Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Document heading doi:10.12980/JCLM.1.2013C42

Occurrence of *Cryptosporidium* species in catfish *(Clarias gariepinus)* Harvested from two lakes and artificial ponds in Zaria, Northern Nigeria

Joy Cecilia Atawodi[1*], Armayau Hamisu Bichi[2]

[1]*Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria*

[2]*Department of Applied Zoology, Bayero University, Kano, Nigeria*

PEER REVIEW

Peer reviewer
Dehai Xu, Ph.D, Research Parasitologist, USDA, Agricultural Research Service, Aquatic Animal Health Research Unit, 990 Wire Road, Auburn, AL 36832-4352, USA.
Tel: (334) 887-3741
E-mai: Dehai.Xu@ARS.USDA.GOV

Dr. BV Maikai, Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.
Tel: +234 802 847 2921
E-mai: Beatt18@yahoo.com

Comments
This is a good pioneer study in which the authors try to look at the significance of consumption of catfish positive for *Cryptosporidium* oocysts. The results are interesting and suggest that catfish can serve as a source for *Cryptosporidium* infection in the study area.
Details on Page 10

ABSTRACT

Objective: To determine the occurrence of *Cryptosporidium* species in catfish (*Clarias gariepinus*) harvested from two lakes and ponds in Zaria, Kaduna State, Nigeria.
Methods: Catfish samples (n=200) from two lakes and (n=200) from two private fish ponds were collected and their gills and gastrointestinal tract samples were analysed for *Cryptosporidium* oocysts using modified Ziehl-Neelsen staining technique was used, followed by microscopy.
Results: Oocysts of *Cryptosporidium* species were found in all the sampling sites with an overall positivity rate of 49.75%. A comparison between the lakes and ponds as sampling sites revealed a statistically significant (P<0.05) higher percentage of occurrence of *Cryptosporidium* oocyst from the lakes (39.25%) than from the ponds (10.50%).
Conclusions: These findings document for the first time the natural occurrence of *Cryptosporidium* sp. in catfish intended for human consumption and therefore, underlines the need for public enlightenment to guard against any possible zoonotic transmission.

KEYWORDS
Cryptosporidium, Oocysts, Catfish, *Clarias gariepinus*, Lakes, Ponds, Nigeria

1. Introduction

Fish is becoming an important component of people's diet in many parts of the world. This is probably due to the many excellent characteristics possessed by fish. Some of these desirable attributes include palatability, leanness, and possession of heart healthy Omega 3 fatty acids. Realizing the importance of fish to human nutrition and its contribution to the Millennium Development Goals makes it imperative to establish and strengthen aquaculture and fisheries programmes[1], this is one of the reasons for the popularity of fish farming in Nigeria and around the world[2]. The African catfish *Clarias garipienus* is a major tropical aquaculture species in Africa and most popular with fish farmers and consumers in Nigeria. Fish consumption is however, not devoid of risks due to the possibility of their harboring infectious or pathogenic microorganisms, particularly if such organisms are zoonotic[3].

Cryptosporidiosis is an emerging zoonotic gastroenteric

*Corresponding author: Joy Cecilia Atawodi, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria, Nigeria.
Tel: +234 (0)8036 090 057
E-mail: joyatawodi@yahoo.co.uk
Foundation Project: Supported by Ahmadu Bello University, Zaria, Nigeria with Grant No. ABU/P16945.

Article history:
Received 29 Jun 2013
Received in revised form 4 Jul, 2nd revised form 7 Jul, 3rd revised form 10 Jul 2013
Accepted 30 Jul 2013
Available online 28 Aug 2013

disease that is known to occur in many animal species[3,4], including wild and farmed fish worldwide and is caused by a protozoan parasite called *Cryptosporidium* spp. Several reports have confirmed the cosmopolitan nature of *Cryptosporidium* species in surface water, groundwater, estuaries, and seawater. *Cryptosporidium* transmission is through its hardy oocysts which are passed in the faeces with water as its major vehicle of transmission. Thus, zoonotic transmission of cryptosporidiosis can occur from consumption of fish obtained from contaminated water[3].

