# Serological responses to *Cryptosporidium* antigens among users of surface- *vs*. ground-water sources

# F. J. FROST<sup>1\*</sup>, T. R. KUNDE<sup>1</sup>, T. B. MULLER<sup>1</sup>, G. F. CRAUN<sup>2</sup>, L. M. KATZ<sup>4</sup>, A. J. HIBBARD<sup>5</sup> and R. L. CALDERON<sup>3</sup>

<sup>1</sup> Lovelace Clinic Foundation, 2425 Ridgecrest Drive S.E., Albuquerque, NM 87108, USA

<sup>2</sup> Gunther F. Craun and Associates, 101 West Frederick Street, Suite 205, Staunton, VA 24401, USA

<sup>4</sup> Mississippi Valley Regional Blood Centre, 3425 East Locust St, Davenport, IA, USA

<sup>5</sup> American Red Cross, 4860 Sheboygan Ave, Madison, WI 53705, USA

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# SUMMARY

*Cryptosporidium* oocysts are commonly detected in surface-derived drinking water. However, the public health significance of these findings is unclear. This study compared serological responses to two *Cryptosporidium* antigen groups for blood donors and college students using chlorinated and filtered river water *vs.* ground-water sources. The surface water received agricultural and domestic sewage discharges upstream. Participants from the surface-water city had a higher relative prevalence (RP) of a serological response to the 15/17-kDa antigen group (72·3 *vs.* 52·4%, RP=1·36, P < 0.001) and to the 27-kDa antigen group (82·6 *vs.* 72·5%, RP=1·14, P < 0.02). Multivariate logistic regression analysis found that the people with a shorter duration of residence or drinking bottled water also had a lower seropositivity for each marker. Use of private wells was associated with a higher prevalence of response to the 15/17-kDa markers. Seroconversion to the 15/17-kDa antigen group was more common in the residents of the city using surface water. These findings are consistent with an increased risk of *Cryptosporidium* infection for users of surface-derived drinking water compared with users of municipal ground-water-derived drinking water. Users of private well water may also have an increased risk of infection.

# INTRODUCTION

*Cryptosporidium* oocysts have been detected in source and treated drinking waters in the United States and elsewhere [1–3]. Enhanced enteric disease surveillance, initiated following detection of oocysts, has not often detected elevated rates of infection or of symptoms compatible with infection at the time [4–6]. Reasons for not finding elevated health risks in these exposed communities are unclear. A previous study suggested that a low rate of drinkingwater-attributable infection (e.g. 1/1000 per year) along with difficulties in diagnosing and reporting cases of cryptosporidiosis probably accounts for low rates of detectable disease [7].

It is possible that many oocysts detected in drinking water are not viable or infectious for humans. Alternatively, *Cryptosporidium* infections may not result in overt or clinically detectable illness for a substantial proportion of the population. If people are regularly exposed to drinking water with low concentrations of oocysts, the risk of symptomatic illness or the severity of illness from infection may even be reduced because of protective immunity [8]. Since surveillance systems for *Cryptosporidium* infections generally focus on the recognition of clinically

<sup>&</sup>lt;sup>8</sup> National Health and Environmental Effects Laboratory, U.S. Environmental Protection Agency, Research Triangle Park, NC 27711, USA

<sup>\*</sup> Author for correspondence.

detected disease (cryptosporidiosis), little information is available on the occurrence of unrecognized and asymptomatic infections.

Estimating the risks of infection and understanding risk factors for illness among those infected are critical to understanding the aetiology of this disease and the public health importance of various environmental factors responsible for the transmission of *Cryptosporidium*. Studies suggest that, even during cryptosporidiosis outbreaks, illnesses may occur in only a small proportion of the people infected [8–12]. Unfortunately, even less is known about the incidence of *Cryptosporidium* infection or cryptosporidiosis in non-outbreak situations.

