Enzyme Immunoassay Detection of Antigen-Specific Immunoglobulin G Antibodies in Longitudinal Serum Samples from Patients with Cryptosporidiosis

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Cryptosporidium parvum is a protozoan parasite that causes diarrheal illness in a wide range of mammalian hosts, including humans. Characteristic serum immunoglobulin G (IgG) antibody responses to antigens in the 27- and 17-kDa size ranges have been shown to develop after infection, and several enzyme-linked immunosorbent assay (ELISA) and Western blot assay formats have been used to measure these IgG levels in human serum. Using a collection of serial samples from laboratory-confirmed cryptosporidiosis patients, we compared the results obtained by using two new ELISAs with those obtained with two different Western blot assays. When assayed with the large-format Western blot, 97% of the 67 patients had a demonstrable antibody response on at least one occasion. The Cp23 ELISA correctly identified 93% of the samples that had a 27-kDa response by Western blot and 100% of the negative samples. The Triton antigen ELISA detected 77% of the samples that had a 17-kDa response by Western blot and 88% of the negative samples. The sensitivity of the Triton antigen assay was higher for samples collected between 16 and 92 days after the onset of symptoms (96%). The minigel-format Western blot did not compare favorably with the large-format blot for the detection of antibodies to the 27-kDa antigen (71% sensitivity). A half-life of about 12 weeks was estimated for antibodies to both the 27- and 17-kDa antigens. We believe the Cp23 and Triton antigen ELISAs will be useful in epidemiologic studies of the prevalence of Cryptosporidium infection in the population.

Cryptosporidium parvum, a protozoan parasite that invades the intestinal epithelium of a wide range of mammalian hosts, causes a self-limiting but sometimes severe diarrheal illness in immunocompetent humans (2). However, in those with compromised immune systems, the disease can be debilitating, chronic, and life threatening (4, 23). Because of its ubiquitous distribution in the environment, its small size, and its resistance to standard chlorination techniques, C. parvum contamination of drinking water may pose a significant public health risk (1, 11, 12). Numerous outbreaks have been traced to contaminated water, and waterborne outbreaks have even occurred in communities served by state-of-the-art water treatment facilities (3, 8, 9, 15). To conduct epidemiologic studies to assess the risks of C. parvum infection that may be associated with drinking water and other potential sources of exposure, new serologic assays that are rapid, sensitive, and specific are needed.

Characteristic serum immunoglobulin G (IgG) antibody responses have been shown to develop in humans after C. parvum infection. As shown by Western blot analysis of serum samples collected from outbreak patients and human volunteers, the antibody response is consistently directed toward two low-molecular-weight antigen families: one in the 27-kDa size range and a second in the 17-kDa size range (16–19, 25, 26).

We recently reported the development of two enzyme-linked immunosorbent assays (ELISAs) for the detection of antibodies to the 17- and 27-kDa antigens (25). The ELISA for antibodies to the 27-kDa antigen uses a recombinant form of the antigen based on the gene sequence reported by Perryman et al. (22), while the assay for antibodies to the 17-kDa antigen uses a Triton X-114 detergent extract of oocysts that is enriched for the native 17-kDa antigen. These assays were shown to be both sensitive and specific for the detection of antigen-specific antibodies compared with the “gold standard” Western blot assay (25). Unfortunately, in our preliminary analysis of the new ELISAs, relatively few serial samples from confirmed cryptosporidiosis patients were available for study.