Cryptosporidium oocysts are very small (4-6 μm) in size and are therefore able to pass through conventional water plant filters. *Cryptosporidium* oocysts are remarkably resistant to common disinfectants. In fact, they are 240 000 times more able to withstand chlorination effects than *Giardia* species, all these characteristics facilitate the water-borne transmission of cryptosporidiosis. With a mean infectivity dose of ten oocysts, a single oocyst is adequate to initiate an infection and cause disease in susceptible hosts[5].

In otherwise healthy individuals, clinical cryptosporidiosis is characterized by watery diarrhea, which can be accompanied by abdominal cramps, loss of appetite, low-grade fever, nausea, vomiting, and weight loss; however, asymptomatic infection occurs frequently[6]. *Cryptosporidium* spp. also causes an opportunistic infection in human immunodeficiency virus (HIV). Infected patients who might experience life threatening infection with profuse, watery, cholera-like diarrhea. In severely immunocompromised patients, disease can progress to cholangitis or pancreatitis and the infection is frequently chronic and can eventually become lethal, second only to tuberculosis, thus, cryptosporidiosis is the main terminal disease in HIV infection[7].

Due to paucity of information on the occurrence of *Cryptosporidium* in most parts of Nigeria, especially in the northern part, this study was designed to investigate the occurrence of *Cryptosporidium* oocysts in catfish obtained from different water sources in Zaria in order to provide some information on cryptosporidiosis status, especially with respect to the incidence of *Cryptosporidium* oocysts in catfish obtained from some lakes and ponds.

2. Materials and methods

2.1. Study area

The study was conducted between January and June, 2010 in Zaria, North central Nigeria. Zaria is located between latitude 11°4′ N and longitude 7°42′ E, and falls into the Guinea Savanna climate with two distinct seasons; wet and dry. The wet season occurs between April and September, while the dry season is from October to March[8].

River Galma is the main drainage channel in Zaria with other rivers and streams discharging into it and the Zaria lake is located on River Galma. Ahmadu Bello University lake is a man-made lake which was constructed on River Kubani (in Zaria) mainly to support the water needs of the university, local fishermen also undertake some fishing activities at these locations to catch fish which they sell to the people residing in the neighbourhood of the university.

Private fish ponds are kept by some residents of the Ahmadu Bello University staff residential quarters, and two of these sited in area F and area G. Samaru are included in the study by convenience sampling.

2.2. Sample collection

A total of 400 live catfish comprising of 100 samples each from the four sources namely, Zaria lake, Ahmadu Bello University lake, area F pond and area G pond, all within Zaria.

The samples were collected in the morning hours by convenience random sampling and placed into clean plastic buckets containing some water from the lake and transported live to the Parasitic Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria for immediate analysis.

2.3. Sample processing and analysis

Prior to analysis the catfish were first euthanized by ice-cold bathing. Using a scalpel blade, each catfish was then cut dorsally just above the anterior dorsal fin and across the spinal cord, an incision was then made on the ventral surface beginning from the operculum to the anal vent through which the gills and gastrointestinal tract were excised separately into appropriately labeled tubes (the contents of each gastrointestinal tract was carefully squeezed out).

Samples were analyzed using the techniques described by Gomez-Couso *et al.*[9] with slight modifications[10-12]. Each sample tube was vigorously hand agitated for 30 seconds and then mixed by vortexes. Aliquot of each sample was carefully smeared over approximately 2.5 cm×3.0 cm area of appropriately labeled, clean, grease-free glass slides. These were air-dried and fixed with 70% methanol for three minutes.

