Occurrence of Cryptosporidium and Giardia in potable water sources in Chandigarh, Northern India
Kjersti Selstad Utaaker, Himanshu Joshi, Anil Kumar, Suman Chaudhary and Lucy J. Robertson

ABSTRACT

Contamination with Cryptosporidium and Giardia from drinking water sources in a city in Northern India was assessed. A protocol modified from a standard ISO protocol, which includes filtration, concentration, separation and detection steps, was tested and showed comparable recovery efficiencies (Giardia mean = 77.4%, Cryptosporidium mean = 61.8% from the modified protocol, compared with Giardia mean = 61.6%, Cryptosporidium mean = 69% from the ISO protocol) at a substantial cost reduction. This protocol was used for analysing 71 samples of potable water from different areas of Chandigarh, where sampling locations were divided into groups according to the population density, which also partially equates with the level of infrastructure. Samples were collected during (n = 29) and outside the monsoon season (n = 42). Of all samples analysed, 16 (22.5%) were Cryptosporidium- and/or Giardia-positive. Parasites per sample were low (1–10 oo) cysts per 10 L, although one sample contained large numbers of Giardia cysts (> 1,000). Polymerase chain reaction analyses on the small subunit ribosomal ribonucleic acid (SSU rRNA), triose-phosphate isomerase (tpi), glutamate dehydrogenase (gdh) and beta-giardin (bg) gene sequences on Giardia-positive samples and SSU rRNA on Cryptosporidium-positive samples tended to be unsuccessful, although Giardia cysts of Assemblages B and C were identified. No association with the season was detected, but an association with the location of water supply was identified. Samples from areas with the lowest infrastructure were not associated with higher levels of contamination, but samples from the middle level were significantly more likely to be contaminated than those from the highest level of infrastructure. Results indicate that even in a city with a well-developed infrastructure, the contamination of potable water with protozoan parasites remains a public health risk.

Key words | Cryptosporidium, detection, drinking water, Giardia, public health

INTRODUCTION

The World Health Organization (WHO) estimates that about 1.1 billion people globally drink unsafe water (Kindhauser & WHO 2003), and the vast majority of diarrhoeal diseases in the world (88%) is attributable to unsafe water, poor sanitation, and general lack of hygiene interventions. Waterborne parasitic protozoan diseases result in 842,000 deaths per year and 1.7 billion cases of diarrhoea (WHO 2002; Checkley et al. 2015), and the morbidity and mortality caused by the waterborne diarrhoeal disease make them one of the planet’s largest environmental health threats to many populations (Gadgil 1998).

Cryptosporidium and Giardia are two of the most common aetiological agents of childhood diarrhoea in developing countries, causing morbidity as well as mortality (Kotloff et al. 2015; Platts-Mills et al. 2015), and an expert elicitation found that waterborne transmission
accounts for, proportionally, 0.37 Cryptosporidium infections and 0.55 Giardia infections in Southeast Asia, making the waterborne route one of the most important exposure pathways (Hald et al. 2016). Between 2011 and 2016, out of 381 waterborne outbreaks of cryptosporidiosis and giardiasis that have been documented, all of them are reported from developed parts of the world, where there have been significant advances in setting up surveillance systems and reporting. The most frequent aetiological agent was Cryptosporidium spp., reported in 63% of the outbreaks, while Giardia was implicated in 37%, making it clear that these parasites contribute substantially to the waterborne diarrhoeal disease (Balodursson & Karanis 2011).

A questionnaire study from Bolivia reported that only 30% of the respondents associated dirty water with diarrhoea, and that diarrhoea was perceived as a normal childhood occurrence (Quick et al. 1997). This perception would indeed substantially contribute to underreporting of outbreaks and disease in developing countries, where these parasites are endemic, the disease burden caused by these pathogens is more common, and waterborne infections are seldom recognized or reported. This is not because they do not occur, but because there is virtually no organized system for the identification, description and analysis of these events (Efstratiou et al. 2017; Rosado-García et al. 2017), and the proportion that is specifically waterborne is seldom identified. Indeed, those regions that are probably most affected by Cryptosporidium and Giardia often do not have the resources to identify or provide any reports of waterborne outbreak events, and nor do they have the resources available to implement routines regarding surveillance and monitoring that can be the basis of risk assessment and introducing interventions where needed. This was the case during the well-known Milwaukee outbreak of waterborne cryptosporidiosis in 1993, which led to improvements worldwide in water treatment, monitoring and regulations (Gradus 2014). Currently, these pathogens are starting to get the attention they deserve in less developed regions: a study from Africa and Asia found Cryptosporidium to be the second most prevalent pathogen responsible for severe diarrhoea in young children (Kotloff et al. 2015), and from 16 outbreaks of waterborne protozoa from 1979 to 2015 in Latin American countries, Cryptosporidium was the most commonly found pathogen (Rosado-Garcia et al. 2017), and a recent study from rural Pakistan showed that children were more likely to get cryptosporidiosis based on drinking water sources, living with domestic animals, season and socioeconomic status (Khan et al. 2019).