In the late spring and summer of 1996, four major outbreaks of cryptosporidiosis occurred in the province of British Columbia in western Canada: 29 laboratory-confirmed cases were identified in the city of Cranbrook (population 18,131) in the East Kootenay region of southeastern British Columbia; 157 laboratory-confirmed cases were identified in the city of Kelowna (population 89,442) in the Central Okanagan region of central British Columbia; 86 laboratory-confirmed cases were identified in Kamloops (population 100,850); and 138 laboratory-confirmed cases were identified in Penticton (population 39,754), also in the Central Okanagan region (21). In three of these communities (Cranbrook, Kelowna, and Penticton), the outbreaks were associated with consumption of water from the municipal supply. From these outbreaks, 67 volunteers with laboratory-confirmed cryptosporidiosis were recruited and were asked to provide multiple serum specimens.
over the course of a 1.5-year study. In this work, we examine the IgG antibody responses to the 17- and 27-kDa \textit{C. parvum} antigens that are found in these serial specimen, and we compare the results obtained with the new ELISAs to those obtained with two different Western blot assay formats.

**MATERIALS AND METHODS**

**Serum specimens.** Serum specimens were collected from 67 adults with laboratory-confirmed cryptosporidiosis who were infected during waterborne outbreaks in British Columbia and Atlanta in the summer of 1996. All patients also met a case definition for cryptosporidiosis based on the occurrence of three or more loose or watery bowel movements within a 24-h period. Informed consent was obtained from volunteers by procedures reviewed and approved by the Clinical Screening Committee for Research Involving Human Subjects at the University of British Columbia. Single specimens were obtained from 26 patients, and sequential specimens (207 sera) were collected from 41 patients at approximately 3-month intervals. The elapsed time between the date of symptom onset and the date of serum collection was recorded for each of the samples collected from the 41 repeat donors and for two of the samples collected from those who donated only once. Five of the individuals who provided multiple serum samples donated their first sample within 15 days of symptom onset. All sera were aliquoted and stored at −20°C until tested. This study was carried out retrospectively on serum specimens that were collected without personal identifiers.

**Crude antigen preparation and Western blots.** A preparation of crude antigen was made from oocysts of the Iowa strain of \textit{C. parvum} by sonication and freeze-thawing as previously described (20). By using the discontinuous buffer system of Laemmli (13), proteins from this crude preparation were resolved by the method of Frost et al. (5, 7). The proteins were then elec-

**Statistical analysis.** The sensitivities and specificities of the ELISAs were calculated from a relative operating characteristic curve by using the consensus Western blot result as the gold standard. Spearman rank order correlation coefficients for the comparison of assay results were calculated with SigmaStat for Windows, version 2.03 (SPSS, Inc.). Antibody half-lives were calculated by probit analysis of the cumulative percent decrease in antibody levels when the number of nonzero responses was sufficient to produce 95% fiducial limits. When samples were limited to two nonzero decreasing antibody responses, the half-life was calculated by interpolating the number of days after onset at which response decreased by 50%. Antibody responses among the different ELISAs during specified intervals after the onset of the infection were analyzed using a log-linear model with a 10-day lag period. The data were analyzed using the Proc GLIMMIX procedure in SAS version 8.2 (SAS Institute, Cary, N.C.).