2.4. Modified acid fast stain

The Kinyoun′s modified acid fast stain also known as cold Ziehl Nielsen stain as described by Nielsen and Ward was used for oocyst detection and counts[13]. This was conducted as follows:

The fixed slides were flooded with cold carbol fuchsin and stained for 3 min following which they were rinsed with tap water. The slides were then drained and decolorized with 3% acid alcohol (a mixture of 3 mL of concentrated hydrochloric acid and 97 mL of absolute ethanol) until no more colour ran from the slides, they were then rinsed with water, drained and counterstained with methylene blue for 2 min. After rinsing with water, the stained slides were air-dried and examined using a Zeiss binocular light microscope with ×10 and ×40 objectives under a bright field.

2.5. Microscopic examination

The visual oocyst count was performed by scanning through 12 microscopic fields using the ×10 objective and looking out for the oocysts, which stain bright pinkish to red against a background of blue-green fecal debris and yeasts. Any such seen were counted and recorded. Oocysts encountered were confirmed using the ×40 objective to observe their round shape and pinkish red coloration. Sometimes, the sporozoites are visible within the oocysts. Some oocysts however, do not take up the stain colour and therefore, do not stain at all, and are called "ghosts"[14].

2.6. Statistical analysis

A test of association between *Cryptosporidium* oocyst and catfish from the different water sources sampled was performed using the Graphpad Prism version 4.0 for Windows. Likewise, association between *Cryptosporidium* oocyst occurrence and the different organs screened (gills and gastrointestinal tract) was also tested.

3. Results

Among 400 catfish samples, the overall prevalence rate of *Cryptosporidium* oocysts was 49.75% (Table 1). There was a significant difference in association between the presence of *Cryptosporidium* oocysts in catfish and all the sites sampled ($P<0.05$).

Table 1
Incidence rate of *Cryptosporidium* oocysts in catfish samples from two lakes and ponds in Zaria, Kaduna State, Nigeria.

Water source	Number of catfish sampled	Number of positive (%)
Zaria lake	100	92 (23.00)
ABU lake	100	65 (16.25)
Area F pond	100	23 (5.75)
Area G pond	100	19 (4.75)
Total	400	199 (49.75)

OR=0.0728 (*CI*=0.451-0.1176).

The comparison between gills and gastrointestinal tract as sampled organs showed that in terms of absolute oocyst count, gastrointestinal tract samples demonstrated a higher oocyst yield of 2305 (94.3%) than gill samples, which yielded 139 (5.7%) oocysts, with those from the lakes having a higher count. The ratio of oocyst occurrence in gills to that in gastrointestinal tract is 1:15 in the samples obtained from the lakes and 1:20 for the ponds.

When assessed according to water source type, samples collected from the lakes had a higher oocyst occurrence rate of 39.25% (Table 2) than those collected from the ponds with oocyst occurrence rate of 10.50% (Table 3). There was a significant association ($P<0.05$) between the presence of *Cryptosporidium* oocysts in catfish and both lakes sampled.

Figure 2 shows a distribution of the oocysts recorded from the different sampling sites. While Zaria lake samples recorded the highest prevalence of 46% and ABU lake samples had a prevalence of 33%, the combined prevalence observed in the samples from both Area G pond and Area G pond was 21% (Figure 2).

Table 2
Prevalence rate of *Cryptosporidium* oocysts in catfish samples from two Lakes in Zaria, Nigeria.

Water source	Number of organ samples	Number Positive (%)
Zaria lake	200	92 (23)
ABU lake	200	65 (16.25)
Total	400	157 (39.25)

$P<0.0001$, RR=3.15 (CI=1.662-5.967), OR=6.192 (CI=2.697-14.22)

Table 3
Prevalence rate of *Cryptosporidium* oocysts in catfish samples from two Ponds in Zaria, Nigeria.

Water Source	Number of catfish sampled	Number Positive (%)
Area F pond	100	23(23)
Area G pond	100	19 (19)
Total	200	42 (21)

$P<0.6029$, RR=1.123 (CI=0.3175-1.545), OR=1.273 (CI=0.6431-2.522).

