The search for *Cryptosporidium* oocysts and *Giardia* cysts in source water used for purification

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Abstract

Cryptosporidium and Giardia are intestinal parasites which cause a self limiting gastro-enteritis, in healthy people but severe and often fatal disease in immuno-compromised individuals. Both organisms are characterised by the ability to survive in an aquatic environment with Cryptosporidium having a high tolerance to most of the drinking water disinfectants. The absence of bacteria associated with feacal pollution does not necessarily indicate the absence of Cryptosporidium or Giardia. Being a common cause of waterborne disease, the requirement for detecting the presence of these organisms in environmental and treated water samples is growing. Although cysts and oocysts can be found in natural waters from any source, the risks of contamination are much greater in surface waters than in ground waters.

A number of waterborne outbreaks of cryptosporidiosis, from potable and recreational water have been recorded. In many cases these outbreaks have occurred from water that complies to current microbiology standards.

Introduction

The potential occurrence of *Cryptosporidiu*m oocysts and *Giardi*a cysts in water supplies is a significant issue for the water industry since outbreaks have been reported in Milwaukee, New Jersey, North West (UK) and more recently in Sydney, Australia. South Africa does not differ in risk from these countries due to high levels of pollution of our rivers and impoundment.

Amongst the protozoan genus, *Cryptosporidium parvum* and *Giardia intestinales* are increasingly recognised as important agents of gastrointestinalis diseases in several species, including humans. Low numbers of (oo)cysts are able to initiate infection that can be life threatening to immuno-compromised individuals, and effective therapy is not available. The widespread occurrence of *Cryptosporidium* oocysts and *Giardi*a cysts in surface water and their resistance to disinfection at the level normally used in the production of drinking water has become a major concern for the water industry (Pezzana et al, 2000).

Rand Water supplies water to more than 12 million people in South Africa over and area of 18 000 km^2. To ensure that the water leaving the treatment works is free of *Cryptosporidium* oocysts and *Giardia* cysts, all sample points from the treatment works are tested on a regular basis. In addition the source water and the catchment area is monitored regularly to determine the levels of *Cryptosporidium* oocysts and *Giardia* cysts.

Turbidity caused by inorganic and organic debris can interfere with the concentration, separation and examination of the sample for C*ryptosporidium* oocysts and *Giardia* cysts. In addition to naturally-occurring debris, such as clays and algae, chemicals, such as iron and alum coagulants and polymers, may be added to finished waters during the treatment process, which may result in additional interference. Organisms and debris that autofluoresce or demonstrate non-specific fluorescence, such as algal and yeast cells, when examined by epifluorescent microscopy, may interfere with the detection of oocysts and cysts and contribute to false positives by immunofluorescent assay (FA). Interferences co-extracted from samples will vary considerably from source to source, depending on the water being sampled. Experience suggests that high levels of algae, bacteria, and other protozoa can interfere in the identification of oocysts and cysts.

In South Africa no definite methodology has been prescribed for the detection of *Cryptosporidium* and *Giardia* (oo)cysts. Substantial variations in the performance of testing have been observed in the detection and enumeration of *Cryptosporidium* oocysts and *Giardia* cysts, and standards are based on performance rather than on prescription. The accepted detection methods for these organisms, involves a concentration step, a purification step and a final detection step. Losses of (oo)cysts occur at every step throughout the testing process. The size of the losses vary greatly and can be influenced by small day to day variations in how the sample is manipulated, differences between analysts performing the test and sample to sample differences such as turbidity and pH. These variations in losses limit the accuracy of the testing methods and contribute to the generation of false –negative results. The recovery of (oo)cysts will vary from sample to sample depending on the type of water sample. From most *Cryptosporidium* and *Giardia* test methods recoveries are lower for more turbid samples. If a range of water types is analysed it is important that recoveries for each water type are assessed.

As no (oo)cysts are detected in the source water from Vaal Dam it was decided that a matrix spike must be done on the source water to determine the recovery rate of *Cryptosporidium* oocysts and *Giardia* cysts in these source waters Sample were taken at different points in the Vaal Dam. The C-K19 a raw water sample point, in the Klip River was chosen as a reference, to compare the recovery rate of the Vaal Dam waters with a raw water from a different source. This river water was used as part of the validation of method 1623 in Rand Water's *Cryptosporidium* laboratory.

