Epidemiol. Infect. (2000), **125**, 87–92. Printed in the United Kingdom

A serological survey of college students for antibody to *Cryptosporidium* before and after the introduction of a new water filtration plant

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(*Accepted 27 April 2000*)

SUMMARY

In April 1997, a large city in the northeastern United States changed their drinking water treatment practices. The city, which previously provided only chlorination for their surface water sources added filtration in addition to chlorination. To assess whether *Cryptosporidium* infections rates declined following filtration, we tested serological responses to 15/17-kDa and 27-kDa *Cryptosporidium* antigens among 107 community college students 1 month before and 225 students 5 months after filtration. Results suggest that levels of *Cryptosporidium* infections did not decline following water filtration. However, seasonal changes in other exposures may have confounded the findings. Swimming in a lake, stream or public pool and drinking untreated water from a lake or stream predicted a more intense response to one or both markers. Residence in the city, not drinking city tap water or drinking bottled water, gender, travel or exposure to pets, young pets, diapers or a household child in day care were not found to be predictive of more or less intense serological responses for either the 15/17-kDa and 27-kDa antigen.

INTRODUCTION

Outbreaks of cryptosporidiosis have been associated with contaminated drinking water, food, day care and recreational exposure to water [1, 2]. Oocysts of the parasite *Cryptosporidium* have been detected in source and treated drinking water in the United States and elsewhere [3–5]. Although enteric disease surveillance studies have not observed an elevated risk of cryptosporidiosis-like illnesses in communities served by oocyst contaminated drinking water [6], recent studies suggest that immunity develops after repeated infections, reducing the severity of illness from subsequent infections [7–9]. If so, evidence of excess illness may not be apparent in populations chronically exposed to *Cryptosporidium* oocysts through drinking water or other routes.

Because of concerns over waterborne *Cryptosporidium* transmission, US utilities not currently filtering surface water have been considering various water filtration technologies. Currently, several large and a number of smaller drinking water utilities in the US still use chlorinated but unfiltered drinking water from surface sources. Some of these water utilities have moderately protected watersheds, with limited sources of faecal contamination from humans or domestic animals [10] while others have very well protected watersheds, with little risk of either human or domestic animal faecal contamination of the source water. However, faecal contamination of the source

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water by wild animals is likely to occur in both types of watersheds.

Because of the US Environmental Protection Agency's (EPA's) Surface Water Treatment Rule (SWTR) [11], some drinking water utilities are currently constructing or upgrading water-treatment facilities. The difficulty in inactivating *Cryptosporidium* oocysts with chlorine levels traditionally used for water disinfectants and the need for multiple barriers preventing pathogens from reaching consumers have been among the primary motivations for the US regulations on water filtration. Planned improvements in water treatment are an opportunity to assess levels of enteric infections and waterborne risks in populations exposed to different water sources and water-treatment technologies before and after the upgrade.

We identified a city with a chlorinated, unfiltered surface water system that planned to install filtration. A water filtration plant to serve the entire city was installed and became operational in April 1997. To ascertain whether water filtration reduces the risk of waterborne *Cryptosporidium* transmission, we compared serological evidence of *Cryptosporidium* infection before and after the initiation of water filtration.

METHODS

Background

The city's surface water is impounded in two chains of upland reservoirs serving two pressure zones of the city. The high zone consists of five reservoirs, and the low zone consists of five reservoirs. Since April, 1997, all surface water is routed through a new water filtration plant. In addition to the surface water sources, the city has two wells which are also chlorinated and are primarily used in the summer to supplement surface water sources. Drinking water is provided to a population of approximately 199000.

The watershed is moderately well protected, with no human dwellings and only a small number of domestic grazing animals. However, elevated levels of turbidity, coliform and trihalomethanes have been periodically observed. Water supplies of neighbouring cities use either filtered or unfiltered surface-derived drinking water.

Serosurveys

To evaluate the health benefits from installing water filtration, two serological surveys of college students were conducted. One was conducted in March 1997, 1 month before filtration was initiated and one in September 1997, 5 months after initiation of filtration. The study protocols were submitted and approved by an Institutional Review Board. Students were recruited from a local commuter college serving the city and surrounding communities. At the time of the blood draw, students completed an informed consent and a two-page questionnaire. Questions were asked about place of residence, the source of drinking water (city or other) and use of tap and bottled water for drinking, making ice and washing food. Students were also asked whether, during the past 6 months, a child in their household attended day care, someone in the household had prolonged diarrhoea and if they were exposed to diapers, grown pets, young pets, livestock or zoo animals and untreated water from lakes or streams, they had swum in a lake, stream or pool, and had travelled outside the United States. Sera were separated from the whole blood shortly after collection, frozen and then shipped to the Lovelace Clinic Foundation in Albuquerque, New Mexico for analysis.