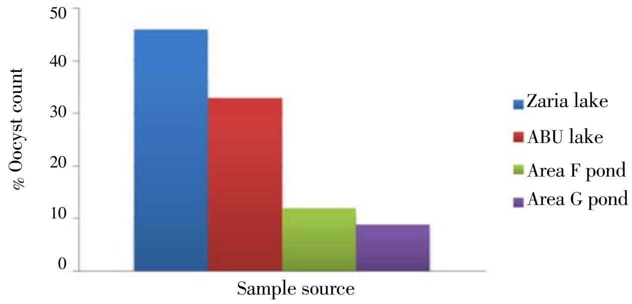

Figure 2. Percentage oocyst count from catfish smaples harvested from two lakes and artifical ponds in Zaria, Nigeria.

A comparison between the ponds (Table 3) indicated that even though out of 100 catfish sampled from each pond 23 (5.75%) were positive for *Cryptosporidium* oocysts from 19 (4.75%) from Area G pond were positive there was no statistically significant association ($P<0.05$) between the presence of *Cryptosporidium* oocysts in the catfish and the ponds sampled in these areas.

The range of oocyst occurrence in gastrointestinal tract and gills per sample was 0–70 and 0–50 respectively with the highest recovery being recorded within the 0–10 range for both organs. This is shown in Tables 4 and 5 respectively.

Table 4

Frequency of *Cryptosporidium* oocysts recovered from gatrointestinal tracts of catfish grown in lakes and artificial ponds in Zaria, Nigeria.

Range of oocyst Recovery	ABU lake	Zaria lake	Area F pond	Area G pond
0–10	100	63	100	100
11–20	0	22	0	0
21–30	0	10	0	0
31–40	0	2	0	0
41–50	0	2	0	0
51–60	0	0	0	0
61–70	0	1	0	0
Total	100	100	100	100

Table 5

Frequency of *Cryptosporidium* oocysts from gills of catfish grown in lakes and artificial ponds in Zaria, Nigeria.

Range of oocyst recovery	ABU lake	Zaria lake	Area F pond	Area G pond
0–10	99	65	100	100
11–20	1	22	0	0
21– 30	0	9	0	0
31–40	0	3	0	0
41–50	0	1	0	0
51–60	0	0	0	0
Total	100	100	100	100

4. Discussion

This investigation involved the evaluation of catfish samples from lakes and ponds in Zaria (intensive system and extensive system) for *Cryptosporidium* oocysts as an index of their potential of transmitting cryptosporidiosis infection to consumers of catfish in the study area. The overall positivity rate of 49.75% as observed in our study indicates a statistically significant association between the presence of *Cryptosporidium* oocysts and catfish obtained from the two lakes and ponds screened. Although earlier workers have reported the presence of oocysts of *Cryptosporidium* species in oysters, clams and mussels from contaminated water[3,10], this finding, to our knowledge, demonstrates for the first time, that catfish in lakes and artificial ponds harbor *Cryptosporidium* oocysts and may therefore, serve as mechanical vectors of this protozoan parasite for zoonotic transmission to humans.

The occurrence of *Cryptosporidium* oocysts in the fish could be a bioindicator of animal and human faecal contamination of ponds, lakes and other water bodies or from wastewater, leaky septic tanks, or recreational activities that occur through run–offs or leaching through the soil that are exposed to such water bodies. Other possible sources of the lake contamination include sewage effluent overflows, waste–water discharges, abattoir waste, direct animal faecal deposition in waterways, indirect deposition through runoff from grazing livestock and/or wildlife, manure and effluent spreading, and storm water runoff[15,16]. These conditions are true for the lakes studied since they are fed by water from polluted waste water originating from the surrounding villages and towns. It was observed that herders bring their cattle to drink directly from these water bodies, and in the process the water gets contaminated with animal waste. Pintar and co–workers reported that in Canada, *Cryptosporidium* species posed a high risk of infection from consumption of contaminated surface water source[17]. Studies have shown that up to 97% of the surface waters serving water treatment plants and 54% of treated (filtered and chlorinated) waters contain low numbers of *Cryptosporidium* oocysts[15,18].

