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PII: S0014-4894(12)00146-4
DOI: http://dx.doi.org/10.1016/j.exppara.2012.04.015
Reference: YEXPR 6438

To appear in: Experimental Parasitology

Received Date: 13 February 2012
Revised Date: 12 April 2012
Accepted Date: 30 April 2012

Please cite this article as: Loganthan, S., Yang, R., Bath, A., Gordon, C., Ryan, U., Prevalence of Cryptosporidium species in recreational versus non-recreational water sources, Experimental Parasitology (2012), doi: http://dx.doi.org/10.1016/j.exppara.2012.04.015

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Prevalence of Cryptosporidium species in recreational versus non-recreational water sources.

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Abstract

Cryptosporidiosis, caused by the protozoan parasite *Cryptosporidium*, represents the major public health concern of water utilities in developed nations due to its small size, resistance to disinfection and ability to be shed in large numbers in faeces. In Australia, recreational access is not allowed on direct supply sources, however, in Western Australia, limited recreational access to drinking water catchments has been allowed, although only in the outer catchment. Recreational activities within 2km of the drinking water body is prohibited. The present study compared the amount, prevalence and species of *Cryptosporidium* in recreational versus non-recreational water catchments in the south west of Western Australia (WA). Recreational water catchments, which allowed swimming and camping had a higher prevalence of *Cryptosporidium* and the majority of samples were the human-associated *C. hominis*. Non-recreational catchments had a lower prevalence and all the samples genotyped were *C. parvum*. Risk analysis identified increasing population as strongly correlated with an increase in the prevalence of *Cryptosporidium* in recreational catchments. This suggests that recreational access to drinking water catchments is a serious public health risk and government policy limiting activities to the outer catchment should be supported.

Keywords: *Cryptosporidium; C. hominis; C. parvum;* recreational water catchment; genotyping.
1. Introduction

*Cryptosporidium* is an enteric parasite that is a leading cause of waterborne outbreaks of gastroenteritis (Karanis et al., 2007). The parasite is particularly suited to waterborne transmission as the environmentally resistant oocysts are shed in large numbers in faeces ($10^8$-$10^9$ oocysts/gram), have a low infectious dose and are highly resistant to chlorine disinfection (Yoder and Beach, 2010). Swimming in untreated recreational waters, such as lakes and dams, is particularly hazardous to public health as there are additional potential sources of contamination, for example contamination from agriculture (Karanis et al., 2007). Of the 23 currently recognised species of *Cryptosporidium*, two species are responsible for the majority of infections in humans; *C. parvum* and *C. hominis* but *C. hominis* which is transmitted anthroponotically, predominates in human cryptosporidiosis infections in Australia (Jex et al. 2008; Waldron et al. 2009; Ng et al., 2010a; 2010b).

The use of drinking water catchments for recreational activities varies across Australia, though in most cases recreational access is not allowed on direct supply sources (Anderson et al., 2008). Despite Australian Drinking Water Guidelines (ADWG) emphasis on maintaining multiple barriers and the protection of drinking water catchments, particularly from human contamination, limited recreational access to Western Australian drinking water catchments has been allowed, though direct access to the water body is prohibited. The aim of the present study therefore was to investigate the
prevalence of *Cryptosporidium* species present in key recreational water and non-recreational water catchments within Western Australia.

2. Materials and methods

2.1 Sampling

A total of 72 10L water samples were collected from three recreational and four non-recreational water catchments. Recreational sites (sites 1 to 3) and non-recreational sites (sites 4 to 6) are listed in Table 1. Of the recreational catchments, only site 1 and site 2 allowed swimming and camping (Table 1). Sampling was conducted between December 2008 and September 2010.

2.2 Sample concentration and purification

Concentration and purification of all samples were carried out as per the USEPA 1623 method (EPA, 2005), using Envirochek filters (Gelman) and a GC Combo Dynabeads-Dynal kit (Invitrogen), following the manufacturer’s instructions. The recovery rates from the Envirocheck filters was analysed by spiking four 10 L water samples with 100 *Cryptosporidium* EasySeed™ oocysts (Biotechnology Frontiers BTF). The recovery rate was calculated by the amount of parasite detected via qPCR as described below.
2.3 DNA extraction and qPCR

DNA was extracted using a Powersoil® DNA kit (MO-Bio) as per the manufactures instruction. Samples were screened and numbers of oocysts determined using a qPCR based on a novel diagnostic locus and genotyped using a nested PCR based on the 18S ribosomal RNA (rRNA) locus as previously described (Yang et al., 2009; Ryan et al., 2003). PCR products were purified using an UltraClean™ DNA Purification Kit (MO Bio) and sequenced using a Big Dye version 3.1 Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, California). Nucleotide sequences were analysed using Chromas v2.3 (http://www.technelysium.com.au/chronas.html) and aligned with reference genotypes from GenBank using Clustal W (http://www.clustalw.genome.jp).