The lack of data around these fundamental questions was the basis for this study in which we investigated the occurrence of Cryptosporidium oocysts and Giardia cysts in potable water samples in different seasons in and around Chandigarh, a city in Northern India.

MATERIALS AND METHODS

Validation and use of a reduced-cost protocol for analysis

Methods of the analysis of water for contamination with Cryptosporidium and/or Giardia cysts have been developed, validated, and adopted throughout many countries in the developed world, and the ISO 15553 standardized method for Cryptosporidium and Giardia analyses of potable water is probably the most commonly used approach in Europe (ISO 2006). This protocol includes a filtration step, and in this study, the water samples were filtered through a membrane with pores of 2 μm. These filters were then washed with a membrane buffer and distilled water, before centrifugation and concentration of the pellet obtained from the suspension of membrane buffer, distilled water and contents on the membrane filter. Immunomagnetic separation (IMS) was used to separate parasites from other debris in the pellet, and the final content from this separation step was stained and examined under the microscope for detection. This is a costly method, and one of the most expensive steps in the protocol incorporates the use of IMS. The IMS procedure is also an essential step in other standard methods of water analysis for these parasites, such as U.S. EPA Method 1623 (U.S. EPA 2012).

As we have previously been successful modifying another ISO Method, 18774 (ISO 2016), which also incorporates IMS, in order to reduce the cost (Utaaker et al.
a similar approach was used here to enable us to have the resources to analyse more samples.

To validate the modified in-house method for use with potable water, ten 10 L samples of tap water were spiked with EasySeed™ (BTF Pty Ltd, Australia) that contains 100 flow cytometer-sorted Cryptosporidium oocysts and 100 Giardia cysts. Five samples were analysed according to the ISO 15553:2006 protocol (for which our laboratory is accredited by the Norwegian Accreditation authority), and the other five samples were analysed by the modified method, in which, rather than using the IMS reagents as in the ISO Method, the IMS was performed according to the method published by Utaaker et al. (2015). In brief, following the concentration of the sample into a 5–7 ml volume following filtration and centrifugation, only 20 μl volume of each type of beads was used, rather than 100 μl, and the buffers were modified such that rather than using 1 ml of each of the buffers provided with the IMS kit (Dynabeads®: Cryptosporidium/Giardia Combo Kit, Idexx Laboratories), a smaller volume was used and augmented with buffers as described in Utaaker et al. (2015).

Field sampling

On sampling occasions during August 2014 to December 2014, each lasting 2–4 months and altogether 8 months in total, which took place during the monsoon and winter season, a total of 71 potable water samples of 10 L each were collected in and around Chandigarh, Northern India.

Chandigarh is divided into sectors (Figure 1), distributed into three phases that are based on the population density, and each phase has a given holding capacity which is 34 persons per acre for phase I, 83 persons per acre for phase II, and 100 persons per acre in phase III (Chandigarh Administration 2016). From phase I sectors, which has the lowest population density, 39 samples were collected. Twelve samples were collected from phase II, which has a higher density than phase I, and 20 samples were collected from phase III and non-sectorial villages surrounding the city.