**RESULTS**

*Western blot analysis.* Sera from 67 laboratory-confirmed cryptosporidiosis patients were assayed for antibodies to the 27- and 17-kDa sporozoite surface antigens with the large-
format Western blot, and a consensus interpretation of the blot results was reached by the Atlanta laboratory group. Of the 26 patients who donated only a single serum specimen, 24 (92%) were positive for antibodies against both antigens by large-format Western blot (data not shown). One of the two patients who lacked antibodies had a serum collection date less than 15 days after symptom onset and therefore may not have had sufficient time to develop an antibody response. The symptom onset date for the other negative patient was not known. Of the 41 patients who donated more than one serum specimen, all were positive for antibodies to one or both antigens at some point during the first 183 days of the study. As represented by the large-format Western blots shown in Fig. 1, the 41 patients who donated more than one serum specimen could be grouped into five general categories. Figure 1A (patient 1) is representative of the 21 patients (51% of those who donated more than one specimen) who were positive for antibodies to both the 27- and 17-kDa antigens at each time point. A gradual decline over time in the total antibody response was apparent from the intensities of the bands on the blots. Of the five patients (12%) who donated their first serum sample ≥15 days after symptom onset, all were initially positive for antibodies to the 27-kDa antigen, but negative for antibodies to the 17-kDa antigen, and all had a detectable peak antibody response to the 17-kDa antigen by the second time point (3 to 4 months later) (represented by patient 2 in Fig. 1B). Ten patients (24%) represented by patient 3 in Fig. 1C were initially positive for antibodies to both antigens, but later became negative for one of the antibodies. Three patients (7%) who had an antibody response to at least one of the antigens later became completely negative (represented by patient 4 in Fig. 1D). Finally, Fig. 1E (patient 5) is representative of two patients (5%) who were positive for antibodies to the 27-kDa antigen at every time point, but who did not have a detectable response to the 17-kDa antigen in any of the specimens tested (average number of tested specimens per patient, 6). No obvious changes were noted in the
intensities of the blot responses to the 27-kDa antigen for these two patients. In summary, changes in the blot responses that were consistent with recent exposure to *C. parvum* were observed for 39 of the 41 patients (95%) who donated multiple serum samples.

When the sera from the outbreak patients were assayed with the minigel Western blot format, we observed that some of the samples previously determined to be positive for antibodies to the 27-kDa antigen when the large-format Western blot was used had responses that appeared to be negative or were very weak and difficult to interpret. The minigel blot results for patients 1 through 3, who were shown in Fig. 1 to have strong antibody responses to the 27-kDa antigen, are presented in Fig. 2. While the 27-kDa antigen responses in samples from patient 1 were clearly positive by minigel blot (Fig. 2A), samples 1 and 7 from patient 2 (Fig. 2B) and samples 2 to 6 from patient 3 (Fig. 2C) were interpreted as negative by both laboratories. This apparent lack of sensitivity was not limited to these two patients: 22 patients had at least one sample in which this discrepancy was observed. Overall, of the 209 samples that were interpreted by both laboratories as positive for antibodies to the 27-kDa antigen by using the large gel blot, 61 were considered negative by both laboratories with the minigel blot. In one of the sets of assays conducted in the Atlanta laboratory, the samples were placed in random wells on the ELISA plate so as eliminate the possibility of positional bias. The mean ELISA responses for the patients whose blot responses were used as the gold standard for comparisons with the ELISA results presented below.

**ELISA results.** To assess the inter- and intralaboratory variability in the assays, the levels of antibodies to the 27- and 17-kDa antigens were analyzed independently by ELISA once in the British Columbia laboratory and twice in the Atlanta laboratory. In one of the sets of assays conducted in the Atlanta laboratory, the samples were placed in random wells on the ELISA plate so as eliminate the possibility of positional bias. The mean ELISA responses for the patients whose blot responses were shown previously in Fig. 1B and C are shown in Fig. 3A and B, respectively. As expected from the positive
correlation coefficients were 0.978 and 0.913, respectively, and the interlaboratory Cp23 and Triton antigen ELISAs calculated for all 233 samples each other: the intralaboratory correlation coefficients for the all of the patient samples were also in good agreement with the blot results. The results of the three sets of assays for sample by Triton antigen assay (solid line), in good agreement with the 27-kDa antigen blot responses in Fig. 1B, the mean ELISA responses for patient 3 (blot responses shown in 17-kDa antigen, and they were followed in sequence by a true positive to negative by Western blot for antibodies to the 10 patients who, by overall blot consensus, converted from false-negative specimens (14 of 40; 35%) were donated by the set from British Columbia did not reveal a significant difference. The specimen type and the set from Atlanta and the set from British Columbia did not reveal a significant difference. The true-negative sample. Thus the low sensitivity may be attributed to a response that rapidly approached the limit of detection for the assay. The low specificity (87%) may be attributed to the fact that six of the seven false-positive serum specimens were donated by a single patient.

