Analysis of water for protozoan parasites is expensive – why not use indicator organisms?

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Why analyse water samples for protozoan parasites?

The protozoan parasites, *Cryptosporidium* spp. and *Giardia duodenalis,* have been associated with hundreds of waterborne outbreaks of disease, some of which have involved hundreds or even thousands of individuals (see: Karanis et al, 2007; Baldersson and Karanis, 2011). In addition to the direct medical issues, such outbreaks are also a substantial economic drain in terms of lost productivity, tracking down and addressing the cause of the outbreak, and also in terms of possible treatment and hospitalization costs.

Although countries with high endemic prevalence of these parasites and with poor infrastructure regarding water supply and sewerage are perhaps more likely to experience outbreaks of waterborne protozoan disease, it seems that no country can consider itself invulnerable, and communitywide outbreaks of waterborne giardiasis and cryptosporidiosis have been reported from Scandinavia in recent years (Robertson et al, 2006; Robertson & Huang, 2012). Our awareness of these outbreaks, as well as the fact that the parasites are difficult to kill or remove from water supplies, has led to the need for analyzing water samples for both *Giardia* and *Cryptosporidium*. Such analyses are not necessarily to determine whether a contamination event has already happened, for by the time the analysis is completed the water might well be already distributed to consumers, but also for a range of other reasons, including to be used for input to risk assessment, to determine contamination sources in catchments, to assess whether sufficient barriers are in place to ensure safe supply to consumers, and to follow up if an outbreak has occurred and to ensure that implemented measures or barriers are working effectively.

Standard Methods: why are they so expensive?

Standard methods for analyzing water for protozoan parasites were first produced in around 1980 in USA and have since been developed and improved; the most commonly used Standard Methods today are US EPA Method 1623.1 (most updated version published in 2012 and developed from earlier versions) and ISO Method 15553 that was published in 2006. These Standard Methods basically consist of 3 stages: concentration (often by filtration), isolation (by immunomagnetic separation – IMS), and detection (by microscopy using specific fluorescent labels to assist in identification). Each of these stages is expensive – some of the filters recommended for the first stage in the analysis cost approximately

600 kr each (around 70 €); there is currently only a single company that supplies an IMS kit that is suitable for isolating both *Cryptosporidium* oocysts and *Giardia* cysts, and the costs are usually over 400 kr (around 50 €) per test, but may be higher for more turbid water samples; and although the fluorescent labels for detection are not expensive, a fluorescence microscope for examining the sample concentrates is, and such analyses are best performed by a properly trained and well-experienced operator. Together, all these factors mean that the analysis of a single water sample is likely to cost, at the very least, around 4000 kr (480 €), and very probably more. In addition, it is that the laboratory that undertakes such analyses is established and respected in this field, with appropriate QC data, successful and regular participation in ring-testing, and, preferably, accreditation for the particular analytical method to be used. All these documentation and competence demonstration steps are expensive, and this cost will be passed onto the customer providing the water sample.

In addition, if parasites are detected, then the water supplier and consumer are likely to be interested in whether the species or genotype is infectious to humans, and thus of public health significance. There are about 25 named species of *Cryptosporidium,* of which two, *C. parvum* and *C. hominis,* are most associated with human infection, and one of which, *C. parvum* is of zoonotic importance, being a cause of diarrhoeal disease in various animals, particularly calves and lambs. Although other species of *Cryptosporidium* can infection humans, and at least one of these, *C. cuniculus* commonly found in rabbits, has been associated with a waterborne outbreak (Chalmers et al, 2009), other species are of limited or negligible public health significance. Similarly, with *Giardia duodenalis,* the species is subdivided into different genotypes or Assemblages that are genetically distinct from each other and also have different host-specificity. Thus, only *G. duodenalis* isolates in Assemblage A or B are infective to humans, but other genotypes are commonly found in other animals; for example cattle and sheep are often infected with *G. duodenalis* of genotype E that is not of public health significance. Therefore, identification of species or genotype of parasites found in water is also of relevance from a water safety perspective, but is an additional procedure and adds further to the cost of the analysis.

The use of indicator organisms with respect to contamination with *Cryptosporidium* and *Giardia*

With analysis of water samples directly for *Cryptosporidium* and *Giardia* being so expensive, it is not surprising that the water industry has long been searching for a more cheaply and readily detected indicator organism that may provide information on when contamination with protozoan parasites is likely to have occurred. The US EPA lists seven criteria to be fulfilled for organisms that may be useful to use as indicator organisms for *Cryptosporidium* and *Giardia*, see table 1.

Criteria for indicator organisms for *Cryptosporidium* and *Giardia*	
1.	The organism should be present whenever *Cryptosporidium*/*Giardia* are present.
2.	The organism should be useful for all types of water.
3.	The organism should have a longer survival time than *Cryptosporidium*/*Giardia*.
4.	The organism should not grow in water.
5.	The organism should be found in warm-blooded animals' intestines.
6.	The testing method should be easy to perform.
7.	The density of the indicator organism should have some direct relationship to the degree of pollution.

Table 1. Criteria for indicator organisms for Cryptosporidium *and* Giardia.

