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Molecular detection of *Toxoplasma gondii* in fresh water fish from major rivers in Nigeria: a preliminary survey

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Abstract

Introduction: *toxoplasmosis, caused by Toxoplasma gondii, is a recognised neglected tropical disease, and a major re-emerging One Health challenge globally. Its prevalence varies from moderate to high across different hosts and regions. The status of toxoplasmosis in fish in Nigeria is not yet sufficiently studied. This study assessed the occurrence of T. gondii in freshwater fish species captured from major rivers in Nigeria to provide data on their toxoplasmosis status and level of environmental contamination with the parasite´s oocysts.* ***Methods:*** *a total of 90 fish species were captured from the River Benue, the River Niger, and the confluence at Lokoja. Their gills and intestines were harvested, homogenised, and tested for T. gondii gene using nested polymerase chain reaction.* ***Results:*** *the DNA of T. gondii was not detected in the gills and intestines of any of the captured fish. This indicates an occurrence of 0% in the fish.* ***Conclusion:*** *this report is the first attempt at detecting the presence of T. gondii in freshwater fish from the River Niger, the River Benue, and their confluence at Lokoja. The results reported may imply that freshwater fish species from these rivers may be a low risk of toxoplasmosis transmission. However, further studies are needed, including other fish species and bivalves, and a larger sample size is recommended across all seasons.*

Introduction

One major re-emerging One Health challenge worldwide is toxoplasmosis, a neglected tropical disease caused by *Toxoplasma gondii* [1]. Globally, over 30% of humans are positive for toxoplasmosis by serological evidence [2,3], while animal prevalence varies depending on the location [4] and the level of exposure to the risk factors [5]. In Nigerian pregnant women, a prevalence of 51.3% was reported in Uyo, a southern state [6], while 25.3% was reported in Benue, a middle belt state [7], and 76.6% was reported in the western part of the country among women of marriageable age living in rural communities [8]. The prevalence in animals varies between 25.5% and 100% [9,10], raising concerns about possible moderate to high environmental contamination with ooysts of *T. gondii*. With a high level of environmental contamination, natural and artificial runoff from agricultural, suburban, and urban land can wash the *T. gondii* ooysts to contaminate the rivers [11]. This can be a public health concern, as some community residents around these rivers are fishermen. Fish harvested from these rivers are also a source of protein for the people. Poor waste disposal practices in water bodies are also a known source of contamination of water bodies with pathogens, including *T. gondii* worldwide [11]. There are reports of aquatic organisms like bivalves, fish, including both filter feeders and benthic feeders, testing positive for *T. gondii*, and this has been associated with contamination of water bodies from run offs and waste disposal into rivers [11-14]. The status of toxoplasmosis in fish and the extent of aquatic spread are yet to be sufficiently studied in Africa [15], particularly in Nigeria. Only a few reports are available on the investigation of pathogenic protozoan contamination of water bodies in Nigeria [14,16]. This study, therefore, investigated the *T. gondii* status of freshwater fish species commonly captured for consumption from River Benue, River Niger, and the confluence at Lokoja, by the use of polymerase chain reaction (PCR).

Methods

Study design: a cross-sectional study was carried out to determine the *T. gondii* status of fish species captured for consumption from the major

rivers in Nigeria, namely River Benue, River Niger, and the confluence at Lokoja.

Study setting and population: Nigeria is a West African country bordering Benin Republic to the west, Niger and Chad to the north, Cameroon to the east, and the Atlantic Ocean to the south. Nigeria has two main rivers, the River Niger and the River Benue. The River Niger is the longest waterway in Africa. The Benue River originates from the Adamawa Plateau in Cameroon and flows westward into Nigeria to join the River Niger at Lokoja, then continues southward into the Niger Delta before reaching the Atlantic Ocean (Figure 1). Both rivers and their confluence at Lokoja are well known for fishing activities [17].

Variables: thirty (30) fish species from each of the River Niger, the River Benue, and Lokoja were randomly selected from the fishermen as they arrived from fishing in each sampling site. The total number of fish was 90. Two tissues (gills and intestines) were obtained from each fish, bringing the total number of tissues to 180. A sampling visit was carried out three times between January and April 2024. Ten samples were obtained on each sampling day. The first fisherman to arrive at the riverbank was sampled on each day. Two of each fish species were selected until a maximum of 10 samples per day was achieved.

