



Original article

Prevalence of Zoonotic *Cryptosporidium* Oocysts in *Clarias gariepinus* Sold for Human Consumption in Maiduguri Metropolis, Northeastern Nigeria

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ABSTRACT

Introduction: Cryptosporidiosis is recognized as a significant water/food-borne zoonosis associated with morbidity and economic losses in farm animals. The zoonotic risk of *Cryptosporidium* via fish-borne infection is emerging as a significant public health concern worldwide.

Aim: This study investigated the incidence of *Cryptosporidium* oocysts among Catfish (*Clarias gariepinus*) sold for human consumption at major wet markets in Maiduguri metropolis, Borno State.

Methods: A cross-sectional survey was conducted from January to April 2015 to select and sample 200 catfish from Custom market (n=100) and Monday market (n=100) of Maiduguri metropolis council. The fishes were dissected to collect visceral contents. The intestinal mucosal scrapings were used to make smears, which were subsequently fixed with Bunsen flame and stained with Ziehl-Nielsen Acid-fast stain for the detection of *Cryptosporidium* oocysts under oil immersion.

Results: In total, 3 (1.5%; 95% CI = 0.39-4.68) samples were positive for *Cryptosporidium* oocysts. Female fish had a non-significantly (χ^2 test: $p>0.05$) higher sex-specific prevalence of 1.92% (95% CI = 0.33-7.45) compared with males 1.04% (95% CI = 0.05-6.49). *Cryptosporidium* oocysts were only recorded in adult fishes (1.62%; 95% CI = 0.42-5.04) and catfishes collected from Custom Market had a slightly high but non-significant (χ^2 test: $p>0.05$) prevalence of 2.0% (95% CI = 0.35 - 7.74) compared to those from the Monday Market (1.0%; 95% CI = 0.05 - 6.24).

Significance: The presence of *Cryptosporidium* oocysts in catfish sold for human consumption in Maiduguri and its environs highlights the potential role of fish as a source of human infection, especially among people who frequently consume roasted or improperly cooked fish.

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Introduction

Fish is an excellent source of animal protein that provides more than 40% of the total protein diet of nearly two-thirds of the world's population (Eyo, 1992; FAO, 1999; Chilima, 2007). Nigeria, with over 1.5 million metric tonnes (MMT) of fish consumption annually, is one of the largest fish consumers in the world, and fisheries make a significant contribution to the country's gross domestic product [GDP] (Imam and Dewu, 2010). Traditionally, fisheries resource is an important sub-sector of the agricultural economy that provides food, employment, and livelihood for coastal communities in Nigeria (Imam and Dewu, 2010). Fisheries have emerged as an important enterprise, which provides a source of livelihood for many people involved in the production, processing, and distribution of fish products, imports, and services in Nigeria in 2021 (Abaggyeh-Igbudu *et al.*, 2021). Fish is an important source of livelihood through food and income generation for households around the Lake Chad basin. But the recent onslaught by armed groups in the Lake Chad region caused reduced access to

fishing grounds and loss of fishing livelihoods with a proliferation of aquaculture production within the metropolis. Despite the presence of large dams, lakes, reservoirs, and ponds, which presents a huge potential for aquaculture and freshwater fisheries development (Sule *et al.*, 2021), Nigeria still imports over 900,000 MT/year, which exceeds domestic production of 650,000 MT/year (Imam and Dewu, 2010). Fish farming is, therefore, an emerging enterprise that has the potential for rapid growth due to the high demand and dwindling natural fisheries resources in Nigeria (Abolarin, 1996). The African catfish (*Clarias gariepinus*) and tilapia are the most widely cultured fish species in Nigeria (Muhammad *et al.*, 2021). The Catfish is a major tropical aquaculture species in Africa due to its high adaptation to artificial ponds and rapid growth rate and it is by far the main choice for most farmers and consumers in Nigeria. However, aquaculture production in Nigeria is constrained by a high rate of poverty, environmental pollution, climate change effects, and the incidence of diseases.