Cryptosporidium galli was used as a positive control and a negative control (no DNA) was included for all reactions. A spike test analysis (addition of 0.5μL of C. galli positive control into each sample) was carried out on randomly selected samples to confirm that negative results were not due to PCR inhibition.

2.4 Risk assessment analysis

A statistical analysis of key environmental (rainfall, temperature, humidity, UV etc) and catchment (water level, use of catchment, population, faecal bacteria, use of land, vegetation etc) risk factors was performed using SPSS version 17.0 (SPSS inc. Chicago, USA) using a one way a linear regression analysis to determine if there were any correlations between the amount of Cryptosporidium and the various risk factors.
analysed. A Pearson correlation was carried out to determine if the correlation was a normal relation or an inverse relation. Metrological data (rainfall, humidity, UV index and temperature) was obtained from remote weather monitoring stations closet to the sampling site from the Bureau of Meteorology (www.bom.gov.au). Data for water levels in catchments were obtained from the Water Corporation (www.watercorporation.com.au). Records for numbers of people attending recreational catchments was obtained from the Department of Environment and Conservation (DEC), Department of Water (DoW) and local rangers. Analysis and quantification of faecal bacteria was carried out by PathWest, on duplicate samples collected as part of the present study. The analysis was confined to Site 1 Brook Dam as insufficient data was available for the other sites.

3. Results

3.1 Recovery rate analysis

The mean oocyst recovery efficiency from the four replicates using envirochek filters and immunomagnetic separation was 45%. The oocyst numbers in the different samples analyzed from recreation and non-recreational sites were re-calculated to factor in recovery efficiency.
3.2 Prevalence of Cryptosporidium species in the different catchments

Overall the prevalence of *Cryptosporidium* in the recreational waters, which allowed swimming and camping was 44.7% with the highest prevalence in site 1 (52.4%). Site 3, which did not allow swimming and camping, had a prevalence of 14.3% (Table 2 and Figure 1). The overall prevalence of *Cryptosporidium* in the non-recreational waters was 16.7% with the highest prevalence in site 5 (33.3%) and the lowest prevalence was in site 6 (16.6%). The difference in prevalence between the recreational and non-recreational catchments was significant (p< 0.05). In the recreational catchments, the majority of positives were detected during the summer months, particularly following public holidays. In the non-recreational catchments, the majority of positives were detected during the winter months.

Genotyping analysis identified all 11 positives from site 1 as the anthropologically transmitted *C. hominis* (Figure 1). At site 2, five of the six positives were *C. hominis* and one was *C. parvum*, whereas in site 3, both positives were *C. parvum*. In the non-recreational catchments, all the positives were *C. parvum*.

3.3 Risk analysis

Statistical analysis identified 4 factors that had a strong correlation with the presence of and amount of *Cryptosporidium* in site 1. These factors were rainfall, temperature, UV index and population. Rainfall had an inverse correlation with the amount of *Cryptosporidium* (i.e. an increase in rainfall was correlated with a decrease in
the amount of Cryptosporidium) while temperature, UV index and population had a positive correlation (i.e. increasing temperature, UV index and population was correlated with an increase in the amount of Cryptosporidium). Population appeared to be highly correlated with the presence of Cryptosporidium ($R^2 = 0.360$, $p<0.004$).

