Serological analysis of a cryptosporidiosis epidemic

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Background  A cryptosporidiosis epidemic occurred among residents and visitors to Collingwood, Ontario, during March 1996. Fifty-five per cent of 36 confirmed cases were Collingwood visitors and 57% of Collingwood resident cases were under 10 years of age. The low level of reported diarrhoeal illness among adult Collingwood residents caused government officials and physicians to question whether an epidemic had occurred in Collingwood.

Methods  To better evaluate the extent of the epidemic, anonymous surplus sera from 89 adult Collingwood residents, collected for routine tests prior to, during and after the epidemic, and from 80 adult Toronto residents were tested using a Western blot assay for IgG antibody response to two Cryptosporidium antigen groups (15/17-kDa and 27-kDa).

Results  For sera collected from 1 January 1996 to 17 June 1996, a higher fraction of Collingwood residents had a detectable serological response (P < 0.002) and the mean intensity of serological responses was higher for Collingwood than Toronto residents (P < 0.001). The mean intensity of serological responses for Collingwood residents was higher in specimens drawn during the 8 weeks following the initial case reports compared to those drawn before or after this period (15/17-kDa, P < 0.02; 27-kDa, P < 0.10).

Conclusions  These elevated serological responses indicate that Cryptosporidium infections among Collingwood residents likely occurred more commonly than illness reports suggested, consistent with a community-wide cryptosporidiosis epidemic. Similar studies should be considered in future suspected cryptosporidiosis epidemic investigations.

Keywords  Cryptosporidium, serological survey, epidemic

Accepted 26 August 1999

Cryptosporidium is recognized as a cause of infectious gastroenteritis. The severity and persistence of symptoms are related to the immunocompetence of the host1 and may be influenced by immune responses from prior infections.1–5 Epidemic cryptosporidiosis has been linked to contaminated food and water.1 During several waterborne epidemics, adult residents of the affected city were at a lower risk of illness than visitors or young child residents, suggesting that adult residents were less frequently exposed or less likely to experience illness from an infection.5 Results from experimental human volunteer studies indicate that prior infection may partially protect against subsequent illness.6

A cryptosporidiosis epidemic was detected by the Simcoe County District Health Unit from illnesses at two Collingwood, Ontario, nursing homes during March 1996. Relatively few other adult Collingwood residents were diagnosed with cryptosporidiosis. Complicating epidemic control efforts was a Collingwood Bulletin newspaper story (6 April 1996) in which local physicians noted that few of their patients were ill with cryptosporidiosis-like symptoms at the time of the epidemic and that this would not be consistent with a community-wide cryptosporidiosis epidemic. Low illness attack rates, however, have been observed among residents of other communities with cryptosporidiosis epidemics.6 As with the Collingwood epidemic, uncommon occurrence of illness among Talent, Oregon residents during a cryptosporidiosis epidemic significantly complicated epidemic detection and control.7
This study was conducted to measure serological responses to two Cryptosporidium oocyst antigen groups in sera from Collingwood residents collected before, during and after the epidemic and in sera from similar individuals not residing in or close to Collingwood. Our hypothesis was that, perhaps because of protective immunity, a large percentage of Collingwood area adults may have been infected, but that the infection resulted in few clinically detectable illnesses. If the occurrence of symptomatic and asymptomatic infections was high among Collingwood residents, then evidence of elevated serological response should be apparent in sera collected during the period immediately following the initial case reports. Sera were readily available and were tested for antibody responses to 15/17-kDa and 27-kDa Cryptosporidium antigen groups. Prior studies indicate that the occurrence and intensity of serological responses to these antigen groups increases following experimental Cryptosporidium infection and that elevated serological responses have been observed in populations involved in prior cryptosporidiosis epidemics.8,9

Methods

The epidemic was identified and investigated by the Simcoe County District Health Unit, Ontario. Reports of diarrhoeal illness were initially received from two Collingwood area nursing homes. Laboratory analysis of five stool specimens from nursing home residents confirmed a diagnosis of cryptosporidiosis in four. Thirty-one of the 36 laboratory-confirmed cryptosporidiosis cases from Collingwood area, non-institutional residents or visitors were interviewed to determine symptoms, the date of onset, age, sex, symptoms, place of residence, work location, the number of children in their household, their source of drinking water, contact with Collingwood and whether they knew others who were ill with diarrhoea around the same time. Cases were asked to list travel destinations, raw milk consumption, Collingwood area party or restaurant exposures, swimming, animal and small children exposures for the two weeks prior to the epidemic.