Western blot procedures

Sera were analysed by immunoblot to measure IgG serological response to the 15/17- and 27-kDa antigen groups [12, 13]. *Cryptosporidium* protein was separated by sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE) using a 15% separating gel in a continuous buffer system. The protein was electrophoretically transferred out of the gel and onto a polyvinylidene fluoride (PVDF) membrane. The PVDF membrane was blocked with PBS/0·3% polyoxyethylenesorbitan 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 was 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 were 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 were incubated with streptavidin alkaline phosphatase at a 1:1000 dilution in PBS/0·3% Tween 20 for 60 min. The

https://doi.org/10.1017/S0950268899004148 Published online by Cambridge University Press

bound antibodies were 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 was digitally analysed by an IS-2000 Digital Imaging System (Alpha Innotech). 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. Serological response to the two antigen groups was based on the measured area under the curve of response intensity for each lane at the expected location of the response for the antigen group.

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 standard positive control serum contained on each blot. The IgG-positive control serum was obtained from individuals with a strong serological response to both antigens, approximating the intensity of responses observed from several individuals with laboratory confirmed cryptosporidiosis. The same positive control serum was used on each blot. Variables were summarized using frequencies and groups compared using the χ^2 test for homogeneity of proportions. We defined a detectable response as one that had an intensity greater than 10% of the positive control.

Statistical analysis

Multivariate analysis of the observed serological response intensities presented a special problem, since the responses cannot be negative and since a disproportionate fraction of the responses clustered at zero intensity. Analytical methods based on the assumption that the data follow a multivariate normal distribution are inappropriate for these data. For this reason, a Tobit [14] model was used to relate responses to risk factor questions with the intensity of serological responses to the two markers. The Tobit model assumes that the expected serological response for individual i (y_i, the dependent variable) can be predicted by a linear combination of risk factors (present or absent, with $x_i = 1$ or 0) based on responses of blood donors to a questionnaire, weighted by a vector of coefficients (B). These results are observed only when the predicted values for y_i (serological responses) are greater than zero. Otherwise, the serological responses are censored at zero. This is represented as follows:

$$\begin{aligned} y_i &= Bx_i + \mu_i \quad \text{if} \quad Bx_i + \mu_i > 0 \\ &= 0 \qquad\quad \text{if} \quad Bx_i + \mu_i \leqslant 0. \end{aligned}$$

The error term (μ_i) is assumed to be normally distributed. The Tobit model was fitted using SAS proc lifereg.

RESULTS

A total of 107 students were screened in the first survey and 225 in the second survey. In both surveys, the majority of participants were female (68% on the first, 67% on the second). Approximately 87% of students in both surveys were white. A higher fraction of students in the first survey (62 *vs.* 21%) were age 21 years and above. Approximately 15% of participants in the second survey also participated in the first survey.

Positivity

For the 15/17-kDa marker, 19% of students had a detectable response in the first survey compared with 24% in the second survey ($P = 0{\cdot}63$). For the 27-kDa marker, 27% had a detectable response in the first survey compared to 41% in the second survey ($P = 0{\cdot}02$).

Tobit analysis

Gender, travel or exposure to pets, young pets, diapers or a household child in day care were not found to be associated with more or less intense serological responses. Residence in the city, not drinking city tap water or drinking bottled water were also not predictive of more or less intense serological responses for either marker (Table 1). Since most participants were ages 18–25 years and white, there was little variation in age and race of participants. Use of bottled water for making ice or washing food was uncommon and its relationship with serological response could not be evaluated. Also exposures to livestock/zoo animals and persons with diarrhoea were uncommon and could not be adequately evaluated by the model.

Swimming in a lake, stream or pool predicted an 18% increase in the intensity of response to the 27-kDa marker, relative to the positive control ($P < 0{\cdot}005$) (Table 1). Swimming also predicted a

https://doi.org/10.1017/S0950268899004148 Published online by Cambridge University Press

Table 1. *Relationship between risk factors and response intensity*

	Estimated increase (as % of positive control)	95% CI	*P*-value	No. of exposed/ total
Swimming				
15/17 kDa	13%	(0%, 29%)	0·12	235/332
27 kDa	18%	(6%, 30%)	0·004	
Untreated water consumption				
15/17 kDa	32%	(9%, 56%)	0·008	24/332
27 kDa	9%	(0%, 28%)	0·36	
Second survey participation				
15/17 kDa	14%	(0%, 31%)	0·12	225/332
27 kDa	16%	(3%, 29%)	0·011	
Residence in city				
15/17 kDa	−10%	(−26%, 6%)	0·23	225/332
27 kDa	−8%	(−20%, 4%)	0·21	
Not drinking city water				
15/17 kDa	20%	(−3%, 43%)	0·09	42/332
27 kDa	15%	(−2%, 32%)	0·07	
Bottled water use				
15/17 kDa	−3%	(−19%, 13%)	0·72	95/332
27 kDa	5%	(−7%, 17%)	0·37	

13% increase to the 15/17-kDa marker but the differences were not statistically significant ($P = 0{\cdot}12$). Alternatively, consumption of untreated water from a lake or stream predicted a 32% increase in the intensity of response to the 15/17-kDa marker relative to the positive control ($P < 0{\cdot}01$). It also predicted a 9% increase for the 27-kDa marker but this increase was not statistically significant ($P = 0{\cdot}36$) (Table 1). Participants in the second survey had a 14% more intense response to the 15/17-kDa ($P = 0{\cdot}12$) and a 16% increase for the 27-kDa marker ($P < 0{\cdot}02$).