Cryptosporidiosis, caused by various *Cryptosporidium* species is an emerging zoonotic gastro-enteric disease that occurs

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worldwide in many animal species including wild and farmed fish (Leal, *et al.*, 2008; Murphy *et al.*, 2009). *Cryptosporidium* species have been reported in freshwater and marine fish globally. Australia, where 58% of global reports on *Cryptosporidium* occurrence is recognised as a hotspot for fish-borne Cryptosporidiosis (Golomazou and Karanis, 2020). *Cryptosporidium* molnari (Alvarez-Pellitero and Sitjà-Bobadilla, 2002), *C. Scophthalmi* (Paparini *et al.*, 2017), and *C. huwi* (Ryan *et al.*, 2015) have been genetically described the three primary species which are specific to piscine hosts. *C. parvum* zoonotic subtypes such as *Cryptosporidium hominis*, *C. parvum*, *C. meleagridis*, *C. felis*, and *C. canis* (Moratal *et al.*, 2020) have been detected in 18 freshwater and marine fish species worldwide (Alvarez-Pellitero and Sitjà-Bobadilla, 2002). The presence of zoonotic subtypes of *Cryptosporidium* species in fish is thought to increase fish-borne zoonotic transmission, especially in areas where raw, roasted, or improperly cooked fish is consumed (Golomazou and Karanis, 2020).

Cryptosporidium is an obligate intracellular parasitic pathogen that has a direct life cycle, which is transmitted via faecal contamination of water with infective oocysts (WHO, 2006). In Nigeria, Joy and Armayau (2013) have documented the presence of *Cryptosporidium* oocysts with an overall prevalence of 49.75% among African catfish from two lakes and artificial ponds in Zaria, Kaduna State. Similarly, Ebere *et al.* (2013) demonstrated the presence of *Cryptosporidium* oocysts with an overall prevalence of 27.59% in bivalve molluscs from Lake Tiga in Kano State. These studies showed that both the fish and aquatic environment harbours *Cryptosporidium* oocysts. Moreover, there is no existing public health regulation or microbial criteria for screening cryptosporidiosis in fresh fish for human consumption in Nigeria. To the best of our knowledge, there is also a paucity of published information on the incidence of *Cryptosporidium* in Maiduguri. This study was therefore designed to investigate the occurrence of zoonotic *Cryptosporidium* oocysts in fresh catfish sold at two major wet markets that supply Maiduguri metropolis, the Borno State capital in Northeastern Nigeria.

Materials and methods

Study area

Maiduguri, also called Yerwa, is the capital and largest city of Borno State located in Northeastern Nigeria between latitude 11°51N and longitude 13°05E (NBS, 2011). Maiduguri lies within the semi-arid zone of Northeastern Nigeria with a Sudan Savannah climate characterised by low rainfall (600mm), which lasts from late June to early October, followed by a prolonged dry season for the rest of the year (Hess *et al.*, 1999). The average monthly temperature of Maiduguri is 35°C. However, during the cooler period (November-February), the average temperature drops to about 20°C. Borno State, with suitable socio-economic and environmental conditions for fish production, is endowed with great potential for both inland fisheries and aquaculture.

Study design and sample collection

A cross-sectional study was conducted between January and April 2015 in Maiduguri metropolis. A total of 200 adult catfish comprising 100 samples each were collected from Custom Market and Maiduguri Monday Market in Maiduguri metropolis. Fishes were euthanized by mechanical stunning dissected to collect visceral contents by making a mid-ventral incision from the operculum to the anal vent using a sharp scalpel blade and the entire gastrointestinal tracts were excised separately into appropriately labelled polythene bags. The sexes of fish were determined by physical examination using the shape of intromission organ on the caudo-ventral surface of the catfish close to the anal vent. Following dissection, the sexes of the catfish were further confirmed by the presence of testes or eggs. A measuring tape was used to measure the length of each fish sample. Based on the length, the fish samples were grouped as juveniles (3-5 cm) or adults (6 -9 cm) (Goselle, 2008). Samples were randomly collected from different selling points and different fish tanks in the morning hours and transported on ice packs to the Parasitology laboratory of the Faculty of Veterinary Medicine, University of Maiduguri for parasitological examination.

Sample processing

The intestinal mucosal scrapings of catfish were gently smeared over approximately 2.5 x 4.0cm area of labelled and cleaned, grease-free microscope glass slides. The slides were dried in air and fixed by passing them 3 times for 3 seconds over the flame of a Bunsen burner.