Although there have been a few reports of natural cryptosporidiosis in teleosts, the only reported case of catfish cryptosporidiosis is of an experimental infection in a freshwater South American catfish, *Plecostomus* spp.[19]. However, no link appears to exist yet between human cases of cryptosporidiosis and ingestion of infected catfish even though such linkages exist between *Cryptosporidium* spp. in other animals and humans. Therefore the occurrence of *Cryptosporidium* oocyst in catfish strongly suggests a possibility that catfish could be a potential source of human cryptosporidiosis. This is particularly important in a country like Nigeria where production and consumption of catfish was estimated to be about 260 million kilogrammes in 2008 and contributing approximately US dollar 1 billion to the country's GDP in 2009[2]. In fact, the implication of this occurrence may be more global since several reports suggest that because of advantages such as its omega–3 content, its leanness and its low saturated fat content, catfish production and consumption is on the increase in other countries like Cameroon, Kenya, Mali, South Africa, Togo and Uganda[20].

Samples obtained from the lakes had a higher prevalence rate (39.25%) than those from the ponds with 10.50% indicating that consumption of catfish raised by extensive farming method may constitute greater public health risk with respect to *Cryptosporidium* infection than those raised by intensive method. The significantly higher occurrence of *Cryptosporidium* oocyst in catfish from the lakes than the artificial ponds could be attributed to bad agricultural practices that encourage faecal contamination of surface waters by livestock. Secondly, lack of proper fencing or embankment may have permitted unrestricted movement of grazing livestock around the immediate lake vicinity, resulting in the indiscriminate defecation around the lake vicinity and it has been reported that faeces of infected animals is a major source of infection for the environmental and humans[20].

Another possible reason for this high oocyst occurrence in catfish obtained from the lakes is the possibility of in–flow of contaminated domestic sewage, outfalls and rainwater runoffs from the neighboring human settlement. This is corroborated by the work of Nnaji *et al.*, who reported that domestic sewage and refuse from many settlements in Zaria, either get leached, discharge directly or runoff from the surface into the river Galma on which the Zaria lake is located[15].

The particularly high oocyst recovery from catfish obtained from Zaria lake may have been facilitated by the lack of integrity at some points of the lake thus allowing the free movement of cattle to the lake banks, making it possible for them to drink directly from the water and it has been documented that *Cryptosporidium* oocyst concentration relates directly to the level of faecal pollution of water bodies. The public health importance of oocyst shedding cannot be overemphasized as contaminated animals can shed massive amounts of oocysts to the environment[21,22], which can be ingested by other animals and even man with the ultimate result of getting infected with *Cryptosporidium*.

The study revealed that more *Cryptosporidium* oocysts were recovered from the gastrointestinal tract samples than from the gill samples indicating that in fish, gastrointestinal tract is a better organ for diagnostic oocyst recovery. This conforms to the report of an earlier work by Leal *et al.*, in which they recorded more *Cryptosporidium* oocysts from the gastrointestinal tract of fish than from other organs screened[3].

The health implication of the high *Cryptosporidium* oocyst occurrence in catfish is far reaching because consuming *Cryptosporidium* oocyst–inundated catfish is capable of causing cryptosporidiosis and mathematical modeling algorithms indicate that some persons could become infected with a dose as low as one oocyst[5].

