Application of Monitoring Data for *Giardia* and *Cryptosporidium* to Boil Water Advisories

Peter M. Wallis,1* Darrell Matson,2 Michael Jones,3 and John Jamieson4

Despite the problems associated with analyzing water samples for *Giardia* cysts and *Cryptosporidium* oocysts, the data can be very useful if their strengths and weaknesses are understood. Two municipalities in northern Ontario, Temagami and Thunder Bay, both issued boil water advisories for *Giardia* contamination. Data from these two cities are compared to show that only one municipality experienced a real outbreak, whereas the other did not. The concentration of *Giardia* cysts was much higher than background during the outbreak at Temagami, and the postoutbreak concentrations of cysts were very similar to the long-term average cyst concentration at Thunder Bay. The waterborne outbreak of giardiasis at Temagami was characterized by consistent positive results from water samples, concentrations two to three orders of magnitude higher than normal, and an obvious increase in the number of cases of giardiasis in the population. No outbreak was experienced at Thunder Bay, but a boil water advisory (BWA) was set in place for more than a year on the basis of a single sample from Loch Lomond in which only two cysts were detected but the sample equivalent volume was low. This gave the impression of a sudden increase in concentration, but 39 of 41 subsequent samples were negative. Additional factors that led to a BWA at Thunder Bay are described, and recommendations are presented to help determine when a BWA is necessary and when it should be rescinded.

KEY WORDS: Risk analysis; waterborne giardiasis; boil water advisory; *Giardia; Cryptosporidium*; monitoring

1. INTRODUCTION

Many municipalities and regulatory agencies monitor raw and treated drinking water for the protozoan parasites *Giardia* and *Cryptosporidium*. Legislation requiring greater levels of water treatment and monitoring is becoming more common, but a worldwide survey of 27 utilities and regulatory agencies(1) found that monitoring is still generally voluntary and the data are only rarely used to make public health decisions. In particular, the application of these data for imposing and rescinding boil water advisories (BWAs) is problematic because their strengths and limitations are not well understood. This article seeks to explore the usefulness and limitations of monitoring data for predicting waterborne disease prevalence by comparing an outbreak and a nonoutbreak situation.

1.1. Giardiasis and Cryptosporidiosis

Waterborne outbreaks of giardiasis and cryptosporidiosis have occurred across North America many times in the past 3 decades as well as in En-
gland, Scotland, Australia, and probably other countries as well. Both of these parasites have environmentally resistant cyst stages that are difficult to remove or inactivate during the water treatment process; thus outbreaks have occurred even when multiple barriers of treatment such as chlorination, clarification, and filtration were present. Most waterborne epidemics, however, have been traced to unusual contamination events or breakdowns in equipment. \(^{(3)}\) Disease can result from improper release of human sewage, but *Giardia* and *Cryptosporidium* are both zoonotic parasites and their presence in water has also been associated with contamination from beavers, cattle, muskrats, and other animals. Watershed management is, therefore, very important, particularly when water treatment is minimal (i.e., chlorination alone) and consumers are accustomed to high-quality tap water. Monitoring data can give advance warning of problems, although there is necessarily a delay of several days between sampling and reporting.

*Giardia* and *Cryptosporidium* infections are characterized by diarrhea, weight loss, anorexia, foul stool, and other symptoms of gastroenteritis that may easily be mistaken for bacterial or viral infections. Diagnosis is usually confirmed by microscopic examination of stool samples, biopsy, and analysis of jejunal fluids, which lead to quite low reporting rates even during epidemics, despite the fact that both diseases are usually notifiable. Because both parasites are transmitted by the fecal–oral route, vehicles other than water can be responsible. There are a number of excellent reviews describing giardiasis and cryptosporidiosis \(^{(3–6)}\) that emphasize the vulnerability of immunocompromised members of the population and the lack of treatment for cryptosporidiosis, which can sometimes be fatal. \(^{(7)}\)

Epidemiological investigation is usually retrospective, and water is often identified as the source of infection without performing any water sample analyses. Monitoring frequently begins only after the number of cases has peaked and the presence of *Giardia* cysts or *Cryptosporidium* oocysts in treated water is needed as evidence for waterborne disease. These situations are usually fairly clear, but sample analysis sometimes reveals the presence of these parasites at low concentrations without any noticeable change in the prevalence of disease in the community. Should action be taken? A careful consideration of all the risk factors that can be identified, such as turbidity spikes, engineering problems, known contamination of the water supply, high runoff, and so forth, can help health authorities interpret the significance of positive monitoring results. Monitoring results should be interpreted in the context of all relevant data if they are to be used to justify a BWA. The following discussion is focused on *Giardia* but is generally applicable to *Cryptosporidium* as well.