Staining procedure

Ziehl-Nielsen Acid-fast staining method was used for the detection of *Cryptosporidium* oocysts (Nielsen and Ward, 1999). Briefly, the fixed smears were flooded with cold carbol fuchsin, and the slide was heated over a Bunsen burner until steam can be seen rising from the surface. After heating, the stained smear was rinsed thoroughly with clean tap water and allowed to drain completely. The stained smear was then decolourized in 3% acid alcohol for 30 seconds and rinsed immediately with tap water. After draining, the smear was counter-stained using malachite green for 45 seconds. The smear was rinsed with tap water and allowed to air dry.

Microscopic examination

Examinations of slides were done using 100x magnification of a phase-contrast microscope under oil immersion. *Cryptosporidium* oocysts were identified as bright pinkish to red coccoid bodies with thick walls measuring about 4-5µm in diameter and containing thin-flat sporozoites on a pale green background of mucosal smear (Soulsby, 1982).

Statistical analysis

The raw data was initially summarised to compute percentages and proportions of infected individuals in Microsoft Excel spreadsheet program version 2020. Further analysis was performed using the Statistical Package for Social Sciences (SPSS) software version 25.0 to determine the associations between the test variables using Fisher's Exact test. A p -value ≤ 0.05 was considered statistically significant.

Results

Overall, there were 96 male, 104 female fishes, of which 185 were adults and 15 were juveniles. In total, 3 (1.5%; 95% CI=0.39-4.68) fishes were positive for *Cryptosporidium* oocysts given. The female fish had a non-significantly (χ^2 test: $p > 0.05$) higher incidence of 1.92% (95% CI=0.33-7.45) as compared with the males 1.04% (95% CI=0.05-6.49). In terms of the age distribution, *Cryptosporidium* oocysts were only recorded in adult fish (1.62%; 95% CI=0.42-5.04) and no infection was recorded among the juveniles' fish 0% (χ^2 test: $p > 0.05$). In terms of the source of fish market, catfish collected from Custom Market had a slightly higher but non-significant (χ^2 test: $p > 0.05$) incidence of 2.0% (95% CI=0.35-7.74) compared to those from Monday Market which had a 1.0% (95% CI=0.05-6.24) (Table 1).

Discussion

This study was designed to investigate the incidence of cryptosporidiosis in catfish sold for human consumption in two major wet markets in Maiduguri metropolis, the Borno State capital. The goal of this study was to highlight, using our results, the potential zoonotic transmission of cryptosporidiosis among the consumers of roasted, fried, and improperly cooked catfish. This study has revealed the presence of *Cryptosporidium* oocysts in catfish sold for human consumption in Maiduguri metropolis.

The presence of *Cryptosporidium* oocyst is epidemiologically significant because infection of fish may directly affect the health and productivity of cultured and free-range fish species due to poor growth rate and mortality (Sitj'a-Bobadilla *et al.*, 2005; Golomazou *et al.*, 2021). Moreover, the consumption of fish contaminated with human and animal wastes was recently highlighted as a new source of human infection with zoonotic *Cryptosporidium* species (Leal *et al.*, 2008; Moratal *et al.*, 2020). Since experimental evidence suggests that human-specific or zoonotic *Cryptosporidium* infection occurs in fish, fish may likely act as mechanical carriers, which plays a significant role in the environmental dissemination of *Cryptosporidium* oocysts (Couso-Pérez *et al.*, 2022). The consumption of contaminated or infected containing even as low as one *Cryptosporidium* oocyst may lead to human infection (Bemamrouz *et al.*, 2012), causing severe abdominal pain and diarrhoea in immunocompromised patients (Moratal *et al.*, 2020).

The overall positivity rate of 1.5% (3/200) recorded among the catfish sampled from Maiduguri wet markets is lower than results from previous studies in Nigeria. Ebere *et al.* (2013) reported a higher prevalence of 27.59% for *Cryptosporidium* oocysts from fish sourced from the famous Tiga Lake in Kano State. Joy and Armayau (2013) further reported an overall prevalence of 49.75% for *Cryptosporidium* oocysts in catfish from Zaria in Kaduna State. The lower incidence of *Cryptosporidium* oocysts among catfish sampled from Maiduguri metropolis could be linked to a predominantly intensive management system of rearing catfish because of disruption in fishing activities from lake Chad and various rivers due to armed conflict and mass displacement in the region. Results obtained from previous studies have shown that fish samples collected from lakes and rivers (under the extensive system) had a higher incidence of *Cryptosporidium* oocyst compared with intensively reared fish from artificial ponds (Joy and Armayau, 2013).