The drinking water supply of Chandigarh is derived from the Bhakra Main Canal and from ground water sources. There are five water treatment plants in

Figure 1 | Areas of Chandigarh where samples were collected.
Chandigarh. Four are situated in sector 39 (phase II), and one in sector 12 (phase I), and the city has 155 tube wells discharging directly into reservoirs underground which are spread over the city. Ground water is mixed and supplied along with the treated water from the Bhakra Main Canal. This water is treated conventionally by aluminium dosing given according to turbidity, chemical mixing, flocculation, sedimentation, filtration and chlorination. The water works also have storing facilities of treated water provided with ventilation and protection from mosquitoes and aquatic insects. The water at the treatment plants is tested in a laboratory set-up in the water works premises in phase II. Water is tested at various stages such as raw water, settled, filtered and chlorinated water. The physico-chemical examination of water is done for eight samples per day, ten bacteriological samples are analysed each day, and water treatment quality parameters such as turbidity, pH and residual chlorine are done on 25 samples every 2 h, six times daily (NEERI 2005). However, reports have revealed that the city loses about 26% water due to leakages (Gupta 2018), and waste dumped in landfill sites is contaminating the surrounding groundwater (Negi et al. 2018), indicating that contamination may take place in the distribution network. In addition, a study from Spain found Cryptosporidium and Giardia (oo)cysts even in conventionally treated water, indicating that these pathogens may escape the commonly used water treatment regimes (Carmena et al. 2007).

The samples were collected into 10 L plastic containers directly from public drinking water sources that were either supplied by a tubewell or from a water tank transported into the area. After collection, the containers were taken directly to the laboratory and processed immediately.

**Analysis of water samples**

The samples were analysed for the presence of Giardia cysts and Cryptosporidium oocysts using the modified protocol of the ISO 15553 (2006) standard, which had been tested prior to use in the same laboratory and provided recovery rates comparable to the ISO Method. The reagents were used according to Utaaker et al. (2015).

The water samples were first filtered within 24 h after collection at the parasitology laboratory at the Postgraduate Institute of Medical Education and Research (PGIMER), Chandigarh through Millipore Isopore membrane filters with a pore size of 2 μm using a Watson-Marlow 520 Bp Profibus pump. Following filtration, the filters were placed in 50 ml centrifuge tubes that were then filled with sample water and stored at 4 °C. These sample tubes containing filters were transported from the PGIMER to the NMBU parasitology laboratory in Norway for the final stages of the analyses (IMS, immunofluorescent antibody testing and any molecular analyses).

In Norway, the filters were washed as according to the 15553 protocol (ISO 2006), and the eluate was poured into 50 ml centrifuge tubes. The tubes were centrifuged at 1,550 rcf (relative centrifugal force) for 10 min, and, following the aspiration of the supernatant, the remaining pellets were concentrated into one tube before the IMS step was performed, using the Dynabeads® kit for the isolation of Cryptosporidium oocysts and Giardia cysts, but using the reagents as described by Utaaker et al. (2015). Following the dissociation of the beads by vortexing under acidic conditions, the final suspension of 50 μl was pipetted onto a single-well slide (Novakemi ab, Sweden) and air-dried at room temperature.

Dried samples were fixed with one drop of methanol and stained with FITC-conjugated monoclonal antibodies (mAbs) against Cryptosporidium oocyst walls and Giardia cyst walls (Aqua-Glo™, Waterborne™, Inc., USA), and nuclei were stained with the fluorogenic DNA intercalator 4′,6-diamidino-2-phenylindole (DAPI) according to Smith et al. (2002). Samples were mounted with DABCO antifade Mounting Medium, then each slide was covered by a glass coverslip and viewed promptly by fluorescence microscopy.

**Microscopy and enumeration**

Microscopy was performed on a Leica DCMB microscope (20×, 40× and 100× objectives), equipped with Nomarski differential interference contrast (DIC) optics. A blue filter block (480 nm excitation, 520 nm emission) was used for the detection of cysts and oocysts labelled with FITC-conjugated mAbs, and a UV filter block (350 nm excitation, 450 nm emission) was used for investigating DAPI staining.
Each well was scanned systematically in an up-and-down or side-to-side manner at 20×, and Cryptosporidium oocysts and Giardia cysts were enumerated. When brilliant apple-green fluorescing ovoid or spherical objects within the appropriate size range for Cryptosporidium and Giardia were observed, magnification was increased to 40× and 100×, and the UV filter block was used for the visualization of DAPI staining. Each (oo)cyst was recorded as DAPI-negative or DAPI-positive according to the presence of characteristic internal light blue staining highlighting the nuclei.

Nomarski (DIC) objectives were used to examine morphological characteristics of the (oo)cysts. A sample was considered positive if the (oo)cyst(s) exhibited typical fluorescence, with correct shape and size, and being DAPI-positive. If internal contents were lacking, but the morphometry was correct and the structure had a typical fluorescence, the (oo)cysts were described as ‘putative’, as they lacked sufficient characteristics for definitive identification.