### 1.2. A Tale of Two Cities

Temagami, Ontario (population 1,000) experienced an outbreak of waterborne giardiasis from February to April 1994 with a symptomatic attack rate of approximately 30%. \(^{(8)}\) Epidemiological investigation showed that adults were more likely to be ill than children, and that infected individuals were broadly distributed throughout age groups and both genders. The asymptomatic infection rate could not be measured. The outbreak was detected when unusually large numbers of local residents went to their doctors complaining of diarrhea and the regional health laboratory confirmed multiple cases of giardiasis by stool analysis. Because the only common factor appeared to be the water supply, samples were submitted to the laboratory for analysis while the outbreak was still underway. High concentrations of *Giardia* cysts were found in the treated water in the initial samples and a BWA was implemented. There are two separate water supplies (both chlorinated and filtered) located on two different lakes that serve the separate communities of Temagami North and Temagami South. These lakes are large and of excellent raw water quality, but there is shoreline development from seasonal residences on both lakes, and the townsites of Temagami South (and the water intake) is situated at the end of a narrow bay. The major source of *Giardia* cyst contamination was believed to be leakage from the storm and sanitary sewage systems aggravated by surface runoff following a winter thaw at Temagami South, because fecal coliforms, normally absent, reached high concentrations for 6 weeks in the raw water immediately afterward. The water treatment plant was found to be suffering from a number of deficiencies, but these were soon rectified and the BWA was rescinded after 2 months.

Thunder Bay, Ontario is a much larger municipality, which also has two water supplies that together supply approximately 107,000 people with drinking water. The two water systems are separate and date back to the period before amalgamation when they served Port Arthur (supplied by Lake Superior and the Bare Point Water Treatment Plant [WTP]) and Fort William (supplied by Loch Lomond). The popu-
2. METHODOLOGY

2.1. Sampling

A total of 40 water samples was collected from raw (12 samples) and treated (28 samples) water at Temagami, Ontario during and after the outbreak that took place in 1994. Samples were taken from the potable water distribution system and the water treatment plants at Temagami North and South. A much larger number of samples from Thunder Bay, Ontario was analyzed from 1993 to 1999 in an ongoing monitoring program for raw and treated water from the Loch Lomond and Bare Point WTPs and from several points in the potable water distribution system. The sampling equipment was the same at all locations and consisted of a portable unit containing a pressure-reducing valve, filter housing capable of accepting 10-inch format 1-μm wound filters, and a water meter (FW5, Hyperion Research Ltd., Medicine Hat, Alberta). The volume filtered was usually 1,000 L although the sample equivalent volumes, meaning the volume equivalent actually examined by microscopy in the laboratory, were often much smaller. Sample equivalent volumes are calculated by multiplying the total volume filtered by the ratio of the volume of the sample clarified for microscopy to the total volume of the filter eluate. Sample filters were shipped over ice by overnight courier to the laboratory in Medicine Hat for analysis.

2.2. Parasite Analysis

The methodology used for analyzing water samples in this study has been described elsewhere\(^{(9,10)}\) and is similar to the protocol specified by the U.S. Environmental Protection Agency (USEPA) for the Information Collection Rule (ICR). Briefly, water is filtered through wound polypropylene cartridges and the filter material is cut from the cartridge core, then hand washed in eluting solution. The eluate is concentrated by centrifugation, clarified using percoll-sucrose discontinuous gradient centrifugation, immunostained with monoclonal antibodies conjugated with the fluorochrome CY3 (Waterborne Inc., New Orleans, LA) and examined by epifluorescence using a Zeiss Axioskop microscope equipped with filter cubes for fluorescein isothiocyanate (FITC), rhodamine, and 4',6-diamidino-2-phenylindole (DAPI). The only major departure from the ICR methodology was the staining of the sample, which was carried out in 15 mL centrifuge tubes rather than on membranes mounted on a Hoefer manifold, and the sample was dried on a well slide (Cel-Line Associates Inc., Newfield, NJ). Nucleic acid staining was performed by flooding the dried sample with DAPI (Sigma Chemical Co., St. Louis, MO) for 5 mins with a working solution prepared by adding 4 μL of 1 mg/mL stock solution to 10 mL of deionized water. The cyst recovery efficiency of this method was measured by spiking known numbers of cysts and oocysts into eluates washed from real water samples and loading the mixture onto filter cartridges using an injection port upstream of a filter connected to a clean, pressurized water supply in the laboratory. This is also a departure from the ICR methodology (which specifies the addition of cyst/oocyst spikes to 10 L of laboratory water in a carboy), but it was felt that spiking cysts and oocysts into material recovered from real samples was much more realistic than trying to recover them from deionized water without normal background material present. It would have been preferable to spike samples taken in the field but this was not possible for logistical reasons (the laboratory is 1,500 to 2,500 km away from both sites), and the undesirability of introducing live parasites into a WTP under any circumstances.
3. RESULTS