Table 1: The prevalence of *Cryptosporidium* Oocysts Amongst Catfish (*Clarias gariepinus*) Sold for Human Consumption in Maiduguri metropolis, Northeastern Nigeria ($n=200$).

Variables	Categories	No. Exam- ined	No. Posi- tive	Prevalence (%)	95% CI*	p -value
Gender	Male	96	1	1.04	0.05, 6.49	> 0.05
	Female	104	2	1.92	0.33, 7.45	
Age	Adult	185	3	1.62	0.42, 5.04	NA
	Juvenile	15	00	00	00	
Source	Custom market	100	2	2.00	0.35, 7.74	> 0.05
	Monday market	100	1	1.00	0.05, 6.24	
Overall		200	3	1.50	0.39, 4.68	

*95% confidence interval with Yates continuity correction on the prevalence; Fisher's exact test; $p < 0.05$ considered statistically significant

Discussion contd.

It was apparent from these studies that the prevalence and concentration of *Cryptosporidium* oocysts in fish depends largely on the level of faecal contamination of water bodies. *Cryptosporidium* species have been detected in marine, freshwater, cultured, captive and ornamental fish populations worldwide (Golomazou *et al.*, 2021). The potential risk for transmitting zoonotic *Cryptosporidium* species through the consumption of freshwater and marine fish contaminated with human and animal wastes were highlighted as a new source of human infection (Leal *et al.*, 2008; Moratal *et al.*, 2020).

It is therefore likely that the consumption wild catfish may constitute a greater public health risk for contracting fish-borne *Cryptosporidium* infection compared with those raised on intensive systems, which are less prone to contamination or infection. In situations where animals are allowed to graze or drink around natural or artificial water pools, they may accidentally discharge faecal material and shed large amounts of oocysts into the water (Galvão *et al.*, 2012; Hofstra *et al.*, 2013). Moreover, the indiscriminate disposal of human and animal wastes into the environment or water bodies may contaminate various sources of natural water and artificial aquaculture systems with infectious materials and increase the risk of human and animal infection with *Cryptosporidium* oocysts (Na-Nakorn and Brummett, 2009). Furthermore, the low prevalence of *Cryptosporidium* oocysts in the present study could be linked to the small sample size and short duration of sampling (January-April) in contrasts with other studies that reported higher prevalence of *Cryptosporidium* in fish using larger sample sizes and longer duration of sampling (Ebere *et al.*, 2013; Joy and Armayau, 2013).

This study has revealed the contamination of catfish meant for human consumption in Maiduguri with oocysts of *Cryptosporidium* species and highlights the potential role of fresh catfish as a new source of human Cryptosporidiosis in Maiduguri and its environs. This finding is especially important in Nigeria where nearly 260 million kilograms of catfish are consumed annually (Bernard, 2009). These findings are very significant since majority of resource-poor farmers rely on fish as a major source of protein (Na-Nakorn and Brummett, 2009). Fishers, fish farmers, fish processors, and food handlers could be directly infected while eviscerating or preparing the captured fish or indirectly via contact with contaminated surfaces or fomites during the processing and storage of the fish (Couso-Pérez *et al.*, 2022). There is a need for further epidemiological investigation using molecular subtyping tools to phylogenetically cluster the *Cryptosporidium* spp recovered from fish into human, animal, fish-specific or zoonotic clusters.

Conclusion

The presence of *Cryptosporidium* oocysts in catfish meant for human consumption in Maiduguri and its environs underscores the potential role of fish in the epidemiology of human infection, especially among people who frequently consume roasted or improperly cooked fish. The Nigerian Public Health Authorities

are advised to come up with a strategy for screening fresh fish and fish products for the presence of *Cryptosporidium* oocysts.

Conflicts of interest

None of the authors have conflict of interest to declare.

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