Cryptosporidium oocysts in *Anodonta* sp. (bivalve mollusc) as indicators of pollution of Tiga Lake ecosystem in Kano State, Nigeria

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Bivalve molluscs are filter feeders and can bioaccumulate oocysts of Cryptosporidium. Tiga Lake in Kano State Nigeria is used for recreational, domestic and agricultural purposes by humans and also serves as a source of drinking water for animals. Bivalve molluscs from the lake are consumed by people. This study was conducted to assess the occurrence of *Cryptosporidium* spp. in Tiga Lake using edible *Anodonta* sp. (fresh water mussels) a bivalve mollusc as sentinel. The samples were examined using modified acid fast staining technique and micrometry of the oocysts. 169 and 150 samples of the molluscs were collected from two locations namely Tashan Idi and Rurum, respectively. The organs examined from each of the molluscs were the gastrointestinal tracts (GIT), gills and haemolymph. The mean oocysts load was higher in the GIT (192.50 ± 173.03) than in the other organs, although the difference was not statistically significant (P > 0.05). Of the two sampling sites, 60 (35.50%) and 40 (26.67%) of the molluscs from Tashan Idi and Rurum respectively were positive for the oocysts. However, the difference was not statistically significant (P > 0.05). The micrometry of the oocysts showed that most of them fell within the size range of 4.0 to 4.3 µm and 4.4 to 4.7 µm suggesting that the oocysts encountered in this work might be those of *Cryptosporidium parvum* and *C. meleagridis* which infect a wide range of animals and also humans. The result of this study reveals that 27.59% of the bivalve molluscs harboured Cryptosporidium oocysts and this may have public health implications if undercooked molluscs are consumed by humans.

**Key words:** Cryptosporidium, bivalve molluscs, Tiga Lake, bioaccumulate, public health.

**INTRODUCTION**

Cryptosporidium is a water-borne zoonotic coccidian protozoan parasite. It invades and then replicates within the microvillus region of epithelial cells, lining the digestive and respiratory organs of vertebrates (Nimri and Hijazi, 1994; Fayer et al., 1999; Xiao et al., 2002). Cryptosporidium is transmitted faeco-orally by ingestion of the sporulated oocysts, and most human cryptosporidiosis outbreaks have been associated with water-borne routes of transmission (Solo-Gabriele and Neumeister, 1996). Cryptosporidium parasites are endemic in many domestic and wild life populations, with young animals often shedding over a million oocysts during initial infection. The infective dose of *C. parvum* in humans can be as low as 10 to 100 oocysts (Fayer et al., 1998). The oocyst stages of *Cryptosporidium* spp. are shed in the faeces of animals and humans which may then enter sewage facilities via agricultural runoffs, wastewater discharges and faecal contamination by wild-
life or persist for a long time in the environment. These oocysts can contaminate surface water, water used for drinking, recreational activities and shellfish production (Graczky et al., 1997). This can pose a serious public health problem especially on aquatic life intended for human consumption. Environmental monitoring for Cryptosporidium spp. Can be problematic, partly because of the dilution effect that occurs as oocysts are disseminated from terrestrial to aquatic ecosystems, and also because particulate matter can inhibit or interfere with Cryptosporidium detection methods (Feng et al., 2003). Filter-feeding invertebrates such as bivalve molluscs, which can filter over 2 L of water/h/shellfish, can act as a natural concentration system (McMahon, 1991). These bivalves can then be collected and tested for pathogens, providing an indication of water quality (Freire-Santos et al., 2000; Graczky, 2003; Miller et al., 2005; Tamburrini and Pozio, 1999).

Bivalve molluscs belong to the Phylum Mollusca, Class Bivalvia, Order Unionoidae, Family Unionidae and Genus Anodonta (Nedeau et al., 2005). They use their gills to capture particulate food from the water. The water current enters the shell from the posterior ventral surface of the animal and then passes upward through the gills and the filtered particles are transported to the mouth. Food entering the mouth is passed to the stomach via ciliary action. Oocysts can be removed by bivalve molluscs from contaminated water and retained on their gills and haemolymph (Freire-Santos et al., 2000; Giangaspero et al., 2007). An approximation of the parasite load of shellfish contaminated naturally indicated that each shellfish could transport more than $10^3$ oocysts (Fayer et al., 1999). Numerous edible bivalve species live burrowed in sand or mud and respire by means of siphons which reach to the surface of water. They are eaten as a delicacy in many parts of the world and are also served in place of red meat (www.wikipedia.org/seafood, 1999; Gomez-Bautista et al., 2000). In developing countries, cryptosporidiosis occurs mostly in children younger than 5 years, with peak occurrence of infections and diarrhoea in children younger than 2 years. Fatalities of this disease occur primarily among patients with Acquired Immune Deficiency Syndrome (AIDS) or other systemic disorders (Bern et al., 2000; Fox and Lytle, 1996; Levy et al., 1997).