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Sample points

The samples points used for this evaluation are:

M-A18	Taken at the sample point at Vereeniging Pumping Station (Vaal Dam water)
M-B5/11	Taken at the sample point at Vereeniging Pumping Station (Vaal Dam water)
M-Canal	Taken at Zuikerbosch pumping station (Vaal Dam water)
C-VD1	Taken at the Rand Water inlet tower at Vaal Dam
C-VD2	Taken at the confluence of the Vaal and Wilge river (Vaal Dam water)
C-VD3	Taken at the Wilge river downstream of Oranjeville (Vaal Dam water)
C-VD4	Taken at the Vaalriver upstream of Vaal Marina (Vaal Dam water)
C-K19	Taken at the K19 weir in the Klipriver

Frequency of sampling

M-A18, M-B5/11, M-Canal and C-K19 were sampled on a bi-monthly schedule and the C-VD1-4 samples were sampled once a month.

Seed samples used

ColorSeed™ C&G

ColorSeed is a vial containing exactly 100 *Cryptosporidium* and 100 *Giardia* that have been permanently labelled with a red fluorescent dye. Each batch of ColorSeed™ C&G is quality controlled by testing 6% of the batch. Tubes spaced evenly throughout the batch are selected. ColorSeed™ C&G is purchased from Biotechnology Frontiers in Australia. (Information from ColorSeed pamphlet).

Rand Water Seed

RWS is a vial containing 100"10 *Cryptosporidium* and 100"10 *Giardia.* Each batch of Rand Water Seed is quality controlled. Seed samples are prepared using a Becton Dickinson flow cytometer. The *Cryptosporidium* oocysts are ordered from Sterling Laboratory, Univesity of Arizona USA, and the *Giardia* cysts are ordered from Waterborn Inc., New Orleans USA. Initial quality control done upon arrival of the (oo)cyasts include brightfield examination, FITC, DIC and DAPI. Age of the (oo)cysts used for seed sample preparation is less than one month. This method is accredited under ISO 17025. The Microbiology section at Rand Water produces their own seed samples.

Methodology

Three carboys from each sample point were received. One carboy was spiked with a vial of ColorSeed (CS), and one carboy was spiked with a vial of Rand Water Seed (RWS). The third sample was the control. All three water samples were analysed according to method 1623, which is incorporated as the routine method for the detection of *Cryptosporidium* and *Giardia* at Rand Water.

Procedure for spiking samples in the laboratory with enumerated spiking suspensions:

- Vortex the tube containing the *Cryptosporidium* oocyst spiking suspension and the tube containing the *Giardia* cyst spiking suspension for a minimum of 2 minutes.
- Add one spiking suspension to the carboy, Allow the spiking suspensions to mix for approximately 1 minute in the carboy.

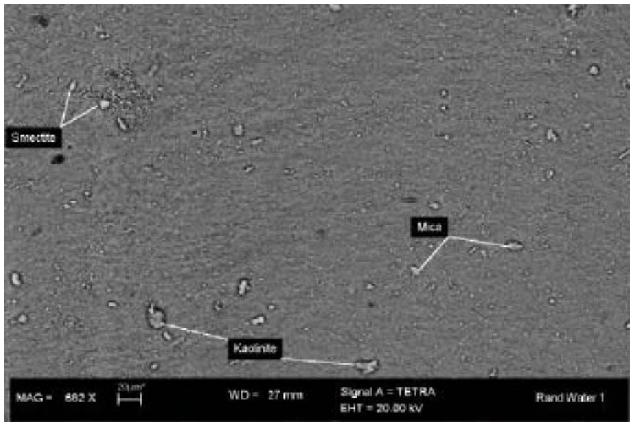


Figure 1
An electron backscatter image of the sample surface at relatively low magnification

- Turn on the pump and allow the flow rate to stabilise. Set flow at the rate designated for the filter under test.
- Allow the pump to pull all the water through the filter and turn off the pump.