The mean recovery efficiency of the filter analysis procedure was $44.0 \pm 4.4\%$ for *Giardia* cysts and $14.1 \pm 4.7\%$ for *Cryptosporidium* oocysts ($n = 78$ control filters). These recoveries compare favorably to the recoveries of $8.8\%$ and $10.8\%$, respectively, reported by Clancy et al.\textsuperscript{(11)} in an interlaboratory comparison, and fit the pattern of recoveries of less than 50% with *Cryptosporidium* being the lowest that was identified by LeChevallier et al.\textsuperscript{(12)}

The analytical results from Temagami North and South and from Thunder Bay, Loch Lomond, and Bare Point, are summarized in Table I. The raw and treated Temagami results are divided into “outbreak” and “postoutbreak” periods to show the marked increase in the number of cysts and the very high concentrations of *Giardia* cysts in both treated and raw water samples from Temagami South during the outbreak. The average concentration of *Giardia* cysts in the South water supply during the outbreak was found to be 0.53 cysts per L in the raw water and 0.84 cysts per L in the treated water. These results show that the treatment plant was ineffectual in removing *Giardia* cysts. The concentration of *Giardia* cysts in the South dropped by three to four orders of magnitude following the outbreak. The concentration of cysts at Temagami North did not change much during and after the outbreak, but the treated water was still approximately an order of magnitude higher than postoutbreak concentrations at Temagami South. Because the North was unaffected by sewage contamination, as shown by negative fecal coliform analyses, these cysts may be of beaver origin. However, of 56 beavers trapped in the surrounding area, only two were positive for *Giardia* (prevalence rate of 3.6%) suggesting that these animals were not an important reservoir. A retrospective examination of conditions at Temagami subsequently revealed that the sewage collection system was notoriously leaky and that a premature thaw 6 weeks before the outbreak had introduced large quantities of water into both the storm and sanitary sewers. The natural outfall of this water was very close to the water intake, because the town is built on a hillside on granitic rock at the end of a bay. Although it could not be proven, it is believed that sewage was the source of the *Giardia* cysts that entered the water treatment plant.

The data from Thunder Bay cover the 6-year period from 1993 to 1998, and are presented for both raw and treated water. It should be noted that even the raw water from both Thunder Bay sources averaged lower *Giardia* concentrations than the postoutbreak conditions at either water treatment plant in Temagami.

The (geometric) mean concentrations of *Giardia* cysts per L (second column, Table I) are similar to long-term averages (last column) that were calculated by dividing the total number of cysts found by the total sample equivalent volume, with the exception of samples from the Loch Lomond treated water system. This difference was caused by a single sample taken at Fort William Gardens, which contained only two *Giardia* cysts, but the sample equivalent volume was considerably lower than usual (8.8 L as com-

