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Short Communication

Molecular detection of *Giardia* contamination in water bodies in a zoo

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**A R T I C L E  I N F O**

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**A B S T R A C T**

We used a combined microscopy-molecular approach to determine the occurrence and identities of waterborne *Giardia* sp. cysts isolated from 18 separate, 10 l grab samples collected from a Malaysian zoo. Microscopy revealed that 17 of 18 samples were *Giardia* cyst positive with concentrations ranging from 1 to 120 cysts/l. Nine (52.9%) of the 17 cyst positive samples produced amplicons of which 7 (77.8%) could be sequenced. *Giardia duodenalis* assemblage A (6 of 7) and assemblage B (1 of 7), both infectious to humans, were identified at all sampling sites at the zoo. The presence of human infectious cysts raises public health issues, and their occurrence, abundance and sources should be investigated further. In this zoo setting, our data highlight the importance of incorporating environmental sampling (monitoring) in addition to routine faecal examinations to determine veterinary and public health risks, and water monitoring should be considered for inclusion as a separate element in hazard analysis, as it often has a historical (accumulative) connotation.

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1. Introduction

Zoos, conservation parks and wildlife-rehabilitation centres which house a wide range of captive wildlife provide ideal places as reservoirs of myriad of pathogens. Captive animals maybe exposed to high-density and high-stress environments which can result in increased susceptibility to infection and disease. They are also exposed to species of parasites that they would not usually encounter in the wild. These infected captive animals may act as potential sources of environmental contamination, especially water (Theron & Cloete, 2002), as most centres also have aquatic features such as lakes and rivers.

Waterborne transmission of giardiasis can affect large numbers of consumers of contaminated drinking water. The transmission of giardiasis can be direct or indirect, and the most commonly recognised indirect route is via water contamination with *Giardia* cysts (Karanis et al., 2007). However, standardised microscopy methods for detecting waterborne *Giardia* cysts are available (Anonymous, 1999) http://www.dwi.gov.uk/regs/crypto/pdf/sop%20part%202.pdf; http://www.epa.gov/microbes/1623de05.pdf), PCR-based molecular methods add value to microscopy findings because of their ability to determine species/genotypes/assemblages and sub-genotypes in morphologically indistinguishable cysts (Mahbubani et al., 1998; Smith et al., 2006, 2007; Smith and Nichols, 2009).

Despite considerable information being available on the occurrence of *Giardia* in water in Malaysia (Lim et al., 2008a), no species or subspecies data are available, limiting the usefulness of these studies for epidemiology and source tracking. Genotyping and sub-genotyping data not only assist in refining the host range and zoonotic potential of known and novel *Giardia* species and sub-genotypes, but also greatly enhance the epidemiological inferences drawn from such studies.

The aim of this study was to investigate the occurrence of *Giardia* in a Malaysian zoo environment by determining waterborne cyst concentrations in the lake, its associated river and tributary and the main water supply tank and determining the molecular signatures of the species detected.

2. Materials and methods

A total of eighteen, 101 grab samples (9 from the lake, 3 from an associated river and 3 from its tributary, and 3 from the main water supply tank) (Fig. 1) taken from approximately 30 cm below the surface of the water were collected. The river and its tributary supply water to the lake and the main water supply tank. Each sample was filtered through a flat bed cellulose acetate membrane (1.2 m, 142 mm dia.) (Anonymous, 1999) and the concentrate was subjected to immunomagnetically separable (IMS) (Dynal, cat. no. 730.02, Oslo, Norway) according to manufacturer’s instructions before being deposited onto a microscope slide and stained with a commercial fluorescein isothiocyanate (FITC)-labelled monoclonal antibody kit reactive with exposed epitopes on *Giardia* cysts (Cellabs Pty Ltd., cat. no. 1K82, Brookvale, Australia) according to the manufacturer’s...
instructions, and the nuclear fluorophore 4′,6-diamidino-2-phenyl indole (DAPI) (Sigma Chemical Co., cat. no. 32670-5MG-F Louis, Missouri, USA). Stained samples were examined by epifluorescence microscopy (400 x) and putative cysts were confirmed by viewing at 1000 x using Nomarski differential interference microscopy to confirm their internal morphologies (http://www.dwi.gov.uk/regs/crypto/pdf/SOP20part202.pdf; http://www.epa.gov/microbes/1623de05.pdf). The number of observed cysts was enumerated three times based on the sampled volume (101).