Chemical composition of source water

X-Ray Diffraction was carried out to determine the bulk mineral composition of the sample. Surface observations at high magnification and X-Ray microanalysis were performed by means of SEM/EDS (scanning electron microscope, a Leica 440 Stereoscan with LINK OXFORD Energy Dispersive System). To determine the relative concentration and distribution of Al and Fe, X-ray maps were generated using the SEM. The sample was carbon coated to minimise electron charging.

Results of XRD

Semi-quantitative estimates, based on selected peak heights proportions reveal that the sample is composed of 62% clay (i.e. illite-smectite interstratification - 34%, smectite - 16%, kaolinite - 8% and mica - 4%), and 38% quartz. The background of the XRD trace is high and noisy, suggesting that the material present is very fine-grained or sub microscopic and/or amorphous.

Results of SEM

An electron backscatter image of the sample surface at relatively low magnification is shown in Fig. 1. The material appears to be very fine grained but distinct grains could be noticed, set in a very fine matrix of which individual particles could not be discerned. All the mineral species, identified by XRD could be confirmed by means of SEM X-ray microanalyses, i.e. smectite, mica, kaolinite.

Results

As can be seen from Tables 1 to 5, the recoveries of *Cryptosporidium* oocysts on the M-A18, M-B5/11, M-Canal. C-VD1, C-VD2, C-VD3 and C-VD4 are very poor. An average recovery between 0-10% was obtained when the sample was spiked with the RWS or ColorSeed seed samples. Recoveries for *Giardia* cysts were higher. An average recovery of between 0 and 30% was obtained for *Giardia*.

The recoveries of *Cryptosporidium* oocysts on the C-K19 samples varied between 14 and 41% and for the *Giardia* cysts between 1 and 52%.

TABLE 1
% recovery of (oo)cysts on M-A18

Colorseed(C)	RWS(C)	Natural(C)	Colorseed(G)	RWS(G)	Natural(G)
5	2	1	38	45	0
10	14	0	32	26	0
12	8	1	12	9	0
3	4	0	20	34	0
1	0	0	22	34	0

TABLE 2
% recovery of (oo)cysts on M-B5/11

Colorseed(C)	RWS(C)	Natural(C)	Colorseed(G)	RWS(G)	Natural(G)
13	5	0	14	20	0
29	47	0	23	38	0
3	13	0	0	23	0
6	4	0	5	21	0
5	2	0	28	52	0

TABLE 3
% recovery of (oo)cysts on M-Canal

Colorseed(C)	RWS(C)	Natural(C)	Colorseed(G)	RWS(G)	Natural(G)
1	13	0	38	45	0
14	8	0	9	24	0
1	10	0	0	24	0
1	1	0	9	44	0
16	3	0	10	12	0

TABLE 4
% recovery of (oo)cysts on C-K19

Colorseed(C)	RWS(C)	Natural(C)	Colorseed(G)	RWS(G)	Natural(G)
35	36	10	57	102	19
29	41	2	52	46	58
21	19	7	37	40	9
26	14	1	1	5	1
22	31	2	2	9	2

TABLE 5
% recovery on different points in the Vaal Dam water

Sample	Colorseed(C)	RWS(C)	Natural(C)	Colorseed(G)	RWS(G)	Natural(G)
C-VD1	0	0	0	0	8	0
C-VD2	1	0	0	2	32	0
C-VD3	2	0	0	1	46	0
C-VD4	2	0	0	16	18	0

The quality control parameters used with method 1623:

- Procedural blank: all results were negative
- Positive sample: A distilled water sample spiked with a RWS seed complied with the minimum of 20 % recovery as stipulated for our laboratory.
- This concludes that no naturally occurring *Cryptosporidium* oocysts in low concentrations will be detected in the Vaal Dam water with method 1623 used at Rand Water. The comparison between the two different types of raw water shows that the recovery of (oo)cysts will vary from sample to sample depending on the type of water sample.