Both putative and confirmed parasites were summed together for inclusion in the results as positive findings. In some of the samples, the (oo)cysts were also quite occluded due to debris when examined under the UV light, making DAPI staining difficult to assess. The recovery efficiencies were not taken into account when (oo)cysts were enumerated, but an estimation of (oo)cyst counts based on the mean recovery efficiency can be found in Table 1.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Season</th>
<th>Source</th>
<th>Microscopy results per 10 L sample; Cryptosporidium oocysts</th>
<th>Microscopy results per 10 L sample; Giardia cysts</th>
<th>Estimated count adjusted to mean values of recovery from spiking trial</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I (lowest population density and highest level of infrastructure)</td>
<td>Monsoon</td>
<td>Sector 9</td>
<td>1 oocyst</td>
<td></td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Sector 14</td>
<td>1 oocyst</td>
<td></td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Sector 27</td>
<td></td>
<td>1 cyst</td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td></td>
<td>Monsoon</td>
<td>Sector 30</td>
<td>10 oocysts</td>
<td></td>
<td>[19–14] oocysts</td>
</tr>
<tr>
<td></td>
<td>Monsoon</td>
<td>Sector 8</td>
<td></td>
<td>1 putative cyst</td>
<td>1 cyst</td>
</tr>
<tr>
<td>Phase II</td>
<td>Summer</td>
<td>Sector 38</td>
<td>1 putative oocyst</td>
<td></td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td></td>
<td>Summer</td>
<td>Sector 38</td>
<td></td>
<td>1 putative cyst</td>
<td>1 cyst</td>
</tr>
<tr>
<td></td>
<td>Monsoon</td>
<td>Sector 38</td>
<td></td>
<td>1 cyst</td>
<td>1 cyst</td>
</tr>
<tr>
<td></td>
<td>Monsoon</td>
<td>Industrial area</td>
<td></td>
<td>2 cysts</td>
<td>[3–2] cysts</td>
</tr>
<tr>
<td></td>
<td>Monsoon</td>
<td>Sector 34</td>
<td></td>
<td>2 cysts</td>
<td>[3–2] cysts</td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Sector 38</td>
<td>136 cysts</td>
<td></td>
<td>[188–165] cysts and Assemblage C MF 150151a</td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Sector 40</td>
<td>1 oocyst</td>
<td></td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td>Phase III and non-sectorial villages (highest population density and lowest level of infrastructure)</td>
<td>Monsoon</td>
<td>Kishangarh</td>
<td>1 putative oocyst</td>
<td></td>
<td>[2–1] oocysts</td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Janta and Kumhar Colony</td>
<td>&gt;1000 cysts</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Winter/Spring</td>
<td>Janta and Kumhar Colony</td>
<td>3 cysts</td>
<td></td>
<td>4 cysts</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Total</td>
<td></td>
<td></td>
<td>16</td>
<td>7</td>
<td>10</td>
</tr>
</tbody>
</table>

*GenBank Accession number.
**MOLECULAR ANALYSES**

**Extraction of DNA from oocysts**

Following microscopy, *Cryptosporidium* oocysts and *Giardia* cysts were retrieved from positive slides and DNA was isolated according to Robertson *et al.* (2009). Briefly, the coverslip from each slide was carefully removed and retained, while 25 μl aliquots of AL lysis buffer (Qiagen GmbH, Germany) were added to the slide wells, which were then scraped using a sterile scalpel blade. The buffer and scrapings were pipetted into a microcentrifuge tube. This process was repeated four times and then the coverslip was replaced onto the slide that was then re-screened. For each slide, no cyst or oocysts could be detected after the procedure.

The contents of each microcentrifuge tube containing slide scrapings were resuspended in Tris–EDTA buffer and held at 100 °C for *Cryptosporidium* oocysts and 90 °C for *Giardia* cysts for 1 h, before the DNA was isolated using the QIAmp DNA Mini Kit (Qiagen GmbH), using an overnight step at 56 °C.

**Polymerase chain reaction**

The samples were run through several polymerase chain reactions (PCRs) using different primer sets listed in Supplementary Table 1 (available with the online version of this paper), and two sequences from the PCR targeting the SSU gene of *Giardia* were obtained. The PCR products were purified using ExoSAP-IT® (Affymetrix USB) and sent to GATC Biotech for sequencing. The sequences were analysed using Geneious 10.2.1©, compared by the NCBI Blast and submitted to GenBank.