Since Cryptosporidium infection elicits a serological response in most infected humans [9], surveys for the presence of this response can be used to estimate the prevalence of previous Cryptosporidium infection in populations [13, 14]. Other studies have tracked serological responses in people intentionally or unintentionally exposed to Cryptosporidium oocysts [8, 15, 16]. Recent studies have focused on responses to a 15/17-kDa and a 27-kDa antigen group [8-19]. Serological responses to these two markers appear to be specific for Cryptosporidium infection [9]. Infection usually elicits a serological response to these antigen groups that peaks [4–6] weeks after infection [17, 18]. The 15/17-kDa marker declines to baseline levels prior to the infection within 4-6 months following infection while the 27-kDa marker remains elevated for 6–12 months [17, 18].

A previous study found a higher prevalence of antibody responses to both antigen groups and a higher rate of seroconversion to both *Cryptosporidium* antigen groups in people who drink water derived from a surface vs. underground source [19]. Questions raised by this previous study were whether this finding applied only to one pair of cites and the characteristics of one water treatment system; and do other factors increase or reduce the risk of infection? This study replicates an earlier study, comparing the prevalence of serological responses to 15/17-kDa and 27-kDa *Cryptosporidium* antigen groups in people who use surface vs. ground water.

#### **METHODS**

#### Subject recruitment

We recruited 184 people residing in a city that uses filtered and chlorinated drinking water obtained from a river that receives animal and treated human faecal waste. We also recruited 309 people from three other cities (n = 108, 81, 183) that use ground-water sources. Participants were recruited from blood donors at volunteer blood collection facilities and a college located in one of the ground-water cities. The city of residence and source of drinking water were elicited. Each study participant signed a letter of consent and completed a questionnaire at the time of enrolment. This questionnaire enquired about demographic characteristics, such as age, gender, residence location, drinking water source (municipal surface water, municipal ground water, or private well water), family size and the number of children and young children in the household. In addition, participants were questioned about other potential exposures to Cryptosporidium, such as untreated water consumption, contact with pets, young pets, livestock, caring for persons with diarrhoea, international travel, swimming, and day care or other child exposures. The questionnaire also asked about the occurrence of diarrhoea in the last 2 months that lasted for 4 or more days. A study identification number was assigned to each participant. Thus, the participant/ sample were anonymous to the study team. Sera were collected at the time the participant completed the first questionnaire. Sera were collected from April to the end of July, 1999.

#### Follow-up sera collection

Sera were again collected at the next visit for blood donation or during a follow-up clinic for blood sampling or donation. Since the delay between blood samples differed by site, only sera drawn within 90 to 180 days of the first blood sample were considered for the seroconversion analysis.

#### Western blot procedures

Sera were analysed by immunoblot to measure IgG serological response to the 15/17- and 27-kDa antigen groups. The methods have been described elsewhere [13, 14]. The intensity of the serological responses to the 15/17- and 27-kDa antigen groups were digitally analysed by an IS-2000 Digital Imaging System (Alpha Innotech, San Leandro, CA, USA). 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. Due to limited resources, it was not possible to blind the microbiologist to the origin of the samples. However, use of the computer to measure detection and the intensity of responses minimizes the risk of introducing operator bias.

#### Statistical analysis

The IgG results for each specimen were standardized by taking the ratio of the response intensity for the unknown sample to the response intensity of a positive control serum contained on each blot. The IgGpositive control serum was obtained from individuals with a strong serological response to both antigens. The response approximated the intensity of responses observed from several individuals with laboratoryconfirmed cryptosporidiosis. The same positive control serum preparation was used for all blots. An extensive quality control effort was conducted analysing replicate samples. We have previously analysed the intensity of responses using a Tobit model. However, it is unclear how to interpret coefficients derived from the Tobit model [19]. To simplify the interpretation we, instead, conducted multiple logistic regression analysis, using four definitions of a positive response, based on the intensity of the response. The positive responses were defined as: >0%, >10%, >20% or >30% of the positive control. The prevalence risk ratio of a serological response was estimated from the relative prevalence of serological responses. Multivariate logistic regression analysis of the observed serological response intensities was conducted using SAS.

