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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