DISCUSSION

Cryptosporidium is recognized as a cause of infectious gastroenteritis. The severity and persistence of symptoms are related to the immunocompetence of the host and may be influenced by immune responses from prior *Cryptosporidium* infections [15]. Because of their widespread distribution [4] and the difficulty in inactivating *Cryptosporidium* oocysts [16], people may be routinely exposed to viable oocysts through drinking water, food and recreational activities. Although waterborne outbreaks have generally been associated with deficiencies in water treatment [17], increasing evidence suggests that elevated levels of endemic infections may be associated with consuming drinking water that meets current water quality standards [18].

This study was part of a pilot evaluation of one natural experiment that occurred when a community installed water filtration for the first time. Prior to April 1997 residents and students who drank college tap water would have been exposed to chlorinated but unfiltered surface-derived drinking water. Results of this study suggest levels of *Cryptosporidium* infections did not decline following water filtration. Although seasonal changes in waterborne transmission of the parasite are possible, the increase in prevalence of serological response to the 27-kDa marker may have resulted from seasonal factors not detected by the questionnaire. For example, sources of food, other recreational activities or even the intensity of recreational activities, such as swimming or untreated water consumption (shown here to be a risk factor for infection), may have changed from early spring to late summer. The data suggest that there may be seasonal changes in the risk of *Cryptosporidium* infections, suggesting that the second survey should

https://doi.org/10.1017/S0950268899004148 Published online by Cambridge University Press

have been done at the same time of the year as the first. There is, unfortunately, very little other information on seasonal changes in the risk of endemic *Cryptosporidium* infection.

Although the sample size was relatively small, given that a significant increase in serological response was detected, it is unlikely that a reduction in risk of *Cryptosporidium* infections was not observed because of the sample size. Based on detecting a statistically significant increase of 16% of the positive control, it is likely that a future serological study of approximately 200–300 individuals should be able to detect changes in the intensity of serological response close to 16%.

The risk of *Cryptosporidium* infection from consuming untreated surface water has been previously noted [19]. The role of swimming in increasing the risk of infection is consistent with outbreaks associated with recreational water exposures [20] and merits further inquiry regarding endemic risks. Swimming reported in the first survey would have included swimming in the autumn and winter when students, because of the cold climate, would have swum in heated indoor pools or on vacation in a warmer climate. The second survey included a period when students might have also swum in local lakes. Elevated serological responses were associated with swimming during both surveys. Additional studies are needed to separately estimate the risks of infection from swimming in pools *vs*. lakes and streams.

Why swimming increased intensity of response to the 27-kDa marker while the untreated water consumption increased response to the 15/17-kDa marker is unclear. It is possible that the duration of intense responses differs between the two markers and that the relative intensities of the two responses provides some information on when the infection occurred. It is also possible that people who are routinely exposed through swimming may not continue to show an elevated response to the 15/17-kDa marker but show an elevated response to the 27-kDa marker. Untreated water consumption, because it is likely to occur episodically, may result in an elevated response to the 15/17-kDa marker but not to the 27-kDa marker.

Failure to detect reductions in seroprevalence to the *Cryptosporidium* markers following drinking water filtration and the increase in seroprevalence for the 27-kDa marker suggest that risks of endemic waterborne *Cryptosporidium* transmission prior to filtration may not have been as important as other routes of exposure. Other community intervention investigations are planned by the EPA to estimate the magnitude of waterborne disease in the United States. Unlike household intervention studies [21], these studies take advantage of natural experiments that are the results of efforts to improve drinking water quality. Serological responses and illness rates will be compared. The EPA studies compare illness rates before and after changes in drinking water treatment. Additional serological and community enteric disease investigations before and after water treatment improvements should be considered in settings outside the United States, especially if a range of source water characteristics, watershed types, sources of contamination, and levels of contamination can be considered. Serological studies such as this can help assess waterborne risks, but studies should be conducted at specific locations and should be of sufficient statistical power to detect small increased risks.

ACKNOWLEDGEMENT

The views expressed in this article are those of the individual authors and do not necessarily reflect the views and policies of the US Environmental Protection Agency or the Centers for Disease Control. The research described in this article has been supported by the United States Environmental Protection Agency through CR 824167-02-1 to the Lovelace Clinic Foundation. It has been subject to the Agency's peer and administrative review, and it has been approved for publication. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

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