**Statistics**

The recovery efficiencies of the samples spiked with *Cryptosporidium* and *Giardia* by the modified method were compared with those obtained by the ISO 15553:2006 method by linear regression.

The results of sample analysis were collated in a Microsoft Excel (2010) database. A chi-square test was used to compare contamination according to the season of the collection of the samples, and a Freeman–Halton test was used to compare contamination according to the location of the site of sample collection.

**RESULTS**

**Recovery efficiency**

The recovery of *Giardia* cysts was significantly higher using the reduced-cost approach (reduced-cost approach; mean = 77.4%; standard deviation [72.47–82.33%], ISO 15553:2006 approach; mean recovery efficiency = 61.6%; standard deviation [51.27–71.93%]; p = 0.017).

*Cryptosporidium* recovery efficiency was not significantly different when the methods were compared (reduced-cost approach; mean = 61.8%; standard deviation [54.14–69.46%], ISO 15553:2006 approach; mean recovery efficiency = 69%; standard deviation [59.1–78.9%]; p = 0.320).

These results were the basis for using the reduced-cost approach for analysing the samples.

The occurrence of *Cryptosporidium* oocysts and *Giardia* cysts in potable water samples from different locations in Chandigarh.

An overview of the results with the location of sampling site, the season of sample collection, the number of parasites per 10 L sample, and the results from molecular analyses are summarized in Table 1.

Of the 71 samples analysed, 16 (22.5%) were positive for either *Cryptosporidium* oocysts or *Giardia* cysts. Seven samples (9.9%) were contaminated with *Cryptosporidium* oocysts, and ten samples (14.1%) were contaminated with *Giardia* cysts. One sample contained both parasites.

A comparison of results according to the season indicated that there was no association of positive results with the monsoon season or any other season (p = 0.995).

However, the location of sample collection area had a significant effect on the probability of a sample being contaminated when the results were compared with a Freeman–Halton test (Pa and Pb > 0.001) (Freeman & Halton 1951), with samples more likely to be positive if obtained from locations in phase II of Chandigarh. Although phase III samples were no more likely to be positive than samples from phase I, in a slum area in phase III.
one highly positive sample was found containing large numbers of *Giardia* cysts. Genotyping of *Giardia* cysts from this location indicated that Assemblage B *Giardia* cysts were present. The two sequences from *Giardia*-positive samples have been issued (accession numbers MF 150151 and MF 150152 in GenBank).

The amplification of the target gene from *Cryptosporidium*-positive slides was unsuccessful.

The volume per sample (10 L) in this study was low compared with the volume recommended by the U.S. EPA (up to 1,000 L) and does not mimic the conditions naturally occurring in treated drinking water.

**DISCUSSION**

The main result of this study is that the potable water samples analysed from in and around Chandigarh were rather likely to be contaminated with *Cryptosporidium* oocysts and/or *Giardia* cysts. Although other studies with a similar design have found varying occurrences, in these reports the positive samples are often from raw and untreated water sources; for example, in Norway, a survey reported a prevalence of positive samples 16.5% for *Cryptosporidium* and 11.5% of *Giardia* (Robertson & Gjerde 2001), but the samples were from untreated surface water. Similarly, a study from Ankara, Turkey, investigated municipal water supply, wells, river water and untreated dams. No contamination was found in the municipal water supply, but the wells, dam and river samples gave an overall occurrence of 5.8% of samples being *Giardia*-positive (Bakir et al. 2003). In countries that are perhaps more comparable with India, a study encompassing the analysis of water samples from Malaysia, Thailand, Philippines and Vietnam reported only a single potable water sample (from the Philippines) containing both *Cryptosporidium* and *Giardia*, whereas neither parasite was detected in potable water samples from the other three countries (Kumar et al. 2016). Nevertheless, earlier studies from North America have reported both *Cryptosporidium* and *Giardia* contamination in potable drinking water; a survey from the USA revealed a 17% prevalence of *Cryptosporidium* and no *Giardia* (Rose et al. 1991), and a survey from Canada reported a prevalence of 18.2% *Giardia* cysts and 3.5% *Cryptosporidium* oocysts (Wallis et al. 1996). These studies had differing designs and protocols, with sample sizes ranging from 2 to 1,000 L of water, but they show that these protozoan parasites are present in water and drinking water worldwide. Although our findings from Chandigarh’s drinking water may give some cause for concern, particularly in relation to results from potable water in other Asian countries, they are not abnormally high compared with some earlier reports from some other places in the world. It should be noted that Chandigarh is a city with a relatively developed infrastructure and has been ranked as one of the most advanced and cleanest cities in India (Chandani 2016). Thus, our findings should not be taken as a representative for the whole country. Other studies investigating the occurrence of *Giardia* and *Cryptosporidium* in Chandigarh in both animals and humans have found differing results. A study on the occurrence of *Cryptosporidium* in humans admitted to a hospital in Chandigarh found a 3.8% prevalence and 25% of the isolates belonged to *Cryptosporidium parvum* (Sharma et al. 2013). One of these genotypes was later found in another study investigating the occurrence of *Cryptosporidium* and *Giardia* in cattle in the same area (Utaaker et al. 2018). *Cryptosporidium* and *Giardia* (oo)cysts has also been found on vegetables from different markets in Chandigarh (Utaaker et al. 2017a, 2017b), and a high prevalence and zoonotic potential of *Giardia* cysts was found in a study done on backyard livestock of goats living close to their owners (Utaaker et al. 2017a, 2017b). *Giardia* infection also had the highest rates reported in India in a low socioeconomic group in Chandigarh in 1991 with a 69.5% prevalence rate (Ramesh et al. 1991), although in another study of the same group from 2004, the prevalence rate was 6% (Bansal et al. 2004).