4 Discussion

In the present study, Cryptosporidium was identified at a higher prevalence in recreational versus non-recreational waters and the majority of samples from the recreational waters were the human-associated C. hominis whereas C. parvum which infects animals as well as humans was identified primarily in the non-recreational waters. Previous studies have shown that water catchment areas are predominantly contaminated by Cryptosporidium from three sources: farm animal sewage, human sewage discharge and contamination from wild animals (Bryan et al., 2009; Xiao, 2010). Molecular epidemiological analysis has improved the understanding of cryptosporidiosis transmission. It is now understood that C. hominis almost exclusively infects humans, whereas C. parvum infects humans and predominantly cattle and sheep (Xiao, 2010). Wild animals are host to a variety of host-adapted genotypes, which are not generally infectious to humans (Xiao, 2010). With the exception of C. cuniculus (formerly the rabbit genotype), which was responsible for an outbreak in the UK (Chlamers et al. 2009), C. parvum and C. hominis are the only species that have been identified during outbreaks of human cryptosporidiosis (Xiao, 2010).
Of the three recreational sites tested, sites 1 and 2 had the highest prevalence of *Cryptosporidium* (52.4% and 35.3% respectively) and were almost exclusively *C. hominis*. Site 3 had a much lower prevalence (14.3%) and the two positives identified were *C. parvum*. However, swimming and camping were only allowed at site 1 and 2 and not at site 3, which has many dairy farms surrounding the catchment. The majority of positives from site 1 were detected during the summer and autumn months. Peak oocyst counts were associated with periods of high visitor numbers during Christmas, New Year and Easter public holidays. Records for site 1 followed similar patterns each year with an average of 31,000 visitors between December to April and just 2,500 visitors between May to December. During public holidays, over 2000 visitors may recreate at site 1 on any one day. During hot weather, over 300 people have been observed to swim in the reservoir. These observations are similar to previous studies of recreational waters and catchments that had an increase in the number of visitors to the catchment during summer (Craun et al., 2005; Lake et al., 2008; McCarthy et al., 2008; Yoder and Beach, 2010).

In the non-recreational sites, the prevalence ranged from 8.3% to 33.3% with the highest prevalence in site 5. Positives were detected during the winter months and only *C. parvum* was detected in the non-recreational sites. A seasonal peak of *Cryptosporidium* in winter months is a common feature among water catchments that have been contaminated by animal wastes (Bodley-Tickell et al., 2002; Kistemann et al., 2002; Keely and Faulkner, 2008; Samadder et al., 2010) and is thought to be associated with winter calving. There are many cattle farms in the non-recreational catchments and the data suggests that the *C. parvum* contamination may have come from cattle in those catchments.
Analysis of the correlation of risk factors and the amount of *Cryptosporidium* at site 1, identified rainfall, temperature, UV index and population as the main factors with the latter three positively correlated. This is in contrast to previous studies that reported that an increase in temperature and UV index resulted in a decrease in the amount of *Cryptosporidium* (King et al., 2005; King et al., 2008; Karanis et al., 2007; King and Monis, 2007; Nasser et al., 2007). This difference is most likely due to an increase in population during these periods. The increases in temperature and UV index coincided with an increase in the number of people visiting the water catchment. Increasing population had the strongest correlation with the amount of *Cryptosporidium* detected and the identification of only *C. hominis* at site 1 provides supporting evidence that increasing human recreation in water bodies is linked to increased *Cryptosporidium* contamination levels at the source.

There are a number of limitations to the current study as it was assumed that the oocyst recovery rate for all the samples, were equal to the recovery rate from the distilled water spike analysis. However, studies have shown that the recovery rate is highly dependent on the quality of the water body being sampled, as samples with high turbidity have been shown to have a lower recovery rate (Feng et al., 2003). It was not feasible to spike the test water samples as the spike used (Easyseed) is *C. parvum* and this would have contaminated all the test samples resulting in *C. parvum* being detected in all samples. As genotyping analysis is becoming routine in water laboratories, this represents an increasing problem, as the only other alternative is to spike a duplicate water sample, which greatly adds to the cost of analysis.
Future studies should be conducted on larger numbers of samples over a longer period of time and with more frequent sampling combined with genotyping and subtyping to more accurately identify the source of contamination and to identify potential methods to minimise contamination of water catchments. However, the detection of a high prevalence of *C. hominis* indicates that recreational access to catchments is a serious public health risk and should only be considered in the outer catchment to avoid contamination of the drinking water supply.

**Acknowledgements**

This study was funded by Water Corporation, Western Australia.
References


catchments in the southwest of Australia. Experimental Parasitology 118(4), 596-599.