As can be seen in the graphs in Fig. 3, the antibody responses to the Cp23 and Triton antigens observed in patients 2 and 3 tended to rise and decay in parallel, and much of the response was lost within the first year after symptom onset. The same downward trends that were observed in the individual patient responses were also apparent when the responses from the 41 patients who donated multiple samples were plotted versus the time after symptom onset. Figure 4A shows the number of samples collected in each 3-month interval after symptom onset. The five samples that were collected within 15 days of symptom onset were grouped separately because they were collected before these patients had developed an antibody response to the 17-kDa antigen. As shown in Fig. 4B and C, when the geometric means for the Triton antigen and Cp23 ELISA responses were calculated for each time interval after symptom onset, a steady decline after the 16- to 92-day interval was shown. Figure 4B and C also give a visual indication of the low levels of intra- and interlaboratory variation that were observed among the three independent sets of assays. Although the repeat set of assays performed in Atlanta yielded a higher geometric mean value for six of nine time intervals for the Triton antigen assay (open circles, Fig. 4B), a statistical comparison by time interval of the two sets of responses from Atlanta and the set from British Columbia did not reveal a significant difference. The P values for the Triton antigen ELISA response comparisons were greater than 0.09 for each of the sample collection intervals (Kruskal-Wallis test with chi-square approximation), and the P values for the Cp23 ELISA response comparisons were greater than 0.53 for each of the sample collection intervals.

The prevalence of the samples that were positive by Triton antigen ELISA and Cp23 ELISA versus the time interval of sample collection is shown in Fig. 4D. The percentage of the patients who were positive by the Triton antigen ELISA demonstrated a downward trend consistent with the conversion of some patients from positive to negative for antibodies to the

### 27-kDa antigen blot response

27-kDa antigen ELISA detection of 27-kDa antigen.

### 17-kDa antigen

17-kDa antigen 24 (92) 24 23' (96) 1' 2 2' (100) 0'

### Sequential (n = 207: 41 patients)

17-kDa antigen 38 (93) 151 111' (74) 40' 56 49' (87) 7'

### Percentage of Western blot-positive samples correctly identified by consensus ELISA result.

| Specimen type | No. (%) of positive patients | True positive | False negative | No. (%) of blot-negative samples | True negative | False positive |
|---------------|------------------------------|---------------|----------------|--------------------------------|---------------|---------------|
| Single (n = 26) | 17-kDa antigen | 24 (92)     | 24             | 23' (96)            | 1'             | 2             | 2' (100)      | 0'             |
|                | 27-kDa antigen | 24 (92)    | 24             | 24' (100)          | 0'             | 2            | 2' (100)      | 0'             |

| Sequential (n = 207: 41 patients) | 17-kDa antigen | 40 (98) | 186 | 172' (92) | 14' | 21 | 22' (100) | 0' |

| 27-kDa antigen | 38 (93) | 151 | 111' (74) | 40' | 56 | 49' (87) | 7' |

| No. (%) of ELISA samples | True positive | False negative |
|---------------------------|---------------|----------------|
| 24 (92)                   | 24            | 24' (100)      |
| 38 (93)                   | 151           | 111' (74)      |

| No. (%) of blot-negative samples | True negative | False positive |
|----------------------------------|---------------|---------------|
| 24 (92)                          | 24' (100)     | 0'            |
| 38 (93)                          | 151           | 111' (74)     |

* Number of patients positive for the given antigen by consensus Western blot result, with the percentage of positive patients in parentheses.

* Consensus of large- and minigel-format Western blot responses.

* Percentage of Western blot-positive samples correctly identified by consensus ELISA result.

* Percentage of Western blot-negative samples correctly identified by consensus ELISA result.

* Triton antigen ELISA detection of 17-kDa antigen.

* Cp23 ELISA detection of 27-kDa antigen.