Seroconversion was defined as a change in the intensity of serological responses greater than 10% of the positive control. Changes were classified as positive if the response increased by more than 10% and as negative if the response decreased by more than 10%. If the follow-up response changed by less than 10% of the positive control, it was classified as remaining the same. Limited data from previous studies suggest that response to both markers increases rapidly following infection, peaking near 4 weeks' post-infection and declining thereafter [20]. However, population surveys suggest that rates of decline may be slower in individuals with repeated previous exposures [14].

### RESULTS

Sera were collected from 496 individuals. Ages ranged from 20 to greater than 60 years with 20% less than

age 30, 16% aged 60 years or greater and 39% aged 45–59 years. Sixty per cent were female and 98% were white. The majority (53%) had some college education and 70% were married. Sera were obtained from four sites. There were 183 participants from the surface-water city (37%) of all participants) and the other three ground-water sites contributed 120 sera (24%), 108 sera (22%) and 81 sera (17%).

The mean intensity of the serological responses was higher for residents from the surface-water city than from the three ground-water cities combined (54·1 vs. 30·3% for the 15/17-kDa antigen group, P < 0.01; and 50·5 vs. 33·2% for the 27-kDa antigen group, P < 0.01). A serological response to the 15/17kDa was detected in 72·3% (95% CI 69·9–74·7) of the people who drank surface water and in 52·4% (95% CI 49·8–55·1) of those who used ground water. The relative prevalence (RP) of a response to the 15/17-kDa antigen group was higher in users of surface-derived drinking water [RP=1.36 (1.10, 3.58), P < 0.001, Fisher's exact test].

Responses to the 27-kDa antigen were detected in 82.6% (95% CI 80.6-84.6) of people who obtained drinking water from a surface source vs. 72.5% (95%) CI 70.1–74.9) of those using ground water [RP = 1.23(1.07, 1.41), P < 0.001, Fisher's exact test]. When the surface-water community was compared to individual ground-water communities, there were differences in the prevalence of serological responses by groundwater site. One ground-water site (n = 183) had a significantly lower prevalence of serological response to both the 15/17-kDa and the 27-kDa antigen groups than the other two ground-water sites (P < 0.001). Another site (n=108) had a significantly lower prevalence of responses to the 15/17-kDa antigen group (P < 0.001) and a lower prevalence of responses to the 27-kDa antigen group, but this difference was not statistically significant (P < 0.06). No significant differences were detected in the prevalence of detectable responses for either antigen group between the surface-water community and the third well-water community (n=81) (P>0.20).

Results of a multivariate logistic regression for the relative prevalence of responses to 15/17-kDa are presented in Table 1. Results for the 27-kDa antigen group are presented in Table 2. Age, sex, family size, contact with pets, young pets, livestock, caring for persons with diarrhoea, international travel, swimming and day care and other child exposures were unrelated to serological responses. For both antigen groups, people residing in a city that obtained drinking water

Factor	Intensity of response as a per cent of the positive control					
	Any response	≥10%	≥20%	≥30%		
Male	1.4	1.3	1.2	1.2		
Age 40-49 years	1.3	1.2	1.5	1.7		
Age 50–59 years	1.4	1.3	1.4	1.5		
Age $60 + years$	1.1	1.0	0.9	1.1		
Children <5 years of age	1.1	1.0	1.1	1.2		
Reside in a city that uses surface water	2·3** (IR)	2·4** (IR)	2·2** (IR)	2·7** (IR)		
Duration of residence <2 years	0·1* (RR)	0·1* (RR)	0·1* (RR)	0·0** (RR)		
Used private well for water	1·9* (IR)	1·9** (IR)	1·7* (IR)	1·7* (IR)		
Regularly drank bottled water	0.5* (RR)	0.6* (RR)	0·5* (RR)	0·5* (RR)		
Swam in last 6 months	0.9	0.8	0.9	0.9		
Had diarrhoea in last 2 months	0.9	0.9	0.9	0.9		
Ate peel of fresh fruits/vegetables	0.8	0.9	1.0	1.1		

Table 1. Relative prevalence of serological response to the 15/17-kDaantigen group (based on a logistic regression model)

\* *P*<0.05; \*\* *P*<0.01.