Data resource and management: each sample was packaged in a nylon bag and transported on an icepack to the laboratory, where identification into the various fish species as described by Nelson *et al.* [18] using morphological traits was carried out. The fish were dissected following standard procedures [19]. The gills and intestines were harvested from each fish and kept preserved in the freezer at -21°C until needed for the DNA extraction, for the detection of *T. gondii.*

DNA extraction: DNA was extracted from each tissue using the Canvax® DNA purification kit (Canvax Biotech, S.L., Cordoba, Spain) according to the manufacturer´s instructions. For the extraction, 20 mg of the tissue was homogenised and resuspended with 2 ml of normal saline. About 25 µl of proteinase K was transferred into the bottom of a 1.5 ml microcentrifuge tube, and 250 µl of the sample homogenate was added. Afterwards, 250 µl of BLU buffer was added and mixed by vortexing. The mixture was allowed to incubate at 55 °C in a water bath for 15 min. Then, 250 µl of ethanol was added. Mixed by vortexing and transferred by pipetting into a mini spin column. The mixture was centrifuged at 8000 rpm for 1 min, and the flow-through solution was discarded. The mini spin was placed in a collection tube, 500 µl WB1 buffer was added, and centrifuged at 8000 rpm. The flow-through solution was again discarded. Next, 500 µl of WB2 buffer was added to the mini spin column, centrifuged at 8000 rpm, and the flow-through discarded. Thereafter, 800 µl of WB2 buffer was again added to the mini spin column, centrifuged at 8000 rpm, and the flow-through discarded. The mini spin column was then centrifuged at full speed for 3 min to dry the spin column. Finally, 200 µl of EB buffer was added to the column, incubated for 2 min at room temperature, and centrifuged at full speed for 1 min to elute the DNA. The extracted DNA was then stored at -21°C until further use.

Nested PCR: the nested primer sets (Inqaba Biotech, SA) were used to amplify the multicopy gene element of *Toxoplasma gondii* as described by Kong *et al*. [20] with slight modifications. The external primers (NF1 and NR1) were 5´-TGACTCGGGCCCAGCTGCGT-3´ and 5´-CTCCTCCCTTCGTCCAAGCCTCC-3´ respectively. While the internal primers (NF2 and NR2) were 5´-AGGGACAGAAGTCGAAGGGG-3´ and 5´-GCAGCCAAGCCGGAAACATC-3´ respectively. All the PCR reactions were performed using a programmable thermocycler (GeneAmp ABI PCR System 9700, United States). The first nested reaction was optimised in a 50 µl reaction volume containing 25 µl 2x MyTaq Mix (Bioline), 0.2 µl primers NF1 and NR1 (both 100 µM), 4 µl extracted DNA, and 20.6 µl Ultrapure molecular grade water. The PCR reaction condition was:

initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C, 1 min, annealing at 58°C for 1 min, and extension at 72°C for 1 min. The final cycle was followed by extension at 72°C for 10 min. The second nested PCR was optimised in a 25 µl reaction volume, using 1 µl of the first nested PCR product (diluted 1: 10 in molecular grade water), 12.5 µl 2x MyTaq Mix, 0.1 µl primers NF2 and NR2 (both 100 µM), and 11.3 µl molecular grade water. The reaction mixture was heated at 94° for 0.5 min, followed by 35 cycles of 94° for 0.5 min, 58° for 0.5 min, and 72° for 0.5 min, with a final extension step of 72° for 10 mins. For both reactions, sterile PCR water was included as a negative control, while DNA extract from RH strains of *T. gondii* (generously provided by Moredun Research Institute, Scotland, UK) was utilised as a positive control. The amplification products were detected by gel electrophoresis using 2% agarose gel in 1x Tris-borate-EDTA buffer, stained with 0.5% ethidium bromide. DNA bands were 164 bp for the positive reaction and visualized under a UV transilluminator (Syngene Bio imaging system, UK).

Statistical analysis: the data obtained were entered into Microsoft Excel. The total number of samples per location and the overall number were computed, and the percentage positive was determined.

Ethical considerations: ethical clearance for this study was obtained from the Animal Ethics and Welfare Committee of the College of Veterinary Medicine, Joseph Sarwuan Tarka University, Makurdi (formerly Federal University of Agriculture, Makurdi) before the commencement of the study. The ethical clearance number is JOSTUM/CVM/ETHICS/2024/1.

