as the TBC: the highest values were during the summer season: 3.1 x10^3 and 1.3 x10^3 CFU in Farm 1 and Burullus Lake, respectively. The lowest values were 1.9 x10^2 and 0.4 x10^2 CFU in Farm 1 and Burullus Lake, respectively, during the winter season. The infection rate was positively correlated with TBC and TCC, except for Burullus Lake.

Table 2. Water temperature, un-ionized ammonia, nitrite, nitrate, total bacterial count and total coliform count

Season	Farm	Temp (^oC)	NH_3 (mg/L)	NO_2 (mg/L)	NO_3 (mg/L)	TBC (CFU)	TCC (CFU)
Winter	Farm1	19.3 ^{C} ±0.2	0.63 ^{C} ±0.03	0.05 ^{C} ±0.03	0.41 ^{B} ±0.05	2.1x10^{3} ^{C} ±10	1.9 x10^{2} ^{B} ± 0.0
	Farm2	18.6 ^{C} ±0.34	0.32 ^{C} ±0.004	0.04 ^{C} ±0.0	0.21 ^{C} ±0.01	1.2x10^{3} ^{C} ±10	1.1 x10^{2} ^{BC} ± 30
	Burullus Lake	20.6 ^{BC} ±0.1	0.22 ^{C} ±0.001	0.09 ^{C} ±0.24	0.18 ^{C} ±0.0	3.6 x10^{2} ^{C} ± 10	0.4 x10^{2} ^{C} ± 80
Spring	Farm1	25 ^{B} ±0.27	1.57 ^{B} ±0.004	0.26 ^{A} ±0.003	0.43 ^{B} ±0.24	2.5 x10^{5} ^{B} ±1000	2.1 x10 ^{A} ±100
	Farm2	25.9 ^{B} ±1.8	1.2 ^{B} ±0.005	0.1 ^{B} ±0.0	0.2 ^{C} ±0.04	1.5x10^{5} ^{B} ±7000	1.9 x10^{3} ^{B} ±60
	Burullus Lake	27.5 ^{B} ±0.31	0.29 ^{C} ±0.00	0.06 ^{C} ±0.001	0.12 ^{C} ±0.06	1.9x10^{4} ^{B} ±200	1.7x10^{2} ^{B} ±20
Summer	Farm1	30.1 ^{A} ±0.28	3.3 ^{A} ±0.05	0.38 ^{A} ±0.03	2.1 ^{A} ±0.00	3.1x10^{6} ^{A} ±1000	3.1 x10^{3} ^{A} ± 200
	Farm2	32.2 ^{A} ±2.01	2.6 ^{A} ±0.3	0.32 ^{A} ±0.24	1.06 ^{A} ±0.05	2.5x10^{6} ^{A} ±20000	1.5x10^{3} ^{A} ±50
	Burullus	29.8 ^{A}	1.47 ^{B}	0.2 ^{A}	0.49 ^{B}	1.9x10^{4} ^{B}	1.3 x10^{3} ^{A}

	Lake	±1.7	±0.001	±0.001	±0.02	±200	± 70
Autumn	**Farm1**	27.5 ^{B} ±2.01	1.5 ^{B} ±0.001	0.14 ^{B} ±0.02	0.35 ^{B} ±0.24	2.8 x10^{5} ^{B} ±1000	4.1x10^{3} ^{A} ±100
	Farm2	26.1 ^{B} ±2.3	0.68 ^{C} ±0.0	0.17 ^{B} ±0.08	0.31 ^{B} ±0.004	1.4x10^{5} ^{B} ±2000	9.2 x10^{2} ^{B} ±30
	Burullus Lake	26.6 ^{B} ±2.01	0.62 ^{C} ±0.00	0.24 ^{A} ±0.05	0.55 ^{B} ±0.0	1.9x10^{4} ^{B} ±200	1.6x10^{2} ^{BC} ±0.0

Note: temp; water temperature, NH_3; un-ionized ammonia, NO_2; nitrite, NO_3; nitrate, TBC; total bacterial count, and TCC; total coliform count. Different superscripts in the same row indicate significant difference (P<0.05).

DISCUSSION

Studies by **Smit *et al.* (2004)** and **Davies *et al.* (2005)** on trypanosomes in freshwater fishes in southern Africa highlight the challenges in identifying and differentiating these parasites on a morphometric basis, particularly with *T. mukasai*. In an initial attempt to address this issue, **Davies *et al. (*2005)** molecularly characterized fish trypanosomes from the Okavango Delta in Botswana, identifying two distinct trypanosome genotypes in that region.

Trypanosoma (haemoflagellate) is a piscine haemoparasite that swims freely in the blood **(Paperna, 1996)**; it has been found and identified in all aquatic systems of Africa in several aquatic animals, for example, the catfish mainly transmitted via leeches in South Africa and Egypt in freshwater fishes **(Smit *et al.*, 2004; Hassan *et al.*, 2007)**.