IR, increased risk; RR, reduced risk.

	Intensity of response as a per cent of the positive control					
Factor	Any response	≥10%	≥20%	≥30%		
Male	1.0	1.0	1.2	1.5		
Age 40-49 years	1.5	1.5	1.3	1.2		
Age 50–59 years	1.6	1.7	1.4	1.3		
Age $60 + years$	0.8	0.9	1.0	0.8		
Children <5 years of age	2.5	1.9	2.0	1.7		
Reside in a city that uses surface water	1·7* (IR)	1·8* (IR)	2·4** (IR)	2·1** (IR)		
Duration of residence <2 years	0·3* (RR)	0·3* (RR)	0·3* (RR)	0·1* (RR)		
Used private well for water	0.8	1.0	1.1	0.9		
Regularly drank bottled water	0.8	0.6*	0.8	0.7		
Swam in last 6 months	1.4	1.3	1.1	0.9		
Had diarrhoea in last 2 months	1.4	1.4	1.1	1.1		
Ate peel of fresh fruits/vegetables	1.5	1.3	0.9	0.9		

Table 2. Relative prevalence of serological response to the 27-kDaantigen group (based on a logistic regression model)

\* *P*<0.05; \*\* *P*<0.01.

IR, increased risk; RR, reduced risk.

from a surface-water source have a higher relative prevalence of a positive serological response, regardless of whether a positive response is defined as any response or a response greater than 10, 20 or 30% of the positive control. Residing at the current address for less than 2 years and drinking bottled water were related to a lower relative prevalence for the 15/17-kDa antigen group (P < 0.05) and residing in the city for less than 2 years was related to lower prevalence of response measured as greater than 10% of the positive control for the 27-kDa antigen group. Users of private well water had a statistically significant increase in the occurrence and intensity of response to the 15/17-kDa antigen but not the 27-kDa antigen.

Among residents of the surface-water city, being married was related to the occurrence of a detectable response to the 15/17-kDa antigen group (P < 0.01, Fisher's exact test) whereas having some college education or a short duration of residence in the city was related to the absence of a serological response to the 15/17-kDa antigen group (P < 0.01, Fisher's exact test). Only some college education was related to a reduced occurrence of a response to the 27-kDa antigen group (P < 0.04, Fisher's exact test). For residents from areas other than the city that used surface-derived drinking water, changing residence was related to lower prevalence of response to the 15/17-kDa and 27-kDa antigen group.

Many factors were not associated with the occurrence of a serological response. These included age, the number of children, diarrhoea in past 2 months lasting for 4 or more days, eating the peel of fruits/ vegetables, washing fruits/vegetables, livestock, pets, small children or exposure to nappies (diapers). Swimming, consumption of untreated surface water, foreign travel and years of education were also not related to the occurrence of a serological response to either antigen group. These factors were tested using logistic regression but were not included in the models in Tables 1 and 2.