Cryptosporidiosis has debilitating effects and can cause high morbidity and mortality in animals and humans. It is usually characterized by diarrhea and general symptoms of gastrointestinal disease[7,14,21]. The cost in terms of treatment and productivity losses due to cryptosporidiosis is usually very high, especially when there is an outbreak as was the case in Milwaukee, Wisconsin, where a massive *Cryptosporidium* waterborne outbreak affected an estimated 403 000 people with 4 400 people hospitalized and left more than 100 people dead. The total cost of outbreak–associated illness was estimated at 96.2 million dollar, including 31.7 million dollar in medical costs and 64.6 million dollar in productivity losses[23]. This situation is bad for the overall gross domestic product of any nation and especially for a country like Nigeria where there is an urgent need to improve national earnings in order to reduce the crunch of the current economic meltdown.

This calls for urgent public health intervention, especially as earlier studies have shown that the infective dose for *Cryptosporidium* oocysts is low[3,10]. Mackenzie *et al.*, also documented the epidemic potential of cryptosporidial diarrheoa and the implications of cryptosporidiosis in HIV/AIDS and immune–suppressed individuals[23].

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We thank the authorities of Ahmadu Bello University, Zaria Nigeria for supporting this work with Grant No. ABU/P16945 to JCA.

Comments

Background

Cryptosporidium is an enteric parasite that causes diarrhoea and other non specific enteric signs. The infection can be debilitating in immune deficient individuals. Consumption of catfish is on the increase in Nigeria because it is a cheap source of protein and can be easily grown. This paper looks at the extent of contamination of catfish meant for human consumption with *Cryptosporidium* oocysts. It compares the contamination of catfish from backyard fish farms (21%) with that of lakes (46% and 33%). This highlights the importance of proper cooking of catfish before consumption.

Research frontiers

Studies are being performed because catfish is the daily meat for the general populace in Nigeria. Knowing that the prevalence of *Cryptosporidium* in catfish in the study area is 49.75% which is of great public health significance. Also the fact that intestinal contents are more contaminated than gills reemphasizes the need to focus on the sampling of intestinal contents for enteric parasites of fish.

Related reports

The data from this study demonstrates for the first time the presence of *Cryptosporidium* oocysts in catfish in the study area. Other studies have found presence of *Cryptosporidium* oocysts in clams, oysters and mussels elsewhere other than study area.

Innovations and breakthroughs

There is no data regarding the prevalence of *Cryptosporidium* oocysts in catfish in the study area. This happens to be the first documented report supporting such claim. Also highlighted in this paper is extent of contamination of catfish in natural source of water and that of artificial source.

Applications

Results of this study suggests the proper cooking of catfish before consumption. It may also be important to know source of catfish before consumption so that extral hygienic measures can be taken if they are from natural sources.

Peer review

This is a good pioneer study in which the authors try to look at the significance of consumption of catfish positive for *Cryptosporidium* oocysts. The results are interesting and suggests that catfish can serve as a source for *Cryptosporidium* infection in the study area.

References

[1] Chilima DM. Democratic Republic of the Congo planning meeting, 5-6 December 2007, workshop report. Malaysia: The WorldFish Center; 2007.

[2] Bernard T. Nigeria: catfish farming-a reliable investment. Cape Town: AllAfrica; 2009. [Online] Available from http://allafrica.com/stories/200904140233.html. [Accessed on 18 September, 2012].

[3] Leal DAG, Pereira MA, Franco RMB, Branco N, Neto RC. First report of *Cryptosporidium* spp. oocysts in oysters (*Crassostrea rhizophorae*) and cockles (*Tivela mactroides*) in Brazil. *J Water Health* 2008; **6**(4): 527-532.

[4] Murphy B, Bradway GD, Walsh T, Sanders GE, Snekvik K. Gastric cryptosporidiosis in freshwater angelfish (*Pterophyllum scalare*). *J Vet Diagn Invest* 2009; **21**: 722-727.

[5] Benamrouz S, Guyot K, Gazzola S, Mouray A, Chassat T, Delaire B, et al. *Cryptosporidium parvum* Infection in SCID mice infected with only one oocyst: qPCR assessment of parasite replication in tissues and development of digestive cancer. *PLoS One* 2012; **7**(12): 51232.