<table>
<thead>
<tr>
<th>Location</th>
<th>Mean concentration cysts per L</th>
<th>Variance</th>
<th>Total sample equivalent volume (L)</th>
<th>Total cysts found</th>
<th>Total concentration cysts per L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temagami</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>North outbreak raw</td>
<td>0.01</td>
<td>—</td>
<td>1,400</td>
<td>14</td>
<td>0.01</td>
</tr>
<tr>
<td>North outbreak treated</td>
<td>0.022</td>
<td>$2.8 \times 10^{-4}$</td>
<td>1,750</td>
<td>43</td>
<td>0.025</td>
</tr>
<tr>
<td>North postoutbreak raw</td>
<td>0.005</td>
<td>$9.2 \times 10^{-4}$</td>
<td>3,420</td>
<td>14</td>
<td>0.004</td>
</tr>
<tr>
<td>North postoutbreak treated</td>
<td>0.02</td>
<td>$1.7 \times 10^{-7}$</td>
<td>5,540</td>
<td>33</td>
<td>0.006</td>
</tr>
<tr>
<td>South outbreak raw</td>
<td>0.53</td>
<td>—</td>
<td>240</td>
<td>128</td>
<td>0.53</td>
</tr>
<tr>
<td>South outbreak treated</td>
<td>0.955</td>
<td>$8.9 \times 10^{-3}$</td>
<td>2,444</td>
<td>2,057</td>
<td>0.841</td>
</tr>
<tr>
<td>South postoutbreak raw</td>
<td>0.004</td>
<td>$4.7 \times 10^{-5}$</td>
<td>2,410</td>
<td>10</td>
<td>0.004</td>
</tr>
<tr>
<td>South postoutbreak treated</td>
<td>0.00002</td>
<td>$3.1 \times 10^{-7}$</td>
<td>8,950</td>
<td>2</td>
<td>0.0002</td>
</tr>
<tr>
<td>Thunder Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Loch Lomond raw</td>
<td>0.0009</td>
<td>$1.2 \times 10^{-4}$</td>
<td>27,009</td>
<td>17</td>
<td>0.0006</td>
</tr>
<tr>
<td>Loch Lomond treated</td>
<td>0.002</td>
<td>$3.4 \times 10^{-4}$</td>
<td>53,480</td>
<td>22</td>
<td>0.0004</td>
</tr>
<tr>
<td>Bare Point raw</td>
<td>0.0017</td>
<td>$3.0 \times 10^{-4}$</td>
<td>19,487</td>
<td>9</td>
<td>0.0005</td>
</tr>
<tr>
<td>Bare Point treated</td>
<td>0.000005</td>
<td>$1.6 \times 10^{-7}$</td>
<td>72,319</td>
<td>5</td>
<td>0.00007</td>
</tr>
</tbody>
</table>
pared to the mean volume equivalent of 400 L); thus, the concentration estimate was very high. The distorting effect of this single sample disappears when average concentration is calculated by dividing the total number of cysts found by the total sample equivalent volume. Nevertheless, this single sample was instrumental in precipitating the BWA at Thunder Bay.

Table II summarizes the number of samples at each location that contained at least one *Giardia* cyst (called a positive sample). All samples were positive at Temagami South during the outbreak, but only 31.3% (5/16) postoutbreak samples (raw and treated) were positive in the 4 months after clinical cases ceased to be reported. At Temagami North, all samples were also positive during the outbreak, and 57.1% (5/14) raw and treated samples were still positive in the postoutbreak sampling interval although the concentrations were much lower than outbreak samples from Temagami South. In Thunder Bay, the percentage of positive samples was much lower, never exceeding 11.5%.

Because the BWA was issued for Loch Lomond in 1997, it is important to look at the pattern of positive results from Loch Lomond to ascertain whether 1997 had an unusual number of positive samples. The number of positive samples and the total number of samples from Loch Lomond (combining samples of raw and treated water, because the numbers are similar) are presented by year in Fig. 1. There was no increase in positive samples in 1997. The proportion of positive samples actually declined from 1996 to 1997 and declined to zero in 1998, further suggesting that there were no unusual events in 1997 in Loch Lomond. There is no obvious relation between the total number of samples taken and the number of positive samples or the concentration of cysts found.

The sample that triggered the BWA contained two cysts. However, this sample also contained higher amounts of algae from the seasonal fall bloom, which limited the amount of material that could be examined under the microscope to a volume equivalent of only 8.8 L. This resulted in a very high concentration estimate of 0.225 cysts per L, compared with a long-term average of 0.0004 cysts per L (based on dividing the total number of cysts found by the total volume filtered in 152 samples). Thirty-nine of 41 follow-up samples taken over the next 15 weeks were negative (the two positive samples contained a single cyst each). Furthermore, a sample analyzed by an independent laboratory (Ministry of Health) found no *Giardia* cysts, and this result was available before the BWA was declared. The large increase in sampling during 1997 is apparent in Fig. 1.