We obtained follow-up sera from 270 individuals between 90 and 180 days of the initial blood sample. There were 50 participants from the surface-water city and 220 from the ground-water cities. These data were displayed in Table 3. Significantly more participants from the surface-water city had an increased intensity of response to the 15/17-kDa marker (P < 0.05) and significantly more participants from the ground-water cities had a decreased intensity of response to the 27-kDa antigen (P < 0.05). Paired city Cryptosporidium study

Marker – change	Ground-water communities	Surface-water community
Cases	220	50
15/17 kDa	n (%)	n (%)
Change less than 10% negative	74 (34)	9 (18)
Change $-10\%$ to $+10\%$	102 (46)	20 (40)
More than 10% positive	44 (20)	21 (42)
P value – any change	<0.01	
P value – decreased intensity	0.26	
P value – increased intensity	0.012	
27-kDa	n (%)	n (%)
More than 10% negative	94 (43)	13 (26)
Change $-10\%$ to $+10\%$	79 (36)	23 (46)
More than 10% positive	47 (21)	14 (28)
<i>P</i> value – any change	0.09	
P value – decreased intensity	0.047	
P value – increased intensity	0.95	

For comparison to previous studies, the per cent of responses by the intensity of the response is provided in Table 4 for the 15/17-kDa antigen group and in Table 5 for the 27-kDa antigen group. In this study, 50% of participants using surface-derived drinking water had a response to the 15/17-kDa marker of 30% or more of the positive control. Other surface-water sites had 19–59% of participants with responses as intense (Table 4). For the 27-kDa antigen group, 64% of participants had a response of 30% or more of the positive control compared to 15–51% for other surface-water sites.

# DISCUSSION

Findings from this study agree with those of two previous studies that compared serological responses of people who live in cities using surface-derived drinking water vs. people living in cities using ground water [19, 20]. In each study, residents of a city using surface-derived drinking water were more likely to have serological evidence of previous *Cryptosporidium* infection. In the current study, the higher

	Intensity of response as a per cent of the positive control				
Site	No. of samples	>0%	≥10%	≥20%	≥30%
Surface United States					
NHANES – SW1 [21]	107	50.5	40.2	28.0	18.7
NHANES – SW2 [21]	502	45.2	41.4	30.7	25.3
NHANES – SW3 [21]	186	72.6	71.1	64.0	58.6
Las Vegas [20]	201	49.8	46.8	41.8	36.3
Medford [13]	504	68·3	57.9	46.4	36.7
This study – surface	184	72.3	71.2	63.6	58.2
Australia – surface water					
Sydney [6]	104	56.7	50.1	36.5	30.8
Melbourne [6]	104	61.5	46.2	38.5	34.6
AIDS cases [23]	298	53.0	49.0	36.2	28.2
Other – surface water					
Russia [22]	108	67.6	67.6	61.1	50.0
Italy [21]	150	84·0	76.0	66.0	56.0
Ground water					
This study – ground water	309	52.4	50.8	43.4	32.4
Albuquerque [20]	201	36.3	34.3	25.3	18.9
NHANES – GW1 [21]	51	47.1	25.5	15.7	13.7
NHANES – GW2 [21]	503	26.0	24.1	17.1	12.5
NHANES – GW3 [21]	120	70.0	68.3	58.3	43.3
NHANES – GW4 [21]	120	39.2	38.3	34.2	24.2

Table 4. Serological responses to 15/17-kDa antigen

Table 5. Serological responses to 27-kDa antigen

	Intensity of response as a per cent of the positive control				
Site	No. of samples	>0%	≥10%	≥20%	≥30%
Surface United States					
NHANES – SW1 [21]	107	49.5	37.4	30.8	25.2
NHANES – SW2 [21]	502	47.6	38.9	25.7	16.3
NHANES – SW3 [21]	186	81.2	75.3	66.1	53.8
Las Vegas [20]	201	55.2	43.8	36.3	31.8
Medford [13]	504	68.5	60.5	54.4	49.2
This study – surface	184	82.6	80.4	75.0	64.1
Australia – surface water					
Sydney [6]	104	60.6	43.3	30.8	24.0
Melbourne [6]	104	65.4	48.1	33.7	23.1
AIDS cases [23]	298	67.1	59.7	45.0	31.9
Other – surface water					
Russia [22]	108	88.9	77.8	65.7	50.9
Italy [21]	150	69.3	54.0	38.0	30.0
Ground water					
This study – ground water	309	72.5	68.6	55.3	44.3
Albuquerque [20]	201	50.8	38.3	26.4	20.9
NHANES – GW1 [21]	51	58.8	52.9	41.2	33.3
NHANES – GW2 [21]	503	35.6	27.0	18.1	12.5
NHANES – GW3 [21]	120	82.5	68·3	53.3	42.5
NHANES – GW4 [21]	120	65.8	49.2	36.7	25.0