Results

Occurrence of *T. gondii* in freshwater fish species: the polymerase chain reaction was successful, and the positive control showed its band on the agarose gel corresponding to about 164 bp of the molecular ladder (Figure 2). However, findings of the detection of *T. gondii* DNA from the gills and intestines of the fish showed that none of the fish species sampled in the study tested positive for the DNA of *T. gondii*, indicating a zero (0%) occurrence.

Freshwater fish species captured from Nigerian major rivers: the fish species commonly harvested from the River Niger, Benue, and Lokoja that were also sampled in this study include wild catfish (*Clarias gariepinus*), Nile tilapia (*Oreochromis niloticus*), Nile squeaker catfish (*Synodontis schall*), silver arowana (*Osteoglossum bicirrhosum*), moon fish (*Citharinus citharus*), elephant snout (*Mormyrus caschive*), milk fish (Chanos), and ningu (*Labeo victorianus*) (Figure 3). In this study, the number of the Nile tilapia was 15, while that of the wild catfish was 35, and the silver arowana was five. The moon fish were 13, Nile squeaker catfish 14, elephant snout 2, milk fish 1, and ningu 5 (Table 1). All the fish species sampled in this study are among the group referred to as benthic feeders, which feed primarily on benthic organisms such as mollusks, crustaceans, and dendritus near the bottom of aquatic systems.

Discussion

This current study focused on the fish species captured in River Benue, River Niger, and their confluence at Lokoja (where the two rivers meet), which is the first attempt at detecting the presence of *T. gondii* in freshwater fish from these locations. Although cases of direct transmission of toxoplasmosis through freshwater fish are rare, the possibility of contamination of freshwater by faecal oocysts of *T. gondii* from infected cats has been implicated [12]. Therefore, freshwater fish could be potential transport hosts of the parasite, highlighting a link between terrestrial and aquatic transmission pathways of toxoplasmosis [21,22]. It has been observed that benthic feeders like the fish species sampled in this study have a higher risk of exposure to sediment-bound oocysts as a result of their feeding habits. This makes

freshwater ecosystems, especially in areas with dense human and animal populations, play a significant role in the transmission of toxoplasmosis [22,23].

In this study, the occurrence of *T. gondii* in fish was zero. This is contrary to a previous report of 34.77% prevalence using light microscopy in Gwagwalada River, Federal Capital Territory, Nigeria [14], which could be due to the different methodologies used in the detection of the parasite in the study. A more recent study [16] reported zero contamination of the Niger Delta River with oocysts of *T. gondii*. This is similar to the result in the present study. Results of the detection of *T. gondii* in rivers can be variable depending on several factors, including geographical location, human activities, and seasonality. Nahnoush *et al*. [15] reported a prevalence of 28.5% in tilapia from the River Nile, while Elmonir *et al*. [13] didn´t record any positive occurrence in tilapia and other fish species from the Nile Delta. The result of zero occurrence of *T. gondii* in this investigation could suggest a probable lack of sufficient contamination with *T. gondii* oocysts in the rivers investigated. Larger sample sizes, seasonality, and investigation of other types of fish (like filter feeders) and bivalves are, however, necessary to substantiate this possibility.

Conclusion

While the observation of zero occurrence of *T. gondii* in fish species captured from River Niger, Benue, and Lokoja may indicate low contamination with oocysts of *T. gondii* and a probably low risk of toxoplasmosis transmission through consumption of fish from these rivers, further investigation to include other seasons in the year, and inclusion of fish species like filter feeders, and bivalves is recommended. We also recommend further studies to assess the status of other water bodies around more densely populated communities, continued surveillance, and an interdisciplinary approach integrating parasitology, environmental science, and public health to mitigate the risk of toxoplasmosis and transmission of other waterborne pathogens through aquatic ecosystems in Nigeria.

Limitations: this study focused only on the major rivers in Nigeria. It also tested only 30 fish species from each of the three rivers. The study had a bias for the common fish species captured and sold in the local communities for consumption, and that may not represent the biodiversity of fish species in the rivers.

What is known about this topic

- *Consumption of undercooked meat is a known transmission route for toxoplasmosis;*
- *The prevalence rate of toxoplasmosis in Nigeria varies from moderate to high across different hosts;*
- *Most investigations of toxoplasmosis in Nigeria focused on warm-blooded animals.*

What this study adds

- *We found out in this study that freshwater fish species in the major Nigerian rivers are not positive for T. gondii;*
- *Our study shows that freshwater fish form these rivers may be low risks of toxoplasmosis.*

Competing interests

The authors declare no competing interests.