Collection of sera

Surplus sera from routine serological tests, conducted for other reasons, were obtained from the Laboratory Services Branch of the Ontario Ministry of Health. Samples were identified from adults with Collingwood listed as the place of residence on their laboratory service request form. Samples from these individuals were frequency age-matched to samples from adults undergoing similar screening with Toronto listed as their place of residence. No information was available on symptoms or other exposures of persons contributing sera. The sera were collected from 31 January through mid-May 1996 and, with identifiers removed, were sent to the Southwest Center for Managed Care Research in Albuquerque, New Mexico for testing.

Western blot procedures

Sera were collected and analysed by immunoblot to measure IgG serological response to the 15/17- and 27-kDa antigen groups.10 Cryptosporidium protein is separated by Sodium Dodecyl Sulfate Polyacrylamide gel electrophoresis (SDS–PAGE) using a 15% separating gel in a continuous buffer system. The protein is electrophoretically transferred out of the gel and onto a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane is blocked with PBS/0.3% Polyoxyethylene sorbitan Monolaurate (Tween 20) for 30 min and placed in an immunoblot screening device. The device creates leak-proof channels to aid in the analysis of different sera samples for the presence of human anti-Cryptosporidium parvum antibodies. Each isolated chamber is exposed to human sera at a 1:50 dilution in PBS/0.3% Tween 20 overnight at 4°C. The bound primary human anti-Cryptosporidium antibodies are reacted with IgG1, IgG2 and IgG4 biotinylated mouse anti-human antibodies at a 1:500 dilution in PBS/0.3% Tween 20 for 60 min on different blots, depending on their immunoglobulin class. Bound secondary antibodies are incubated with streptavidin alkaline phosphatase at a 1:1000 dilution in PBS/0.3% Tween 20 for 60 min. The bound antibodies are visualized with 5-Bromo-4-Chloro-3-Indolyl Phosphate as substrate and Nitro Blue Tetrazolium as the chromogen. The intensity of the serological responses to the 15/17- and 27-kDa antigen groups on the immunoblots are digitally analysed by an IS-2000 Digital Imaging System (Alpha Innotech). The image is captured using a high performance CCD camera and the system calculates the pixel density of the manually selected band of the immunoblot. This allows the intensity of the serological response on the immunoblot to be quantified.

The IgG results for each specimen were standardized by taking the ratio of the response intensity for the unknown sample to the response intensity for a positive control serum contained on each blot. The IgG positive control serum was derived by mixing sera from several individuals with strong serological response to both antigens. No detectable responses were observed with intensities of <5% of the positive control and the vast majority of detectable responses had an intensity of >10% of the positive control. Statistical analysis was conducted using SPSSPC® version 5.0. Results are reported as the ratio of the unknown to the positive control for the specific blot for the IgG. An analysis of variance on the ratios of the responses to the positive controls was conducted.

Results

Ova and parasite testing identified 36 laboratory-confirmed cases of cryptosporidiosis among people who were not nursing home residents. Of 31 cases interviewed, 18 (58%) were under age 10 and 17 (55%) resided outside of Collingwood. The per cent of cases under age 10 was almost identical for Collingwood residents and visitors (57% versus 59%). Of the 17 visitor cases, 7 were one-time visitors while 10 were regular visitors. No single restaurant was identified as visited by more than 3 of the 10 visitor cases who recalled where they ate. Of the infected one-time Collingwood visitors, six of seven had visited between 1 March and 13 March. The time of illness onsets peaked during the week of 4 March, declining to background levels by 25 March (Figure 1).