### Table 1. Agreement between consensus ELISA and Western blot results for single and sequential specimens

| Specimen type | No. (%) of positive patients | True positive | False negative | No. (%) of blot-negative samples | True negative | False positive |
|---------------|------------------------------|---------------|----------------|--------------------------------|---------------|---------------|
| Single (n = 26) | 17-kDa antigen | 24 (92)     | 23' (96)    | 24' (100)          | 2             | 2' (100)      | 0'             |
|                | 27-kDa antigen | 24 (92)    | 24' (100)   | 24' (100)         | 2             | 2' (100)      | 0'             |

| Sequential (n = 207: 41 patients) | 17-kDa antigen | 40 (98) | 186 | 172' (92) | 14' | 21 | 22' (100) | 0' |

| 27-kDa antigen | 38 (93) | 151 | 111' (74) | 40' | 56 | 49' (87) | 7' |

| No. (%) of ELISA samples | True positive | False negative |
|---------------------------|---------------|----------------|
| 24 (92)                   | 24            | 24' (100)      |
| 38 (93)                   | 151           | 111' (74)      |

| No. (%) of blot-negative samples | True negative | False positive |
|----------------------------------|---------------|---------------|
| 24 (92)                          | 24' (100)     | 0'            |
| 38 (93)                          | 151           | 111' (74)     |

* Percentage of Western blot-positive samples correctly identified by consensus ELISA result.

* Percentage of Western blot-negative samples correctly identified by consensus ELISA result.

* Triton antigen ELISA detection of 17-kDa antigen.

* Cp23 ELISA detection of 27-kDa antigen.
17-kDa antigen by Western blot, while the percentage of patients who were positive by the Cp23 ELISA was relatively constant, as expected from the Western blot results for the 27-kDa antigen described earlier.

**Antibody half-life determination.** A sufficient number of nonzero Cp23 ELISA responses were available from 25 patients to allow the calculation of the antibody half-lives by probit analysis. A mean half-life of 87 days (median, 72 days; range, 50 to 181 days) was obtained for Cp23 reactivity. From the responses of 15 patients, a mean half-life of 84 days (median, 69 days; range, 47 to 215 days) was estimated for the Triton antigen response. The half-lives for the two antibody responses were not significantly different ($P = 0.514$). For those patients’ responses that could not be analyzed by the probit procedure, a half-life estimate was calculated assuming a linear rate of decay. These estimates (mean of 83 days for the Cp23 assay with 10 patients; mean of 68 days for the Triton antigen assay with 17 patients) did not differ significantly from those described above.

**DISCUSSION**

Several different assay formats have been used to assess the levels of human serum IgG antibodies to *C. parvum* antigens: an ELISA that uses a crude antigen preparation (29), a large-gel-format Western blot that requires the separation of parasite antigens on a gradient SDS-polyacrylamide gel (6, 17–19, 25), a minigel-format Western blot (5, 7, 10), and ELISAs that use purified recombinant or native antigens (25). A number of laboratories have noted that the results of the large-gel-format Western blot and those of the crude antigen ELISA were not well correlated (6,18, 19, 25; F. J. Frost and G. F. Craun, Letter, Infect. Immun. 66:4008–4009, 1998). In general, the large-gel-format Western blot appeared to be more sensitive than the crude antigen ELISA for the detection of IgG antibodies: more than half of the crude antigen ELISA-negative individuals in the study of Frost et al. (5; Frost and Craun, Letter) were found to be antibody positive by the large-gel-format Western blot. Unfortunately, because of its complexity and expense, the large-gel-format Western blot is not suitable for use in large-scale epidemiologic studies of the prevalence of *C. parvum* antibodies in the general population. To accomplish this type of work in a more reagent- and cost-efficient manner, several groups have turned to the minigel-format Western blot. While the minigel assay compared favorably in our hands with the large-gel-format blot for the detection of antibodies to the 17-kDa antigen, it failed to detect 29% of the samples that were positive for antibodies to the 27-kDa antigen. Recent studies have suggested that roughly one-third to one-half of those individuals who are positive for antibodies by the large-gel Western blot assay only have an antibody response to the 27-kDa antigen (6, 25). Thus the minigel-format blot (at least in the format currently used) may significantly underestimate the proportion of the population with prior *Cryptosporidium parvum* exposure. No data yet exist on whether the minigel assay would perform better if more antigen was used.