Figure 1. *Cryptosporidium hominis* and *C. parvum* oocysts numbers detected in recreational and non-recreational water sites. *=* *C. parvum*. All other samples are *C. hominis*. 
Fig 1
Cryptosporidium hominis and C. parvum oocysts numbers detected in recreational (sites 1 and 2) and non-recreational water sites in Western Australia from December 2008 to September 2010. *= C. parvum. All other samples are C. hominis.

Graphical abstract
Research Highlights

- One of the 1st studies to genotype Cryptosporidium from recreational catchments
- Cryptosporidium hominis detected only in recreational sites
- Non-recreational catchments had a lower prevalence
- All non-recreational samples genotyped were C. parvum.
- Increasing population was correlated with an increase in Cryptosporidium.
<table>
<thead>
<tr>
<th>Site</th>
<th>Water Usage</th>
<th>Recreational usage</th>
<th>Activities allowed.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recreational</strong> Site 1</td>
<td>Recreational/Agricultural</td>
<td>Heavy</td>
<td>Swimming, walking trails, canoeing, fishing, water skiing, marroning, camping and caravan sites.</td>
</tr>
<tr>
<td>Site 2</td>
<td>Recreational/Agricultural</td>
<td>Heavy</td>
<td>Swimming, walking trails, canoeing, fishing, marroning, water skiing, camping and caravan sites.</td>
</tr>
<tr>
<td>Site 3</td>
<td>Agricultural</td>
<td>Moderate</td>
<td>Walking trails, marroning, fishing and canoeing</td>
</tr>
<tr>
<td><strong>Non Recreational</strong> Site 4</td>
<td>Agricultural</td>
<td>Light</td>
<td>Limited campling/walking trails allowed in outer catchment</td>
</tr>
<tr>
<td>Site 5</td>
<td>Drinking</td>
<td>Light</td>
<td>Limited campling/walking trails allowed in outer catchment</td>
</tr>
<tr>
<td>Site 6</td>
<td>Drinking</td>
<td>Light</td>
<td>Limited marroning/fishing, camping</td>
</tr>
</tbody>
</table>
Table 2: *Cryptosporidium* species and genotypes identified in sampled water catchment areas at the 18S and diagnostic locus

<table>
<thead>
<tr>
<th>Location</th>
<th>No sampled</th>
<th>No. Positive</th>
<th>Prevalence % (CI)</th>
<th>C. <em>hominis</em></th>
<th>C. <em>parvum</em> oocysts per 10 L (based on 45% recovery rate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site 1</td>
<td>21</td>
<td>11</td>
<td>52.4 (31-73.7 CI)</td>
<td>11</td>
<td>-</td>
</tr>
<tr>
<td>Site 2</td>
<td>17</td>
<td>6</td>
<td>35.3 (12.6-58 CI)</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Site 3</td>
<td>14</td>
<td>2</td>
<td>14.3 (0-32.6 CI)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>19</td>
<td>36.5 (23.5-49.6 CI)</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>Recreational</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Site 4</td>
<td>12</td>
<td>1</td>
<td>8.3 (0-24 CI)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Site 5</td>
<td>6</td>
<td>2</td>
<td>33.3 (0-7171 CI)</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Site 6</td>
<td>6</td>
<td>1</td>
<td>16.6 (0-45.4 CI)</td>
<td>-</td>
<td>1</td>
</tr>
<tr>
<td>Total non-recreational</td>
<td>24</td>
<td>4</td>
<td>16.7 (1.8-31.6 CI)</td>
<td>-</td>
<td>4</td>
</tr>
<tr>
<td>Total</td>
<td>76</td>
<td>23</td>
<td>31.9 (20-40.6 CI)</td>
<td>16</td>
<td>7</td>
</tr>
</tbody>
</table>
Table 3: Statistical analysis of key environmental and catchment risk factors correlated with the prevalence of *Cryptosporidium*.

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Strength of correlation (R²), degrees of freedom and significance value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rainfall</td>
<td>0.174 (1,20) p&lt;0.040</td>
</tr>
<tr>
<td>Temperature</td>
<td>0.273 (1,20) p&lt;0.010</td>
</tr>
<tr>
<td>UV index</td>
<td>0.197 (1,20) p&lt;0.040</td>
</tr>
<tr>
<td>Population</td>
<td>0.360 (1,20) p&lt;0.004</td>
</tr>
</tbody>
</table>