Finding an organism that fulfils all of these criteria is not easy. For example, *Cryptosporidium* oocysts are notoriously robust, surviving weeks or months in damp, cool conditions and also surviving exposure to various disinfectants to which other microorganisms are susceptible. Thus, many of the most common faecal indicator organisms do not fulfill criterion number 3. For example, a study was conducted in Spain in which four water reclamation facilities were monitored over 2 years to determine the occurrence and concentrations of a set of microbial indicators (total coliforms, *Escherichia coli,* enterococci, spores of sulphite reducing Clostridia, somatic coliphages, and some phages), along with those of two pathogens, one of which was *Cryptosporidium* (Costan-Longares et al, 2008). The results obtained demonstrated that several regression models and discriminant functions that were able to predict both the presence and concentrations of enteroviruses. However, neither index functions nor a predictive relationship were observed between any of microbial indicators and viable *Cryptosporidium* oocysts (Costan-Longares et al, 2008).

Another potential indicator organism, which is also more robust than those used in the study of Costan-Longares et al, (2008) is *Clostridium perfringens,* as the spores of this species of bacteria are very robust and can withstand various environmental p ressures, including high temperatures. It is also a normal component in the environment and is found in the intestinal tract of humans and other animals. However, the conclusion from a three-year study in Europe was that defining *C. perfringens* as a conservative indicator for total faecal pollution monitoring in water was no longer justified; although *C. perfringens* was shown to be a conservative indicator for faecal excreta from non-herbivorous wildlife and human-associated sewage, it was not suitable for herbivorous wildlife (Vierheilig et al, 2013). Due to the zoonotic nature of both *Cryptosporidium* and *Giardia,* and that species and genotypes of public health significance of both parasites occur in herbivorous wildlife, these findings indicate that *C. perfringens* may not fulfil the first criterion in table 1, and therefore is not necessarily appropriate as indicator of contamination or potential contamination with *Cryptosporidium* and/or *Giardia*.

In the paragraphs above, specific publications have been used to demonstrate reasons why there are currently no appropriate indicator organisms for *Cryptosporidium* and/or *Giardia*. However, consideration of more studies may provide more convincing data. In a publication by Wu et al (2011), data were collected on the relationships between indicator organisms and pathogens (including *Cryptosporidium* and *Giardia)* published in scientific journals between 1970 and 2009 (over a 40 year period). These data were then sorted, with only data retained where the methods of statistical analysis, correlation coefficients and p-values were reported and where correlation analyses were not grouped across water types. In addition, due to the low detection of pathogens in groundwaters and treated drinking waters, these data were excluded from the study. This resulted in 92 data points for *Cryptosporidium* and 59 for *Giardia*; logistic regression analysis demonstrated that of the 92 *Cryptosporidium* cases, 69 (75%) found no correlation with indicators and only 23 (25%) found a correlation, while for *Giardia* 40 cases (68 %) were uncorrelated and 19 (32%) were correlated. Although *C. perfringens,* faecal coliforms and total coliforms were suggested as possible indicators for protozoan parasites, they were not significantly better than others (Wu et al, 2011). The study concludes that indicator organisms cannot with certainty signal the presence of pathogenic contamination for a water sample, but long-term monitoring may indicate the potential degree, and thus a relative risk score. However, much of the problem is due to insufficient data and sizeable site-specific monitoring efforts are necessary to define local public health risk accurately (Wu et al, 2011).

The role of faecal source tracking

As all *Cryptosporidium* and *Giardia* in water originate from human or animal faeces, faecal source tracking has also been a field that has been

considered with respect to water monitoring for these parasites. In a review article considering faecal source tracking, indicators and water quality (Field and Samadpour, 2007), the authors make the point that emphasis on faecal indicator bacteria in legislation has resulted in the pathogens themselves being overlooked. The authors suggest that a more rational approach to regulating water quality with respect to contamination would start with using the available epidemiological data to identify pathogens of concern in a particular water body (e.g. cattle operations might suggest a potential for *C. parvum* contamination). When levels of the appropriate indicators rise, then targeted monitoring for pathogens could be undertaken, thus minimising expensive shotgun approaches, but ensuring that health threats were being monitored. In addition, targeted source tracking could be used as required to identify the probable source of pathogens, again avoiding expensive shotgun approaches (Field and Samadpour, 2007). Targeted pathogen monitoring coupled with targeted faecal source tracking and baseline monitoring of indicators would become just one tool among many.

Conclusion

Although monitoring of water for protozoan contaminants has improved in efficacy over the last decades, with improved filtration methods, new isolation techniques, and molecular characterization possibilities readily available at many laboratories, the analytical technique has not become cheaper and there is still a need for trained and experience laboratory personnel and proper quality control and accredited use of methods. Although the use of an indicator organism to replace the requirement for such analyses remains elusive, this does not mean that such approaches should not be used when considering public health and safe drinking water within the context of contamination with *Cryptosporidium* oocysts and/or *Giardia* cysts. A shotgun approach is both expensive and inefficient, and a more useful way of considering any drinking water catchment with respect to these protozoan parasites is first to identify potential sources of contamination, using both catchment knowledge and epidemiological data, and then, in the light of a structured risk assessment, use baseline surveying of indicators, targeted faecal source tracking, and specific pathogen monitoring when appropriate to monitor the risk of contamination under different scenarios. Our arsenal of approaches to address this issue of waterborne protozoan transmission is increasing, and includes not only the approaches described above but also more advanced molecular techniques, use of Geographic Information Systems, and improving monitoring of infection in the human and animal communities within the water catchment area.

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