Using a large collection of serial samples from laboratory-confirmed cryptosporidiosis patients, we have demonstrated that the recombinant Cp23 and Triton antigen ELISAs can be used to monitor changes in the antibody responses to two specific *C. parvum* surface antigens. The high sensitivities and specificities of the Cp23 and Triton antigen ELISAs in the analysis of the single specimens (>96%) and those of the Cp23 ELISA in the analysis of the sequential specimens (>93%) are similar to what we have previously reported (25), but the Triton antigen results are somewhat lower for the sequential specimens than previously reported (74 and 87% versus 90 and 94%, respectively). The sensitivity of the Triton antigen assay was higher for samples collected between 16 and 92 days of symptom onset (96%) than in subsequent samples, and many of the false-negative samples were donated by patients who eventually converted to negative for antibodies to the 17-kDa antigen by Western blot. We think the lower sensitivity is simply an artifact caused by the repeat sampling of patients who have borderline-positive Western blot responses in samples collected many months after the clearance of the infec-
tion. Similarly, the lower specificity of the assay can be attributed to one patient who donated six of seven false-positive specimens. Interestingly, this patient had a very high level of antibodies to the 27-kDa antigen, and a small amount of 27-kDa antigen is normally found in the Triton extract used in the assay (the amount of 27-kDa antigen is 10- to 20-fold lower than that of the 17-kDa antigen) (25). When these sera were checked by Western blotting with Triton-extracted antigen, only the 27-kDa antigen was apparent (data not shown). Thus, it is possible that the Triton antigen ELISA is not absolutely specific for antibodies to the 17-kDa antigen when high concentrations of antibodies to the 27-kDa antigen are present. In practice, however, very few patients have been found with a high 27-kDa-antigen response and no 17-kDa-antigen response.

We noted that the antibody response to both antigens increased and decreased in parallel in a time frame consistent with *C. parvum* infection near the time of symptom onset. The peaks of the responses most likely occurred between 16 and 92 days of symptom onset, but, because of the 3-month interval between sample collections, they could not be further pinpointed. In a recent study in which human volunteers were fed *C. parvum* oocysts, the peak of the response most likely occurred between 11 and 32 days after ingestion of oocysts (19). We conclude that it is highly unlikely that the antibody responses observed in our study could have resulted from cross-

FIG. 4. ELISA analysis of sera from cryptosporidiosis patients who donated sera on more than one occasion. Panel A shows the total number of serum samples collected during each time interval after symptom onset from 41 confirmed cryptosporidiosis patients who donated more than one specimen. Triton antigen (B) and Cp23 (C) ELISAs were performed with these serum samples on two occasions in Atlanta to assess the intralaboratory variation (Atlanta, random and Atlanta, repeat) and on one occasion in British Columbia to assess the interlaboratory variation (British Columbia). For the assays represented by the “Atlanta, random” results, the duplicate serum samples were placed in random locations on the ELISA plate to eliminate the possibility of positional bias. The geometric means (log scale) of the ELISA responses are plotted for each interval of sample collection, and the threshold for positivity is indicated in each graph by a dotted line. Panel D shows the fraction of the samples from each time interval positive for antibodies in the Triton antigen (black bars) and Cp23 (grey bars) ELISAs. A serum sample was considered ELISA positive if at least two of the responses from the three independent assays were above 35 AU for the Triton antigen assay and above 86 AU for the Cp23 assay.
reaction with antibodies from a concurrent or previous infection with another organism. This conclusion is further supported by the observation that most of the antibody response to these two antigens is directed against the protein component of the antigen rather than against a carbohydrate component (24, 25; J. W. Priest and P. J. Lammie, unpublished observations). Robbins et al. (27) have suggested that serum antibodies to the carbohydrate epitopes of surface antigens, especially those whose acquisition is age-related, may result from exposure to cross-reacting species found on normal enteric and respiratory flora, while serum antibodies that recognize surface protein epitopes are most often elicited by infection with the specific pathogen. We believe that the Cp23 and Triton antigen ELISAs are specific for Cryptosporidium infection in humans, and despite our efforts and those of other laboratories, no evidence of cross-reaction has been found to implicate other human parasites, including Toxoplasma gondii, Giardia lamblia, and Isospora sp. (14, 29; Priest and Lammie, unpublished).