One obstacle to surveying water supplies for these parasites in developing countries or in projects with limited budgets is the expense of the technique. In this study, the ISO standard and a modified alternative were compared prior to sample collection, and the modified version was found to perform with comparable recovery rates at a lower cost and was thus used in the survey. This method could be further tested and validated in multi-laboratory ring trials and could offer a cheaper alternative for laboratories or research projects with constrained budgets.
Climate is considered to be likely to impact the occurrence of protozoan parasites as contaminants in drinking water, particularly with respect to extreme weather events. A meta-analysis indicated that the likelihood of the contamination of fresh surface water with Cryptosporidium oocysts and Giardia cysts was significantly increased during extreme weather events (Young et al. 2015), and especially surface water sources are more prone to contamination during the monsoon season (Gopal 2007). Thus, it may be expected that during the monsoon season, contamination would be increased. In Chandigarh, there are three distinct seasons; summer with hot, dry weather and occasional heat waves; monsoon is when Chandigarh receives moderate to very heavy rainfall, with a peak in precipitation in July and August. The winter also receives a small part of the rainfall. Spring offers mild temperatures and less precipitation.

In this study, the seasons had no apparent effect on contamination, and samples taken in the monsoon season, in particular, were no more likely to be positive than those taken during other seasons. However, this result may reflect the unstable precipitation during the period of sampling as during this period, the seasons and precipitation differed to that which is normal for Chandigarh. During the monsoon season in 2014, the Chandigarh district only received about 45% of its normal rainfall, and 2014 was ranked as ‘dry to extremely dry’ (India Meteorological Department 2015). Furthermore, 2015 was the third warmest year in India recorded since 1901, and although the year was considered within the normal range in terms of overall precipitation, the rain came in unexpected seasons. During the summer, Chandigarh received three times more than the normal rainfall, but during the usual monsoon season, the precipitation was 50% less than expected, and the winter season was below normal in terms of rainfall (India Meteorological Department 2016).

Nevertheless, from our data no specific season appeared to be more associated with contamination than others, and the risk of contamination is known to be associated with many variables. It is possible that season and precipitation are not major drivers for contamination in this area or that insufficient samples were analysed to reveal a seasonal pattern, particularly in the light of the unusual and unexpected weather patterns during sampling.

Although season was not associated with the contamination of drinking water, the location where the water was collected from did affect the likelihood of the sample being contaminated. Chandigarh city is an area planned for a specific number of inhabitants and, additionally, was originally planned for a differential pattern of density. Like most cities in India, Chandigarh is overpopulated, with a current population density of approximately 9,300 persons per km² (Government of India 2011). Citizens of Chandigarh are unevenly distributed throughout the city, in so-called phases numbered from I to III, with increasing densities from phase I, situated in the northern part of the city, to phase III, which is at the south end. The city is also divided into sectors, in a grid pattern based on the roads, and beyond those, there are so-called non-sectorial villages and slum colonies. These villages and slum colonies lack much of the infrastructure and sanitary facilities of the planned sectors. Due to cheaper housing, the phase III areas and non-sectorial villages are characterized by high population pressure, with consequences such as unsanitary conditions, flood problems, poor garbage disposal, disposal of livestock dung into open drains, and discharge of untreated sewage into drains or small rivers surrounding the city (Chandigarh Administration 2016).