[6] Chalmers RM, Davies AP. Minireview: clinical cryptosporidiosis. *Exp Parasitol* 2010; **124**: 138-146.

[7] O'connor RM, Shaffie R, Kang G, Ward HD. Cryptosporidiosis in patients with HIV/AIDS. *AIDS* 2011; **25**: 549-560.

[8] Kaduna State Government. Zaria master plan. Kaduna State, Nigeria: Ministry of Lands and Survey; 2000.

[9] Gomez-Couso H, Freire-Santos F, Ortega-Inarrea MR, Castro-Harmida JA, Ares-Mazas ME. Environmental dispersal of *Cryptosporidium* parvumoocysts and cross transmission in cultured bivalve mollusca. *Parasitol Res* 2003; **90**: 140-142.

[10] Leitch GJ, He Q. Cryptosporidiosis-an overview. *J Biomed Res* 2012; **25**(1): 1-16.

[11] Tang J, McDonald S, Peng X, Samadder SR, Murphy TM, Holden NM. Modelling *Cryptosporidium* oocysts transport in small ungauged agricultural catchments. *Water Res* 2011; **45**(12): 3665-3680.

[12] Casemore DP, Armstrong M, Sands RL. Laboratory diagnosis of cryptosporidiosis. *J Clin Pathol* 1985; **38**(12): 1337-1341.

[13] Nielsen CK, Ward LA. Enhanced detection of *Cryptosporidium* parvum in the acid-fast stain. *J Vet Diagn Invest* 1999; **11**: 567-569.

[14] White Jr. AC. *Cryptosporidium* species. In: Mandell D, Bennett JE, Dolin R, editors. *Principles and practice of infectious diseases*. 7th ed. Philadelphia: Churchill Livingstone, Elsevier; 2010, p. 3547-3560.

[15] Feng Y, Zhao X, Chen J, Jin W, Zhou X, Li N, et al. Occurrence, source, and human infection potential of *Cryptosporidium* and *Giardia* spp. in source and tap water in Shanghai, China. *Appl Environ Microbiol* 2011; **77**(11): 3609-3616.

[16] Nnaji JC, Uzairu A, Harrison GFS, Balarabe ML. Effect of pollution on the physico-chemical parameters of water and sediments of River Galma, Zaria, Nigeria. *Res J Environ Earth Sci* 2011; **3**(4): 314-320.

[17] Pintar KD, Fazil AA, Pollari FF, Waltner-Toews DD, Charron DF, McEwen SA, et al. Considering the risk of infection by *Cryptosporidium* via consumption of municipally treated drinking water from a surface water source in a Southwestern Ontario Community. *Risk Anal* 2012; **32**(7): 1122-1138.

[18] Smith HV, Nichols RAB. *Cryptosporidium*: detection in water and food. *Exp Parasitol* 2010; **124**(6): 61-79.

[19] Muench TR, White MR. Cryptosporidiosis in a tropical freshwater catfish (*Plecostomus* spp.). *J Vet Diagn Invest* 1997; **9**: 87-90.

[20] Na-Nakorn U, Brummett RE. Use and exchange of aquatic genetic resources for food and aquaculture: *Clarias* catfish. *Rev Aquacult* 2009; **1**: 214-223.

[21] Galvão ALB, Ortiz EG, Ferreira GS, de Vasconcellos AL, Vieira MC, Bresciani KDS. Importance of cryptosporidiosis as zoonosis. *Arch Vet Sci* 2012; **17**(2): 18-28.

[22] Hofstra N, Bouwman AF, Beusen AH, Medema GJ. Exploring global *Cryptosporidium* emissions to surface water. *Sci Total Environ* 2013; **442**: 10-19.

[23] Kothavade RJ. Potential molecular tools for assessing the public health risk associated with waterborne *Cryptosporidium* oocysts. *J Med Microbiol* 2012; **61**(8): 1039-1051.