Our results indicate that antibodies against both the 17- and 27-kDa antigens are cleared at about the same rate, with half-lives of approximately 12 weeks each, and that both antibody responses reach a fairly constant level about 1 year after the infection. This working estimate will be important for future studies of the prevalence of cryptosporidiosis in the population. To our knowledge, only one other group has attempted to establish an estimate of the duration of the human serum antibody response following Cryptosporidium infection. In a recent work, Frost et al. (5) collected paired serum samples from cryptosporidiosis patients from a waterborne outbreak in Jackson County, Oreg., at 6 months and then again at 2.5 years after the end of the outbreak and analyzed these samples by using the large-gel- and minigel-format Western blot assays. They observed that the antibody response to the 27-kDa antigen declined by 46% (as measured from the blots by densitometry) over this 2-year period, while the response to the 17-kDa antigens declined by only 9%. Their conclusions that the response to the 17-kDa antigen may have declined to baseline levels before the beginning of the serum collection and that the antibody response to the 27-kDa antigen may remain high for an extended period of time appear to be supported by our results. Indeed, had sera been collected from the patients in our study 6 months after symptom onset (184 to 274 days) and again 1 year later (548 to 638 days) (Fig. 4), a pattern similar to that reported by Frost et al. would likely have been observed, since the largest fluctuations in the antibody responses appear to occur within the first 6 months after symptom onset.

Our work supports the suggestion that the postinfection level of the antibody response is higher (relative to the threshold of antibody detection) for the 27-kDa antigen than for the 17-kDa antigen: eight patients with a persistent 27-kDa antigen response became negative by overall blot consensus for antibodies to the 17-kDa antigen, while only one patient with a persistent 17-kDa antigen response became negative for antibodies to the 27-kDa antigen over the course of the study. The geometric means of the responses to the 27-kDa antigen in the various time intervals of our study were consistently above the cutoff threshold, but this was not the case for the responses to the 17-kDa antigen (Fig. 4). We also observed that the five patients who provided a serum sample <15 days after symptom onset either had a very early 27-kDa antigen response or had a preexisting 27-kDa antigen response from a previous infection, since no response to the 17-kDa antigen was apparent until the following time point. In our studies of sera collected from individuals with no known previous exposure to Cryptosporidium, we have, in fact, observed that a significant proportion of the population has an antibody response only to the 27-kDa antigen (25; Priest and Lammie, unpublished). We do not yet understand why the response to the 27-kDa antigen should persist, but it may in some way be related to repeated exposure to the parasite.

In conclusion, we have demonstrated that the Triton antigen and Cp23 ELISAs are useful for monitoring the changes in antibody responses associated with infection with C. parvum and that this technology can be transferred to other laboratories. Given that antibodies to the 27-kDa antigen may persist above the limit of detection, we believe that the Cp23 ELISA may be better suited to the assessment of historic exposure to Cryptosporidium, while the Triton antigen ELISA may be a better choice for the detection of recent infections. For both assays, a high antibody response is likely to be indicative of a recent infection.

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