In the present study, *Trypanosoma* sp. was identified as *T. mukasai,* which had the highest infection rate of 30 and 28% in the summer season in catfish and the Nile tilapia, respectively. At the same time, *Trypanosoma* spp was isolated only in the spring season with a percentage of 4.3% in *O. aureus,* without being detected in the catfish **(Shagar, 2004)**. Several studies confirmed that *T. mukasai* is the most distributed *Trypanosoma* sp. throughout Africa, and it has been isolated from many fish species for a century **(Ferreira & Avenant-Oldewage, 2013)**. At least sixteen *Trypanosoma* species are distributed around Africa, but in the late 1990s, they were declined to six species. Finally, using molecular techniques, it was obvious that only two genotypic groups were identified as *T. mukasai* **(Smit *et al.*, 2020)**. In addition, they added that a high parasitemia (>108/ml) in the Nile tilapia resulted in 70% mortality within 20 days of infection. Other species could infect the Nile tilapia and cause mortality outbreaks, for example, *Trypanosoma epinepheli* isolated from *Lates calcarifer*, which was confirmed in the 95 and 98% in sequenced **Jesus *et al.* (2018)**. *Trypanosoma carassii*, also known as *T. danilewskyi*,

infects the freshwater goldfish (*Carassius auratus*) and could be cultivated *in vitro* **(Bienek & Belosevic, 1997; Chen *et al.*, 2022)**.

In this work, NH_3, NO_2, and NO_3 were higher during summer compared to the winter season, which was the lowest in examined farms (3.25 and 0.8mg/ L) and Burullus Lake (2.2 and 0.27mg/ L). In agreement, **Alabaster and Lioyed (1982)** mentioned that ammonia concentration increases in the summer due to water's increasing temperature and pH and the metabolic activity of fish and other aquatic organisms. In addition, **Moussa (2004)** found an increase of NH_3, NO_2, and NO_3 in Burullus Lake. Additionally, it was noticed that both fish farms were higher than Burullus Lake in the four seasons. These results may be explained by the increasing supply of organic fertilizer and increased organic compound decomposition with increasing water temperature. **Boyd (1990)** and **Randall and Tsui (2002)** reported that the primary source of ammonia in aquatic environments is the decomposition of biological waste. The NH_3 concentrations in the examined fish farm water were directly proportioned to the low water temperature values. **Abd Al-Aziz and Ibrahim (2003)** stated that NH_3 increased with poultry manure to 1.1mg/ L, while it was 0.16mg/ L before the addition. High ammonia compound was attributed to the high rate of microbial activity associated with high organic compounds and, in turn, high nitrogen content **(Abdel-Baky & Zyadah, 1998; Koussa, 2000)**.

Data concerning the bacteriological examination of water showed that the TBC of water was higher in the water of farms using organic fertilizer weekly as fish fed (mixed with ration) when compared with Burullus Lake. **Gharieb (2003)** and **Mousa *et al.* (2005)** proved a higher TBC during summer and low count during winter. These results might be attributed to the accumulation of organic and inorganic nutrients and relatively high temperatures, which induce active proliferation of bacteria **(Sherif *et al.*, 2022b, c; Sherif *et al.*, 2023c, d, e; Tawfeek *et al.*, 2023; Okasha *et al.*, 2024)**. These results supported those of **Noga (1996)**, who mentioned that using manure to fertilize fish ponds has at least a dual action, making fish more susceptible to infestation. It increases the bacterial load in water and decreases water quality.

The TCC had the same trend as the total bacterial count, which increased in farms than in Burullus Lake. Moreover, it increased during summer and decreased to a minimum during winter. These results supported **Poikolain *et al.* (1993)**, who mentioned that total coliform was found in water enriched with poultry and animal manure droppings. In addition, **Abd El-Rahman *et al.* (2004)** noted that fish ponds treated with organic fertilizer (chicken manure) had the highest most probable number (MPN) per gram, at $4.5 \times 10^{\wedge 8}$ CFU, while the water in these ponds contained $1.4 \times 10^{\wedge 6}$ CFU. In contrast, untreated ponds had water with only $6.0 \times 10^{\wedge 3}$ CFU. Additionally, the infection rate was notably high during the spring season. This may be attributed to the immune system being affected by stress from changes in water temperature, which can decrease antibody synthesis **(Woo, 2006; Sherif *et al.*, 2023a)**. It is well known that both innate

and acquired (adaptive) immunity play crucial roles in protecting fish from parasitic diseases **(Woo & Jones, 1989; Jones, 2001)**.

CONCLUSION

There were positive correlations between water (nitrogenous compound and bacteriological status) and the infection rate of *T. mukasai*. From the results of this study, it is evident that the infection rate of *T. mukasai* increased significantly by increasing the pollution of nitrogenous compounds, TBC, and TCC, and fish's health was affected significantly by organic pollution. A positive correlation between temperature and infection rate was noticed, and a temperature rise resulted in a high infection rate. This trend did not match with the Nile tilapia in Burullus Lake, where the highest infection rate was recorded in autumn. Therefore, it is recommended to monitor water parameters, particularly temperature, as trypanosome infections could be expected.

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