Tiga Lake of Kano State, Nigeria, receives agricultural run-offs and is used by livestock and wildlife. These may bring Cryptosporidium into the lake. The lake also has a lot of molluscs that are harvested for human consumption.

**Statement of the problem**

In Nigeria, there is paucity of literature on the status and prevalence of Cryptosporidium infection from bivalve molluscs. Globally, there is an increase in sourcing of food especially protein and consumption of less cholesterol meat. Varying consumer vogue like roasting, frying and undercooking of molluscs to retain natural taste; pre-contamination of infected molluscs with Cryptosporidium oocysts on hands of the handlers and cooking utensils can pose a risk for food borne transmission of Cryptosporidium from bivalve molluscs intended for human consumption (Doyle, 2003).

The paper assessed the occurrence of Cryptosporidium oocysts in Tiga Lake of Kano State, Nigeria using Anodonta sp. as sentinel to determine: the occurrence of Cryptosporidium oocysts in Anodonta sp., the load of Cryptosporidium oocysts in Anodonta sp., and the sites where Cryptosporidium oocysts are mostly found in the molluscs (gills, haemolymph and gastrointestinal tract).

**METHODOLOGY**

Tiga lake is situated in Kano State between Latitude 11° 151' to 11° 29° North and Longitude 8° 16' and 8° 38' East. It is a man-made lake in Northern Nigeria with a surface area of 17,806 hectares and was the second largest man-made lake before the creation of the Jebba and Shiroro Reservoirs in 1983 in Nigeria (Ita et al., 1983; Galkowski and Galkowski, 1980). The lake was formed primarily for irrigation purposes with hydroelectric power generation as a secondary consideration and with recreation and conservation as other objectives. Other activities include, cattle grazing and supply of drinking water and human activities including washing, bathing, swimming and fishing (Galkowski and Galkowski, 1980). Sampling sites in the lake was Tashan Idi and Shiroro which are tributaries of the lake. Bivalve molluscs are some of the fauna found in the lake.

A total of 319 Anodonta sp. were collected by hand picking between the periods of December 2009 through April 2010 by convenience random sampling from the two sampling sites in Tiga Lake (169 molluscs from Tashan Idi and 150 from Shiroro). Fifty samples were collected weekly. Each collection was kept on ice and transported to the Parasitic Zoonoses Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria immediately for examination. The anterior and posterior muscles of each mollusc were cut with a scalpel inserted gently below the anterior and posterior ends of the molluscs (Graczky et al., 1997; Rowett, 1970). The shell was opened. The haemolymph, approximately 0.5 ml, was collected with a pipette from the adductor muscle into a clean well labeled test tube. The gills and GIT were excised from each bivalve and each of these organs was placed individually in 2 ml of phosphate buffered saline (PBS) pH 7.4 in an appropriately labeled test tube, washed and left for 30 min. The organs were then vortexed using a vorter mixer and removed. A drop of the sediment from each mollusc was carefully smeared on a properly labeled clean glass slide. The slides were air-dried overnight and stained using modified acid fast staining technique. Oocysts from the positive samples were further measured with a micrometer.

The modified acid fast staining technique was conducted as Nielson and Ward (1999). The prepared slides were examined by covering about 8 to 12 fields with a binocular microscope using x40 objective under a bright field. Measurement of the oocysts from the positive samples was estimated by using a calibrated microscope, knowing the size of Cryptosporidium oocysts to be between 4 to 6 µm (Sreter et al., 2000). A calibrated eye piece was inserted into the micrometer; the slide containing the oocysts was placed on the stage of the microscope and focused while viewing from the eye piece. The oocyst was moved towards the calibration with help of the adjustment knob of the microscope until the calibration was in alignment with the oocyst. The zero point was placed at the tip of the oocysts and measurement was taken from one end of the
oocyst to the other end. Each calibration represented a micrometer.

This was done in the Helminthology Laboratory of the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria, Nigeria. One drop (approximately 0.02 ml) of the prepared sediment was placed on a clean slide. An approximate estimate of the oocysts was calculated as follows: Total volume of solution divided by 0.02 ml multiplied by number of oocysts present in a drop of the sample expressed in millions; as modified by Mc master oocysts counting technique (Soulsby, 1968).

The oocysts from the samples of the gills, GIT and haemolymph were enumerated separately to determine the concentration and where the oocysts are trapped most, in the different organs. One-way analysis of variance was used to compare the mean number of oocysts detected in the different organs. The t test was used to determine the difference in mean oocyst load according to the site of collection and values of P < 0.05 were considered significant.