Authors' contributions

Samuel Agwu Ode, Mathew Adamu, Benjamin Emikpe: study design, and manuscript revision; Samuel Agwu Ode: sample collection and draft manuscript writeup; Samuel Agwu Ode, Husseini Ularamu, Oyekan Odediran Olumuyiwa, Elizabeth Ene Williams: DNA extraction and PCR. All the authors have read and agreed to the final manuscript.

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Table and figures

Table 1: fish species captured from major rivers in Nigeria and their *Toxoplasma gondii* status

Figure 1: map of Nigeria showing the major rivers and sample locations

Figure 2: agarose gel electrophoresis showing negative samples and positive control

Figure 3: fish species captured from major rivers in Nigeria

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Table 1: fish species captured from major rivers in Nigeria and their *Toxoplasma gondii* status

Fish species	Location	Sample count	% Positive
Nile Tilapia (*Orechromis niloticus*)	River Niger	13	0
	River Benue	2	0
	Lokoja	0	0
Wild Catfish (*Clarias gariepinus*)	River Niger	16	0
	River Benue	9	0
	Lokoja	9	0
Silver arowana (*Osteoglossum bicirrhosum*)	River Niger	0	0
	River Benue	0	0
	Lokoja	5	0
Moon fish (*Citharinus citharus*)	River Niger	0	0
	River Benue	1	0
	Lokoja	12	0
Nile squeaker catfish (*Synodontis schall*)	River Niger	0	0
	River Benue	0	0
	Lokoja	14	0
Elephant snout (*Mormyrus caschive*)	River Niger	0	0
	River Benue	2	0
	Lokoja	0	0
Milk fish (*Chanos chanos*)	River Niger	0	0
	River Benue	1	0
	Lokoja	0	0
Ningu (*Labeo victorianus*)	River Niger	0	0
	River Benue	6	0
	Lokoja	0	0

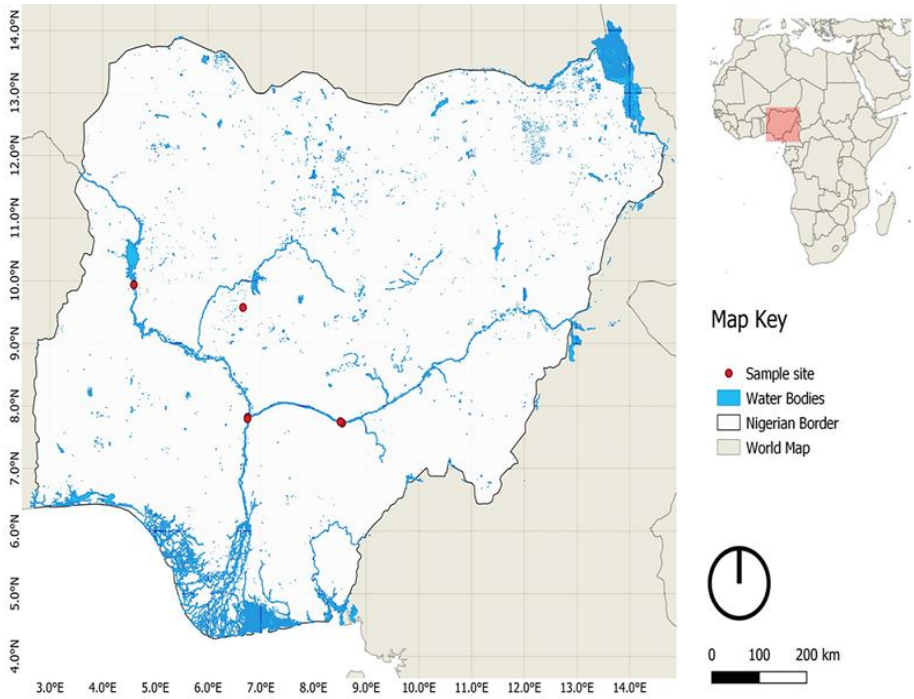

Figure 1: map of Nigeria showing the major rivers and sample locations

Figure 2: agarose gel electrophoresis showing negative samples and positive control

Figure 3: fish species captured from major rivers in Nigeria