DNA was extracted from IMS-isolated Giardia cysts using the Qiagen DNA Mini Kit (Qiagen, cat. no. 51306; Germany) before a nested PCR assay was used to amplify a partial region of the SSU rRNA gene (OLYMPUS Stream, cat. no. R0192, Ontario, Canada), 2 mM MgCl2 (Qiagen, cat. no. R0971, Ontario, Canada), 5% dimethyl sulfoxide (DMSO) (Sigma, cat. no. 673439, USA), 0.2 mg/ml gelatin and 400 mg/ml bovine serum albumin (BSA) (New England Biolabs, cat. no. BS14, Ipswich, USA). Two µl of DNA template was used in both primary and secondary PCRs. In both amplifications, samples were incubated in a MyCycler thermal cycler (Bio-Rad, cat. no. 170-9705, Hercules, USA) under the following conditions: denaturing at 95 °C for 2 min, followed by 35 cycles of denaturing for 20 s at 96 °C, annealing for 20 s at 59 °C and extension for 20 s at 72 °C, followed by a final extension at 72 °C for 7 min. PCR products were electrophoresed on 2% agarose gel and stained with 0.5 µg/ml final concentration of ethidium bromide (AMRESCO inc, cat. no. X328, Solon, Ohio, USA).

PCR products were purified using the QiAquick Gel Extraction Kit (Qiagen, cat. no. 20704, Germany) according to the manufacturer’s instructions and purified DNA was sequenced using ABI Prism® BigDye® terminator v3.0 Ready Reaction Cycle Sequencing Kit (Applied Biosystems, USA) in both directions using Giar-F and Giar-R primers (Read et al., 2002). The sequences obtained were aligned with those sequences reported in this paper are available in the GenBank database under accession numbers FJ668856-FJ668862.

3. Results and discussion

A total of 17 of 18 (94.4%) samples from the lake, its associated river, its tributary and main water supply tank in this zoo (Fig. 1) were positive for Giardia cysts, with concentrations ranging from 1 to 120 cysts/l (Table 1). The highest concentrations of Giardia cysts were detected in the main water supply tank (42.3 ± 67.4 cyst/l; range: 6.8–120), followed by its associated river (35.3 ± 56.1 cyst/l; range: 1–100), river tributary (16.7 ± 10.9 cyst/l; range: 4.6–25.6) and finally the lake (10.3 ± 2.7 cyst/l; range: 7.2–12.4).

While 17 samples were positive for Giardia cysts by microscopy, only 9 (52.9%) produced amplicons of which 7 (77.8%) could be sequenced. Possible reason for this discrepancy is the presence of humic and fulvic acids in the water which inhibits PCR even when they are present at low concentrations (Mayer and Palmer, 1996). Six sequences displayed the sequence reported for G. duodenalis assemblage A and one for assemblage B. Of importance, only G. duodenalis assemblages A and B infect humans (Olson et al., 2004; Thompson and Monis, 2004; Smith et al., 2007; Cacciò and Ryan, 2008) however, assemblage A

Table 1

<table>
<thead>
<tr>
<th>Water type</th>
<th>Detection of Giardia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Numbers of sample examined</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Main drinking water supply tank</td>
<td>3</td>
</tr>
<tr>
<td>Associated river</td>
<td>3</td>
</tr>
<tr>
<td>River tributary</td>
<td>3</td>
</tr>
<tr>
<td>Lake</td>
<td>9</td>
</tr>
<tr>
<td>Overall</td>
<td>18</td>
</tr>
</tbody>
</table>