Water table elevation studies have revealed that the flow of ground water is from the north to the south within the city (Singh et al. 2012), which, in turn, means that the groundwater flows from phase I through phases II and III. Thus, not only does phase III have the highest population density, but also the groundwater has run through the other two sectors before reaching this stratum. This stratification within the city also results in the social layers being more exposed and accessible for investigation, as particular social groups tend to cluster together in terms of where they live in a city, and this is probably particularly demarcated in India, which was a hierarchical society. Although our results indicate significant differences in the likelihood of the contamination of the potable water according to the phase, our expectation that phase III areas and non-sectorial villages would have higher rates of contamination of drinking water compared with other areas was not the case. This is in contrast to the findings of Taneja et al. (2011), where the
drinking water from the semi-urban and rural areas of Chandigarh were more likely to be positive for faecal contamination. Despite the factors of poor infrastructure and lack of basic hygiene, the phase III areas and villages did not have a significantly higher contamination rate than those in phase I. Nevertheless, it should be noted that this study involved a limited number of samples which may have affected the results, and one sample with a very high level of contamination was from a slum colony.

The similarity in contamination between phase I and phase III may reflect the fact that the city as a whole has a relatively high population density, such that the demarcations between the phases of the city are not very clear, and the water sources are distributed on a relatively small area. However, this does not explain why sampling sites in phase II were significantly more likely to show contamination.

Previously, the water supply of Chandigarh was based on tubewell sources alone. As the population has increased, so has the demand for water, and the underground source alone is currently not sufficient to meet the city’s requirements. This gap is met by tapping surface water from the Bhakra Main Line. The non-sectorial villages and phase III are not supplied with water from waterworks, according to the City Development Plan, and thus, these non-sectorial villages do not have access to piped drinking water supply, sewer systems or storm water drainage (Chandigarh Administration 2016). Thus, drinking water supplies may be driven in to non-sectorial villages and phase III.

As the highest contamination was found in a water tank in a phase III slum area, it is likely that individual tanks have the potential to be hot-spots of contamination and sources of infection. They are more prone to on-the-spot contamination, as they are stagnant, enclosed sources of drinking water and removal of contamination due to flow-through may be difficult. However, in this study, only eight tanks were sampled, of which two samples were positive. We were unable to obtain information about the water source for the water tanks and if this water is tested or inspected. Further studies on the routines regarding these water tanks are recommended.

It was hoped that molecular methods would provide further information on the source of contamination and also on the likelihood of potential to infect people. Due to logistics between the collaborating laboratories, the period between the filtration of the water sample until the isolation of (oo)cysts and the examination of slides was often prolonged. Parasites may have degenerated during storage, and the formation of other microorganisms may have had a deteriorating effect on the DNA in terms of degeneration and formation of inhibitors. In addition, water samples often contain PCR inhibitors such as debris, fulvic acids, humic acids, metal ions and polyphenols (Abbaszadegan et al. 1993; Ijzerman et al. 1997), which may explain the lack of results in most of the PCRs.

Although most of the PCR studies did not result in DNA amplification, the two successfully genotyped samples were of *G. duodenalis*, Assemblages B and C. Whereas Assemblage B is usually associated with human infection, Assemblage C is commonly associated with canids and is not associated with human infection. This Assemblage C *Giardia* was identified in a contaminated public water source in a religious temple. The public frequently visits these temples, and it is common to wash and drink from the taps. This means that the source could have been someone handling an infected dog, or could have come directly from one of the many dogs in Chandigarh, the population of which is currently increasing (Víctor 2013).

**CONCLUSIONS**

A significant contamination of drinking water with *Cryptosporidium* and *Giardia* occurs in Chandigarh, and it seems that phase II of the city receives most of the contaminated water. Although the results of our study do not implicate the potable water supply as a source of intestinal protozoan infections for the citizens of Chandigarh or provide evidence that it is a major transmission vehicle, they do indicate that it can be a potential source of infection, particularly in less wealthy areas of the city. Further research that identifies how and where contamination occurs would be of value, such that appropriate barriers can be implemented.

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**COMPETING INTERESTS**

The authors state that they have no competing interests.

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