A total of 89 sera were collected from Collingwood residents undergoing routine serological tests and from 80 Toronto residents. Most of these Collingwood (84%) and Toronto (84%) residents were women undergoing prenatal screening. Analysis of the sera showed that a higher fraction of Collingwood sera had a detectable serological response to the IgG-15/17 (P < 0.002) and 27-kDa (P < 0.001) antigen groups than Toronto sera (Table 1). The intensity of IgG responses to both the 15/17-kDa
and the 27-kDa serological markers were statistically significantly elevated in sera from Collingwood versus Toronto residents ($P < 0.001$) (Table 1). The intensity of IgG serological responses to the two Cryptosporidium antigen groups are displayed for two 8-week periods (1 January to 26 February and 27 February to 21 April) and two 4-week periods (22 April to 19 May and 20 May to 17 June) (Table 2). The first 8-week period includes sera collected prior to the epidemic while the second 8-week period includes sera collected at the time of the epidemic and 8 weeks following the initial case reports. The combined response of the Toronto individuals is presented for comparison. Serological response to the 15/17-kDa marker was more intense for sera collected during the 8-week period that included and followed the epidemic than for sera collected during all other periods ($P < 0.02$). Similarly, the intensity of the 27-kDa marker was higher during this same time period ($P < 0.08$). The intensity of response to each antigen group was lowest during the initial period, peaked following the epidemic, and declined thereafter (Table 2). However, the intensities of neither marker declined to levels of the Toronto sera.

**Table 1 Serological responses by city**

<table>
<thead>
<tr>
<th>Marker</th>
<th>Collingwood</th>
<th>Toronto</th>
<th>$P$-value</th>
</tr>
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<tbody>
<tr>
<td>Fraction with a detectable IgG serological response</td>
<td></td>
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</tr>
<tr>
<td>15/17-kDa</td>
<td>69%</td>
<td>45%</td>
<td>0.002</td>
</tr>
<tr>
<td>27-kDa</td>
<td>88%</td>
<td>45%</td>
<td>0.001</td>
</tr>
<tr>
<td>Mean IgG serological response (mean % of a positive control)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15/17-kDa</td>
<td>42.9%</td>
<td>15.2%</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>27-kDa</td>
<td>46.9%</td>
<td>22.9%</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

$^a$ Detectable defined as >5% of the positive control value.
$^b$ 89 Collingwood sera analysed.
$^c$ 80 Toronto sera analysed.

**Table 2 Mean serological response by time of sera collection. Collingwood and Toronto residents**

<table>
<thead>
<tr>
<th>Time</th>
<th>Collingwood</th>
<th>Toronto</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgG 15/17-kDa (N)</td>
<td>IgG 15/17-kDa Mean (SE)</td>
</tr>
<tr>
<td>1 January–25 February</td>
<td>(12)</td>
<td>26.4% (13.46)</td>
</tr>
<tr>
<td>26 February–21 April</td>
<td>(37)</td>
<td>57.8% (8.80)</td>
</tr>
<tr>
<td>22 April–19 May</td>
<td>(19)</td>
<td>39.9% (9.49)</td>
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<tr>
<td>20 May–17 June</td>
<td>(21)</td>
<td>30.5% (6.65)</td>
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**Discussion**

Few studies have evaluated serological responses to epidemics among people not specifically diagnosed with cryptosporidiosis.\textsuperscript{14,16} This study found statistically significant elevations in both the frequency and the intensity of serological responses in Collingwood compared to Toronto residents. The study also found that the intensity of serological responses to both IgG markers was elevated for the 8-week period including and following the initial reported cases compared to the periods prior to the epidemic or following this 8-week period.