Concentration estimates based on low sample volumes are unreliable. In general, the samples contain only one or two cysts and almost 90% of all the samples are negative. Negative samples should be reported as less than the detection limit but this really means that no cysts were found in whatever sample equivalent volume was examined. Larger sample equivalent volumes generate more confidence because the detection limit is lower.

Fig. 2 shows all the positive samples from Loch Lomond (raw and treated systems combined). When the sample volume equivalent is small, the estimate of concentration is high. In contrast, when the volume is the maximum 1,010 L, a lower estimate is pro-

<table>
<thead>
<tr>
<th>Location</th>
<th>Total no. of samples</th>
<th>No. of positive samples</th>
<th>% Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temagami North outbreak raw</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Temagami North outbreak treated</td>
<td>3</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>Temagami North postoutbreak raw</td>
<td>5</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>Temagami North postoutbreak treated</td>
<td>9</td>
<td>6</td>
<td>67</td>
</tr>
<tr>
<td>Temagami South outbreak raw</td>
<td>1</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>Temagami South outbreak treated</td>
<td>5</td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td>Temagami South postoutbreak raw</td>
<td>5</td>
<td>3</td>
<td>60</td>
</tr>
<tr>
<td>Temagami South postoutbreak treated</td>
<td>11</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Thunder Bay Loch Lomond raw</td>
<td>80</td>
<td>9</td>
<td>11.3</td>
</tr>
<tr>
<td>Thunder Bay Loch Lomond treated</td>
<td>152</td>
<td>13</td>
<td>8.6</td>
</tr>
<tr>
<td>Thunder Bay Bare Point raw</td>
<td>61</td>
<td>7</td>
<td>11.5</td>
</tr>
<tr>
<td>Thunder Bay Bare Point treated</td>
<td>104</td>
<td>2</td>
<td>1.9</td>
</tr>
</tbody>
</table>
duced. It is clear that the concentration estimates are an inverse function of the volume equivalent, and are largely unrelated to the number of cysts detected. Large concentration estimates are only found when the volume equivalent is low. Thus, concentration estimates tend to be higher when the sample volume is low. As an aside, the hypothesis that *Giardia* concentrations are in fact higher when algae concentrations are high can be rejected because the average volume equivalents for positive and negative samples were comparable. If algae are misidentified as cysts by inexperienced analysts, however, there can be an apparent correlation.

Although 30% of the population of Temagami experienced symptoms of giardiasis during the outbreak (established by survey), the number of laboratory-confirmed cases in Thunder Bay remained low or declined throughout the period 1994 to 1997 (Table III). Furthermore, the number of cases of giardiasis in Thunder Bay was actually higher in the north end of the city served by Bare Point (full conventional treatment including filtration) than in the south supplied by Loch Lomond (chlorination alone). The total population of Thunder Bay is about 107,000, distributed 62:38 between the two water treatment systems. The figures for Ontario and Canada in Table III are rates per 100,000 population; thus, the data are fairly comparable. The BWA was established in Thunder Bay on October 9, 1997 in response to monitoring data showing a single high concentration of *Giardia* cysts in Loch Lomond treated water on September 17, 1997. It is important to note that the number of stool samples submitted to the laboratory for parasite analysis increased sharply from about 200 to over 500 per month after the BWA was proclaimed. The number of confirmed cases, however, only increased from three in September to six in October and then dropped back to three again for both November and December.

*Cryptosporidium* oocysts were not detected in any of the Temagami samples and in only seven of the Thunder Bay samples. Five of the *Cryptosporidium* positive samples were from the Loch Lomond system and two were from the Bare Point system. All of the *Cryptosporidium* concentrations in the positive samples were very low, and few of the oocysts observed could be identified with confidence. No background prevalence data are available for Temagami for either *Giardia* or *Cryptosporidium*.

### 4. DISCUSSION

The data reveal that the BWA for Thunder Bay was a false alarm. There was no outbreak of *Giardia* in 1997. The two factors that appear responsible for the BWA decision were (1) the very high concentration estimate from a low volume equivalent, and (2) one of the cysts was deemed to be viable. In order to prevent these factors from producing further false alarms, it is important to understand the limitations of current methodologies.