Optimisation of method

Principle of the recovery process

A known number of *Cryptosporidium* oocysts and *Giardia* cysts are seeded into 10 litre water samples and the water is pumped through an Envirochek filter using a peristaltic pump. Oocysts and cysts are then removed in an elution process that involves pre-treatment with sodium polyphosphate and washing with a laureth 12 buffer. The washings are concentrated by centrifugation and the oocysts and cysts removed from the concentrate using immunomagnetic separation (IMS). During the IMS process, oocysts and cysts are dissociated from the IMS beads by using 0.1 N hydrochloric acid. Oocysts and cysts are spotted onto microscope slides containing 1.0 N sodium hydroxide to neutralise the acid, dried, fixed and stained with monoclonal antibodies tagged with a fluorescent dye before counting using immunofluorescent microscopy.

Optimisation of the process involves seeding water samples of 10 litres with a known number of oocysts and cysts and attempting to recover as many as possible. As an additional check, participation in an external quality assurance scheme provides evidence of the correct performance of the analytical technique. Typical recoveries for this technique should be between 40 - 60%. Occasionally recoveries may fall to below 30%, usually because the technique is not optimised or the analyst has not been diligent in following the procedure.

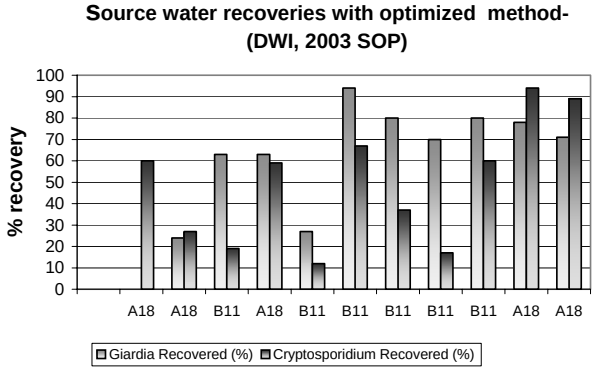
In a recent study for Pall Life Sciences in the United Kingdom (Boynton et al., 2002) the removal of bound particulate material on a filter was shown to be improved by warming all the elution solutions to 37°C and increasing the shaking speed from 600 to 900 oscillations per minute. Improved recoveries, particularly with dirty filters, were obtained during the study. The standard methods used in the United Kindgom (DWI- Standard operating protocol for the monitoring of *Cryptosporidium* oocysts in treated water supplies, part 2. 2003) incorporates these modifications.

The approved American methods for the technique are known as methods number 1622 and 1623 and the United Kingdom (UK) method, defined by the Drinking Water Inspectorate (DWI) is published as a Standard Operating Protocol (SOP). There are slight differences in the two methods but both are based on proven inter-laboratory trials using the types of water samples intended for the method use.

Results

Sample reference	Pellet Volume (mℓ)	*Giardia* recovered (%)	*Cryptosporidium* recovered (%)
A18	<0.5	ND	60
A18	<0.5	24	27
B11	>0.5<1.0	63	19
A18	<0.5	63	59
B11	2.5	27	12
B11	<0.5	94	67
B11	<0.5	80	37
B11	<0.5	70	17
B11	<0.5	80	60
A18	<0.5	78	94
A18	<0.5	71	89

ND= not done
M-A18: Taken at the sample point at Vereeniging Pumping Station (Vaal Dam water)
M-B5/11: Taken at the sample point at Vereeniging Pumping Station (Vaal Dam water)



Discussion

- When analysing the samples using method 1623 the source water (A18/B11) show very low recoveries for *Cryptosporidium* and *Giardia* (Tables1-5)
- Turbidity caused by inorganic and organic debris proofs to interfere with the concentration, separation and examination of the sample for C*ryptosporidium* oocysts and *Giardia* cysts.
- Improved recoveries, particularly with dirty filters, were obtained when the optimised method was used.

Conclusion

Following the SOP (Drinking Water Inspectorate. 2003) improves the recoveries of *Cryptosporidium* and *Giardia* (oo)cysts.

The pre-treatment step eliminates the interference of turbidity caused by inorganic and organic debris which can interfere with the concentration, separation and examination of the sample for C*ryptosporidium* oocysts and *Giardia* cysts.

References

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