relative prevalence for residents of the surface-water city as well as the lower relative prevalence for people residing in the area for less than 2 years, and for people using bottled water, suggest a local environmental source of infection. Except for bottled water use, the findings were consistent for responses to each antigen group and independent of the definition of a positive serological response (any response, >10%, >20%or >30% of the positive control). It is possible that, since the responses were more intense than observed in other studies, the influence of non-drinking water risk factors may not be apparent. This could occur if intense responses from drinking water exposures mask responses from other risk factors. The increased risk of infection for private well-water users could be due to well-water contamination or to exposure to other sources. Although the private wells in the study area are from deep aquifiers, it is possible that the wells were subject to contamination from surface water runoff or other sources. We were unable to obtain detailed information about the private wells and future studies should consider this possibility. Alternatively, many of the private wells served rural families with pets or domestic animals. These could have also increased the risk of infection.

This study also agrees with a previous study [19] that found that increased intensity of serological responses, consistent with recent infection or seroconversion, occur relatively often and also occur more often in users of surface-derived drinking water compared to non-users. Years of repeated infections may result in high levels of serological response that remain elevated for considerable periods of time. The follow-up testing of 270 individuals over a 3–6 months period in this study indicates that 24% had an increased intensity of serological response to the 15/17-kDa antigen group of more than 10% of the positive control and that 42% of surface-water users had an increase.

This study supports the hypothesis that users of surface-derived drinking water are at higher risk of endemic waterborne *Cryptosporidium* infection. However, no relationships were observed between the occurrence of symptoms consistent with cryptosporidiosis during the past 6 months and serological response to either marker in this or in the previous paired city study. It is possible that mild illness may have been associated with a serological response but that these symptoms would not have been recalled at the time of blood sampling. Alternatively, it is possible that the more intense serological responses to the *Cryptosporidium* antigens among residents of the surface-water city indicate an increased level of protection from clinically significant cryptosporidiosis due to higher levels of endemic infections. In a review of cryptosporidiosis outbreaks, we suggested that previous infections may provide some level of protection against symptomatic infections, resulting in fewer outbreaks among users of water normally contaminated with *Cryptosporidium* oocysts [10]. Given the widespread use of surface-derived drinking water, the role of protective immunity and the potential implications of reducing low-dose exposure to oocysts merits further investigation.