#### 4.1. Sample Volumes

Cysts and oocysts are rare particles in pristine surface water such as the Thunder Bay and Temagami drinking water sources, except in cases of gross contamination, and their distribution is not necessarily random. Clumping of microorganisms is a well-known problem and their distribution may approximate the binomial rather than the Poisson. In order to max-
imize the probability of detection, a large volume of water typically from 100 to 1,000 L, is filtered. The filter elution process homogenizes the mixture and breaks up clumps so that the distribution of cysts is probably more random (Poisson) in the filter eluate than in the original water sample. Because the volume filtered is often much larger than the laboratory can actually process and examine, much of this material may be discarded in the analysis. If too small a volume is filtered, on the other hand, there is a risk that no cysts will be detected even though clumps of infectious microorganisms may be present.

The ICR methodology for the analysis of *Giardia* cysts and *Cryptosporidium* oocysts is imprecise because the organisms must be recovered from water and counted under the microscope against a background of silt, algae, and other microorganisms. These parasites are protozoans, unlike coliform bacteria, and cannot be grown in culture, so they must be trapped on filters and eluted in the laboratory. The three major sources of error are mistaken identification and unknown viability status (which lead to overestimation) and incomplete recovery (which leads to underestimation). In risk models, these sources of error are often assumed to cancel each other out, at least partially. Mistaken identification is not a great problem for *Giardia* cysts, because they are elliptical and relatively large; but *Cryptosporidium* oocysts are smaller and round, which makes them harder to spot and recognize. If *Cryptosporidium* oocysts are mistakenly identified, there may be large numbers reported, because the algal cells with which they may be confused are usually abundant. Cysts and oocysts are preferentially concentrated from the sample by density gradient centrifugation or immunomagnetic separation and selectively stained using fluorescent monoclonal antibodies, but the final count is invariably incomplete because some are lost at each stage. Density gradient centrifugation is particularly prone to error because a high degree of skill is required to properly judge how much of the sample to load onto the gradient. These problems compromise the accuracy and precision of the analysis so that recovery rates for *Giardia* cysts and *Cryptosporidium* oocysts are typically only 35% and 10%, respectively, using wound filters and conventional methodology (i.e., the ICR method).

The amount and nature of the background material in the water being sampled is crucial because it can obscure the target cells and limit the amount of the sample that can be feasibly examined under the microscope. Algal cells are a major interference in the ICR method and their concentration rises and falls seasonally in blooms that occur naturally in pristine surface waters and are enhanced in eutrophic water bodies. This means that the sample equivalent volume (i.e., the amount of the prepared sample that is actually examined microscopically as opposed to the total volume filtered) changes from sample to sample because the concentration of background particles can vary considerably. Theoretically, the amount examined could be held constant, but the sensitivity of the analysis increases with the sample equivalent volume and the analyst always strives to examine as much of the sample as possible. The minimum number of cysts that can be observed is one, so the detection limit becomes one in the sample equivalent volume. Results are typically reported in cysts per 100 L, however, so the detection of a single cyst can be magnified into an alarming number if the sample equivalent volume is small. This problem is discussed in some detail by investigators who advocate the use of similar sample equivalent volumes to reduce this error. This is a difficult thing to do with dissimilar water types, however, and care must be taken when comparing data based on analysis of samples using very different sample equivalent volumes because the sample equivalent volume is dictated by the amount of background, interfering particles.

The interpretation of monitoring data must consider the sample equivalent volume and the units that are used for reporting. If the sample equivalent volume is unusually low and cysts and/oocysts are found, the conversion to a concentration per 100 L can produce unusually high concentrations. In the case of Thunder Bay, the observation of two *Giardia* cysts in a sample equivalent volume of 8.8 L was reported as 22.5 cysts/100 L, a concentration far higher than the average. If subsequent sampling fails to substantiate high concentrations of cysts or oocysts, as was the case at Thunder Bay, the cessation of a BWA should be considered unless other risk factors such as high turbidity or engineering breakdown that contributed to the original decision have not been resolved. Turbidity associated with rainfall has been shown to be positively correlated with *Giardia* cyst and *Cryptosporidium* oocyst concentrations in river water and could be a short-term cause of contamination.