**Figure 1** Laboratory-confirmed non-institutional cryptosporidiosis cases in the Collingwood outbreak
The sera used in this study were collected from Collingwood area adult residents without a diagnosis of cryptosporidiosis. Since 16% of non-nursing home laboratory confirmed cryptosporidiosis cases were adult Collingwood residents, one might conclude that few adult Collingwood residents were infected. The finding of an increased intensity of serological responses during the 8 weeks following the initial case reports among Collingwood adult residents who submitted sera for routine medical tests suggests an increased occurrence of infection throughout the community. If so, the low number of confirmed cryptosporidiosis cases among Collingwood residents likely resulted from a reduced risk of illness rather than from a reduced risk of infection.

In a prior study, Moss et al. examined persons with confirmed *Cryptosporidium* infections and showed increases in the intensity of IgG responses to the 15/17-kDa and 27-kDa oocyst antigen groups. People experimentally infected with *Cryptosporidium* showed a peak serological response to the 15/17-kDa and 27-kDa antigen groups at 32 days post-exposure or approximately 4 weeks post-illness. Protection from illness but not infection was also observed among those individuals experimentally infected who had prior serological responses to 15/17-kDa or 27-kDa antigen groups.

Since prior infection may provide some immunity to illness, the occurrence of over half of the laboratory-confirmed cases in individuals under the age 10 is possibly related to their immune status. If endemic occurrence of *Cryptosporidium* infections were common in Collingwood prior to the epidemic, the reduced rate of illness among Collingwood area adult residents at the time of this epidemic might be expected. A reduced rate of illness was also observed among residents of Talent, Oregon during a waterborne cryptosporidiosis epidemic in which the Talent drinking water supply was implicated as the source of infection. Evidence of increased serological responses to *Cryptosporidium* antigen groups among Talent residents was still apparent 3 years after the Talent epidemic. Although analysis of the 12 Collingwood sera samples collected prior to the epidemic suggests that the serological responses of adult residents prior to the epidemic were elevated compared to Toronto residents, the differences were not statistically significant (P > 0.20).

The role of cross-reactions in either initiating or continuing a serological response to *Cryptosporidium* oocyst antigen has been suggested as an important consideration and may explain the extended duration of serological response to certain antigen groups. However, cross-reactions are unlikely to explain the more intense serological responses to two antigen groups following an epidemic as observed in this study. Additional work is needed to track temporal trends in serological responses before, during and after a cryptosporidiosis epidemic and to compare changes in serological responses between persons who became ill and those who did not. Since some evidence of prior infection is commonly found in many populations, changes in the mean serological responses over time, rather than the presence or absence of the markers, might provide a convenient means to monitor the time course of the epidemic and the populations affected.

One of the unfortunate limitations of evaluating non-standard approaches to epidemic investigations is the limited availability of biological specimens collected at the time of the epidemic. This study was made possible by delays in the Ontario Ministry of Health discarding routinely processed sera. The sample size was limited by the availability of routinely collected sera from this relatively small town. Unfortunately, no sera were collected from nursing home residents or other residents at or near the time of the epidemic. The sera available from the Ontario Ministry of Health may not represent the serological responses of all Collingwood residents, since of these a large fraction of sera were from women undergoing prenatal screening. Since the fraction of sera from women undergoing prenatal screening was comparable between Collingwood and Toronto, there is no reason to believe that characteristics of the population tested biased the comparisons.

It is likely that there are significant differences in the baseline serological responses between communities. Differences between communities in the seroprevalence to *Cryptosporidium* antigens may result from differences in the quality of drinking water, source of food or from local differences in the occurrence of animal-to-human or person-to-person transmission of the parasite. For this reason, baseline sera should be collected, if possible, from each community involved in epidemic. Alternatively, since serological responses to *Cryptosporidium* decline over time, paired sera collected at the time of the epidemic, one or 2 months after the epidemic and 6 months later can provide useful information on the extent of infections during the epidemic by examining declines in serological response over time. Sera should be considered as a routine part of future *Cryptosporidium* epidemic investigations.

References