#### REFERENCES

- McClellan P. First Interim Report, Possible Causes of Contamination. Sydney Water Inquiry, New South Wales Premier's Department, August 1998 (http:// www.premiers.nsw.gov.au/pubs.htm).
- Lisle JT, Rose JB. *Cryptosporidium* contamination of water in the USA and UK, a mini-review. J Water SRT-Aqua 1995; 44: 1003.
- Rose JBN, Gerba CP, Jakubowski W. Survey of potable water supplies for *Cryptosporidium* and *Giardia*. Environ Sci Technol 1991; 25: 1393.
- Craun GF, Hubbs SA, Frost F, Calderon RL, Via SH. Waterborne outbreaks of cryptosporidiosis. J Am Water Works Assoc 1998; 90: 81–91.
- Dawson A. Cryptosporidium surveillance: isolation from human stools and water supplies. In: Proceedings of 1994 Workshop on Cryptosporidium in Water Supplies (Dawson A, Lloyd A, eds.). London: Her Majesty's Stationery Office.
- Frost FJ, Muller TB, Fairley CK, Hurley JS, Craun GF, Calderon RL. Serological evaluation of *Cryptosporidium* oocyst findings in the water supply for Sydney, Australia. Int J Environ Health Res 2000; 10: 35–40.
- Perz JF, Ennever FK, LeBlancq SM. Cryptosporidium in tap water: comparison of predicted risks of observed levels of disease. Am J Epidemiol 1998; 147: 289–301.
- Frost FJ, Craun GL, Calderon CL, Hubbs SA. So many oocysts, so few outbreaks. J Am Water Works Assoc 1997; 89: 8–9.
- Moss DM, Chappel CL, Okhuysen PC, et al. The antibody response to 27-, 17- and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. J Infect Dis 1998; **178**: 827–833.
- Frost FJ, Craun GF. Serological response to human Cryptosporidium infection [Letter]. Infect Immun 1998; 66: 4008.
- Okhuysen PC, Chappell CL, Sterling CR, Jakubowski W, DuPont HL. Susceptibility and serological response of healthy adults to re-infection with *Cryptosporidium parvum*. Infect Immun 1998; 66: 441–443.

- Frost FJ, Muller T, Craun GF, et al. Serological analysis of a cryptosporidiosis epidemic. Int J Epidemiol 2000; 29: 376–379.
- Frost FJ, de la Cruz AA, Moss DM, Curry M, Calderon RL. Comparisons of ELISA and Western blot assays for detection of *Cryptosporidium* antibody. Epidemiol Infect 1998; 121: 205–211.
- Frost FJ, Calderon RL, Muller TM, et al. A two-year follow-up of antibody to *Cryptosporidium* in Jackson County, Oregon following an outbreak of waterborne disease. Epidemiol Infect 1998; 121: 213–217.
- Moss DM, Bennett SN, Arrowood MJ, Hurd MR, Lammie PJ, Wahlquist SP. Kinetic and isotypic analysis of specific immunoglobulins for crew members with cryptosporidiosis on a U.S. Coast Guard cutter. J Eukaryot Microbiol 1994; 41: 528–558.
- Moss DM, Bennett SN, Arrowood MJ, Wahlquist SP, Lammie PJ. Enzyme-linked immunoelectrotransfer blot analysis of a cryptosporidiosis outbreak on a United States Coast Guard cutter. Am J Trop Med Hyg 1998; 51: 110–118.
- Priest JW, Li A, Khan M, et al. Enzyme immunoassay detection of antigen-specific immunoglobulin G antibodies in longitudinal serum samples from human

cryptosporidiosis patients. Clin Diagn Lab Immunol 2001; 8: 415-423.

- Muller TB, Frost FJ, Craun GF, Calderon RL. Serological responses to *Cryptosporidium* infection. Infect Immun 2001; 69: 1974.
- Frost FJ, Muller T, Craun GF, Lockwood WB, Calderon RL. Serological evidence of endemic waterborne *Cryptosporidium* infections. Ann Epidemiol 2002; 12: 222–227.
- Frost FJ, Muller T, Craun GF, Calderon RL, Roeffer PA. Paired city *Cryptosporidium* serosurvey in the southwest USA. Epidemiol Infect 2001: 126: 301–307.
- Frost FJ, Muller T, Kunde T, Craun GF, Calderon RL. Seroepidemiology. In: Hunter PR, Waite M, Ronchi E, eds. Drinking water and infectious disease: establishing the links. CRC Press, 2002: 167–168.
- 22. Egorov A, Frost F, Muller T, Naumova E, Tereschenko A, Ford T. Serological evidence of *Cryptosporidium* infections in a Russian city and evaluation of risk factors for infection. Ann Epidemiol (in press).
- Friedman ND, Frost F, Caputo C, Horrocks M, Fairley CK. One year follow up of antibodies to *Cryptosporidium* among individuals with HIV infection. Venereology 2001; 14: 21–24.