**RESULTS**

The overall infection rate of 319 Anodonta sp. obtained from Tiga Lake of Kano State was 88 (27.59%). Table 1 shows that 100 of the 957 organs of the molluscs examined were infected with Cryptosporidium oocysts. Out of 319 gastrointestinal tracts (GIT) of molluscs examined, 40 (12.54%) were positive. Thirty one (9.72%) of 319 gill samples were positive while 29 (9.02%) of the 319 samples of haemolymph examined were positive for Cryptosporidium oocysts. The contamination rate in the GIT (12.54%) was higher than in the other organs (Table 1). However, the difference was not statistically significant (P > 0.05). The mean oocyst load was higher in GIT (192.50) than in any of the organs, though the difference was not statistically significant (P > 0.05) (Table 1). Table 2 shows that the single organ contamination with Cryptosporidium oocysts was more common than the multiple organ contamination. Out of 169 samples of the Anodonta sp. from Tashan Idi examined, 60 (35.50%) were contaminated with Cryptosporidium oocysts, and of the 150 samples of molluscs collected from Rurum, 40 (26.67%) was contaminated with the oocysts. The difference in contamination rate between the two locations (Tashan Idi and Rurum) was not statistically significant (P > 0.05) (Table 3). Oocysts with the size range of 4.0 to 4.3 µm were more in number 35 (45.45%), followed by 17 (22.08%) oocyst that fell in the range of 4.4 to 4.7 µm (Table 4).

**DISCUSSION**

The presence of Cryptosporidium oocysts in the bivalve molluscs examined (27.59%) in Tiga Lake of Kano State showed that the molluscs are indicators of faecal contamination as seen with the findings of Gomez-Couso (2006) in which 184 mussel sample (29.30%) were positive for Cryptosporidium oocysts, though

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**Table 1. Frequency of Cryptosporidium oocyst and the Mean oocyst load in the GIT, gills and haemolymph of Anodonta spp in Tiga Lake of Kano State, Nigeria.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number of oocysts examined</th>
<th>Number positive</th>
<th>Percentage positive (%)</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Lower bound</th>
<th>Upper bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>GIT</td>
<td>319</td>
<td>40</td>
<td>12.54</td>
<td>92.50</td>
<td>±173.03</td>
<td>137.16</td>
<td>247.84</td>
</tr>
<tr>
<td>Gills</td>
<td>319</td>
<td>31</td>
<td>9.72</td>
<td>67.74</td>
<td>±132.63</td>
<td>119.09</td>
<td>216.40</td>
</tr>
<tr>
<td>Haemolymph</td>
<td>319</td>
<td>29</td>
<td>9.02</td>
<td>41.38</td>
<td>±111.86</td>
<td>98.83</td>
<td>183.93</td>
</tr>
<tr>
<td>Total</td>
<td>957</td>
<td>100</td>
<td>31.28</td>
<td>70.00</td>
<td>±145.30</td>
<td>141.17</td>
<td>198.83</td>
</tr>
</tbody>
</table>

95% confidence interval for mean.

**Table 2. Single and mixed organ contamination with Cryptosporidium oocysts in Anodonta spp in Tiga Lake of Kano State, Nigeria.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Number positive</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single organ contamination</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>GIT only</td>
<td>33</td>
<td>10.35</td>
</tr>
<tr>
<td>Gills only</td>
<td>25</td>
<td>7.83</td>
</tr>
<tr>
<td>Haemolymph only</td>
<td>20</td>
<td>6.27</td>
</tr>
<tr>
<td>Double organ contamination</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Gills and Haemolymph</td>
<td>2</td>
<td>0.63</td>
</tr>
<tr>
<td>Gills and GIT</td>
<td>2</td>
<td>0.63</td>
</tr>
<tr>
<td>Haemolymph and GIT</td>
<td>4</td>
<td>1.25</td>
</tr>
<tr>
<td>Triple organ contamination</td>
<td>2</td>
<td>0.63</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
<td>27.56</td>
</tr>
</tbody>
</table>
this was statistically significant, isolation and detection some-times play a significant role in the recovery and enumeration of the oocysts (Downey and Graczyk, 2007). The gastrointestinal tracts (GIT) of the molluscs examined were more contaminated with the oocysts than other organs. This is probably because most of the filtered particles from the surface water end up in the GIT for digestion before being circulated to other parts of the body (Sreter et al., 2000). However, Downey and Graczyk (2007) found that haemolymph exa-mined harboured more oocysts. They attributed this to the fact that shortly after exposure, oocysts may be caught in the gills and other tissues of the digestive system but with time, the haemocytes scavenge these particles in an attempt to clear them from the gills and digestive tracts. Though the recovery of the oocysts from the GITs was higher than the haemolymph and gills, this was not statistically significant (P > 0.05) indicating that these organs have an equal chance of being contaminated. Multiple-organ contamination observed in this study agrees with the findings of Fayer et al. (1997) in which oysters removed Cryptosporidium parvum oocysts from artificially contaminated water and retains them in haemocytes, gills and within the body for at least one month. Multiple organ contamination indicates a higher chance of contracting cryptosporidial oocysts since these oocysts are trapped all over the body of the molluscs. Also filter feeders such as bivalve molluscs may be of value as biological monitors for the presence of Cryptosporidium oocysts and other water borne pathogens in river and marine water and in particular contaminating bivalves used for consumption which has obvious public health implications (Chalmers et al., 1997).