*Fig. 1. Map of the zoo depicting the various sampling points (indicated by numbers 1–6) and a selection of some animals on display. G. duodenalis assemblage A were detected in the lake (1,2,3)*, an associated river (4)* and the main supply tank (6)* whilst G. duodenalis assemblage B was only detected in the river tributary (5)*.
parasites also infect slow loris, livestock (cattle, sheep, pig, water buffalo), horse, deer and moose, cats, dogs, foxes, beavers, muskrats, voles, guinea pigs, ferrets, while assemblage B parasites also infect monkeys, siamung, slow loris, livestock (cattle, sheep), horses, chinchillas, dogs, cats, beavers, muskrats, voles, rats and marmoset (Smith et al., 2007; Cacciò and Ryan, 2008). Most of these hosts are exhibited in the zoo and the majority (~80%) are indigenous to Malaysia.

Assemblage A cysts were detected in the lake, the associated river and the main water supply tank, whereas assemblage B cysts were detected in the river tributary, only (Fig. 1; Table 1). As both assemblages A and B cysts are infectious to humans, their occurrence in this setting and their sources are of public health interest. The occurrence of assemblage B cysts in the river tributary is likely to be due to human contamination from villagers living at the upper stream (in relation to the zoo) of the tributary, as previous studies indicate a preponderance of G. duodenalis assemblage B in Malaysian communities (unpublished data). Determining the potential sources of assemblage A cysts is less easy. Clearly, to understand the likely significance of risk to both the customers, staff and inhabitants of this zoo, further species, sub-species and source tracking investigations using multilocus genotyping (e.g., beta-giardin, glutamate dehydrogenase and triose phosphate isomerase genes) should be performed using recommended criteria (Smith et al., 2006, 2007).

There are identifiable risks of infection to susceptible, captive animals imposed by this regimen of water usage. The highest cyst contamination was detected in the main water supply tank followed by the river, the river tributary and lastly the lake water (Table 1). Sources of water supplying the main supply tank include river and rain water, and the cyst-contaminated river water will contribute Giardia cysts to this closed system source. Only treated drinking water, supplied by Department of Water Supply, Malaysia, is used as drinking water for all captive animals in the zoo, but untreated water from the contaminated main supply tank is used for cleaning and bathing animals and washing animal compounds. Although this contaminated water is not consumed directly, captive animals may still be exposed to waterborne cysts following its deliberate or accidental ingestion following washing, or its aerosolisation. Mammals groom by licking their fur after they are washed, risking the accidental ingestion of any cysts deposited on their fur. Aerosols containing Giardia cysts, from cages cleaned by spraying, may also be another transmission route.

The river supplies water into the lake and simultaneously the lake also receives wastewater discharged from the zoo following gravity settlement in the lake. The lake, situated at the south-west of the zoo (Fig. 1), receives both wash water from cage washing performed at various locations in the zoo and all faecal material remaining on cage floors channelled into the lake for settlement. In addition, cyst (and other infectious disease) contributions from water-dwelling birds and other aquatic animals inhabiting the lake are to be expected. Minimal work has been carried out to de sluice the lake since the establishment of the zoo in 1963, mainly because of lack of funds, which will add to the reservoir of cysts.

Here, we highlight the commonness of Giardia assemblages A and B cyst occurrence in this zoological setting (94%; 17 of 18 samples), yet our other, contemporaneous, study, analysing faecal samples from captive animals in this zoo for intestinal parasites, did not detect Giardia cysts (Lim et al., 2008b). Reasons for this include the possibility that zoo animals were not the source of our waterborne Giardia cysts; the method (i.e., trichrome stain) we used to detect faecal cysts was insufficiently sensitive (and zoo animals may have contributed to our waterborne cysts); and other (unidentified) sources (human/mechanical vectors for assemblages A and B cysts) were responsible for the waterborne contamination. Dependant on the setting, the investigation of environmental (soil, water, etc.) contamination often provides added value to protozoan parasite risk assessment as it offers a further element, often with a historical (accumulative) connotation, for analysis, and should be included as a separate element in hazard analysis.

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References