One of the major limitations of the ICR method of water analysis for *Giardia* cysts and *Cryptosporidium* oocysts is carryover of background particles from the sample at the clarification step, which limits the amount of the sample that can be examined microscopically and results in a low sample equivalent vol-
4.2. Viability and Infectivity

Even if cysts or oocysts are detected in water there is no easy way of knowing if they are alive or infective to humans. Closely related species of both parasites that are not human infective are nearly identical in appearance and a recently deceased cyst looks very similar to a live one. Analysts customarily use nucleic acid dyes along with fluorescent antibodies based on the principle that the dye should not penetrate living cysts. Unfortunately, dead cysts are often empty, so there is no DNA to stain. Water treatment studies have shown that viability testing by nucleic acid staining is not well correlated with animal infection experiments at high levels of water treatment and low numbers of cysts. Dye exclusion can, however, be a good indicator of viability in the absence of disinfectants. Because penetration of cells by nucleic acid dyes demonstrates abnormal membrane permeability rather than the status of metabolic activity, a cell that admits dye may not yet be dead and could cause an infection if ingested. Animal infection models have proven to be more sensitive indicators of viability than nucleic acid staining and the results are not ambiguous. Not all strains of parasite are equally infective to test animals such as gerbils, and their routine use would be very expensive; thus, they are most commonly used only when it is important to confirm viability or to collect isolates for culture. Viability assays by dye exclusion are, therefore, useful but not completely reliable. A conservative approach is to assume that all cysts and oocysts detected are viable and this overestimate of infectivity is offset to some degree by the low recovery efficiency of the analytical method. This assumption is commonly made in risk models.

The treated water sample from Loch Lomond that triggered the BWA contained two cysts in the sample equivalent volume examined. One of these was judged to be alive based on nucleic acid staining, and this factor weighed heavily in the decision to set a BWA in place. Unfortunately, this particular sample was not tested by animal infection, but large equivalent volumes from several subsequent samples failed to produce infections in gerbils. The single cyst that was resistant to dye penetration caused considerable alarm because it was found in a treated water sample. This situation is the same as that discussed by Labatiuk et al. who concluded that viable cysts as indicated by fluorogenic dyes may not be able to complete their life cycle and produce an infection. The actual risk of giardiasis infection presented by this possibly viable cyst must have been low, because there was no detectable increase in human cases of giardiasis in the months following the report of the positive sample. The predominantly negative results from follow-up samples make a convincing case for the safety of the Loch Lomond water supply even though Giardia cysts are occasionally detected.

4.3. Characterizing a Waterborne Outbreak

A monitoring program that samples unfiltered surface waters can be expected to include at least some positive results if enough samples are taken. However, concentration estimates based on single, low-volume samples should be treated with caution. Similarly, determinations of viability of cysts are also subject to error. A comparison of the two incidents indicates that a waterborne outbreak of giardiasis is characterized by (1) consistent positive samples, (2) concentrations of cysts two to three orders of magnitude above background, and (3) an obvious increase in the number of cases in the population served.

The data from Temagami show that positive samples occurred regularly and at relatively high concentrations during the outbreak of giardiasis that took place in 1994. In contrast, most of the samples taken at Thunder Bay were negative for both Giardia cysts and Cryptosporidium oocysts. Water monitoring during the Temagami outbreak showed a clear improvement when the problems with the water treatment system were corrected. This improvement was a useful indicator to support the rescinding of the BWA.
In conclusion, a comparison of these two cases shows that water monitoring of parasites can be very useful for detecting the large changes in concentration that occur during outbreaks and for determining when to rescind the BWA after the problems are corrected. However, BWA decisions based on small concentration changes in estimated parasite concentrations are more prone to error. Wallis et al.\(^{(10)}\) reached a similar conclusion based on the relation between parasite concentrations and outbreaks of giardiasis in a number of Canadian municipalities. They proposed that concentrations of *Giardia* less than three to five cysts per 100 L should not be justification for issuing a BWA. However, repeated samples yielding concentration estimates above five cysts per 100 L should trigger a BWA. Haas and Rose\(^{(2)}\) proposed higher action thresholds for *Cryptosporidium* (10 to 30 oocysts per 100L). If a sudden sharp increase in concentration is observed, its significance can be regarded as minimal if (1) only a small number of cysts or oocysts are observed in a small sample volume equivalent, (2) subsequent samples are negative, and (3) there are no other risk factors such as plant breakdown or high turbidity. Analytical methodology is imperfect, but low parasite recovery efficiency is offset to some extent by the fact that parasite viability, a difficult property to measure, is invariably less than 100%.

**REFERENCES**