In Tiga Lake where large scale fishing activities takes place, bivalve molluscs which are in abundance are used as baits to catch fish. Contaminated molluscs when used for such purposes may serve as a route for cryptosporidial oocysts infection in fishes. This is possible as Graczyk et al. (1996) reported an infection in fishes, amphibians and reptiles. Thus aquatic animals other than bivalve molluscs might be potential sources of waterborne Cryptosporidium oocysts.

The two sampling sites investigated, showed that molluscs harvested at Tashan Iddi had a higher number of molluscs positive for the oocysts than Rurum. This may be attributed to the activities of the surrounding villages such as swimming, bathing, farming and fishing. Also cattle owners around bring their herds for grazing and drinking of water. These activities may serve as routes in which Cryptosporidium oocysts are discharged into the water body. These agree with findings of Fayer et al. (1997) and CDC (2000) in which oocysts can contaminate surface water directly from human faeces, urine, recreational activities and indirectly from runoffs.

In micrometry of the oocysts most of them fell within the range of 4.0 to 4.8 µm, suggesting that the oocysts might be C. parvum or Cryptosporidium meleagris. These species are of zoonotic importance and their sizes fall in line with that of Sreter et al. (2000) and Xiao et al. (2004).

To date there are no published reports of human cryptosporidiosis cases resulting from the consumption of bivalve molluscs, despite a growing body of scientific literature on the recovery of Cryptosporidium oocysts

### Table 3. Distribution of Cryptosporidium oocysts in Anodonta sp. according to the site of sampling in Tiga lake of Kano state, Nigeria.

<table>
<thead>
<tr>
<th>Site of collection</th>
<th>Number of molluscs examined</th>
<th>Number positive</th>
<th>Percentage positive (%)</th>
<th>Mean oocysts load (per 2 ml)</th>
<th>Standard deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tashan Iddi</td>
<td>169</td>
<td>60</td>
<td>35.50</td>
<td>166.67</td>
<td>±145.75</td>
</tr>
<tr>
<td>Rurum</td>
<td>150</td>
<td>40</td>
<td>26.67</td>
<td>157.50</td>
<td>±127.88</td>
</tr>
</tbody>
</table>

### Table 4. Size range of Cryptosporidium oocysts in Anodonta sp. in Tiga lake of Kano State, Nigeria.

<table>
<thead>
<tr>
<th>Oocyst size range (µM)</th>
<th>Frequency of oocyst</th>
<th>Percentages (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.6 – 3.9</td>
<td>4</td>
<td>5.2</td>
</tr>
<tr>
<td>4.0 – 4.3</td>
<td>35</td>
<td>45.45</td>
</tr>
<tr>
<td>4.4 – 4.7</td>
<td>17</td>
<td>22.08</td>
</tr>
<tr>
<td>4.8 – 5.1</td>
<td>8</td>
<td>10.39</td>
</tr>
<tr>
<td>5.2 – 5.5</td>
<td>4</td>
<td>5.2</td>
</tr>
<tr>
<td>5.6 – 5.9</td>
<td>8</td>
<td>10.39</td>
</tr>
<tr>
<td>6.0 – 6.3</td>
<td>1</td>
<td>1.3</td>
</tr>
<tr>
<td>Total</td>
<td>77</td>
<td>100</td>
</tr>
</tbody>
</table>
sporidiosis of oocysts in molluscan shellfish. The long incubation period, that is, 7 to 10 days makes it difficult to associate the infection with a particular exposure (Graczyk and Schwab, 2000).

Conclusion

This study has established the presence of Cryptosporidium oocysts in *Anodonta* sp. in Tiga Lake of Kano State. It is established that these molluscs can effectively remove and retain oocysts of *Cryptosporidium* from contaminated water bodies. Due to the zoonotic potentials of the infection, threats to human life should not be underestimated especially when considering susceptible groups such as newborn infants, the elderly, patients on immunosuppressive drugs, people infected with HIV who are at a higher risk of infection. There is a need for a more in depth molecular investigation to highlight and identify the *Cryptosporidium* species recovered from *Anodonta* sp.

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