Serological responses to *Cryptosporidium* antigens among women using riverbank-filtered water, conventionally filtered surface water and groundwater in Hungary

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ABSTRACT

We compared serological responses to *Cryptosporidium parvum* antigens using surplus sera from females undergoing routine screening for pregnancy from three counties in Hungary where bank-filtered surface water, conventionally filtered and disinfected surface water, and groundwater from either a karst or confined aquifer are commonly used for drinking water. The primary purpose was to determine whether the prevalence and intensity of serological responses, indicators of prior *Cryptosporidium* infection were similar for these populations. Women using groundwater from a confined aquifer had significantly lower mean serological responses for both the 15/17-kDa and 27-kDa ($p < 0.0001$) antigen groups than women using conventionally filtered and disinfected surface water or karst well water. This is suggestive of less frequent infections. Women using bank-filtered water also had lower mean responses for both antigen groups. Among women using bank-filtered water, the mean intensity of response for both antigen groups was almost one-third of the mean response observed for women using conventionally filtered and disinfected surface water. These findings suggest that riverbank filtration may be an effective alternative to conventional treatment for reducing *Cryptosporidium* exposures and infection from surface drinking water sources.

Key words | *Cryptosporidium*, riverbank filtration, serology, waterborne disease

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INTRODUCTION

Bank filtration has been used for centuries to purify surface water. In its most simple form, a well is drilled near a body of water such as a stream or a lake. Vertical wells can be dug or drilled at varying distances from the surface water source and horizontal wells (also called collector wells) can be drilled under the stream or lake. Stream or lake water flows through the sediments before entering the well. Depending on the soil characteristics, hydrological conditions and the distance the water travels, high levels of pathogen removal can be achieved (Kuehn 2000). Water treatment efficiency for removing specific pathogens depends on the specific hydrogeology of each bank filtration well field (Schijven *et al.* 2001).

Although used extensively in Europe (Kuehn 2000; Ray *et al.* 2003), bank filtration has not been widely used in the United States. In Germany, bank filtered surface water is the second leading source of drinking water, accounting for approximately 16% of all drinking water in the country (Ray *et al.* 2003). It is also used extensively in the Netherlands (Ray *et al.* 2003). In Hungary, about 30% of the population is supplied from bank filtration wells, mostly in populations situated near the river Danube.

Bank filtration offers some advantages over the conventional filtration of surface water, including reliability and low cost of operation (Kuehn 2000). But there are also some disadvantages. Local conditions affect the effectiveness of

bank filtration (Ray *et al.* 2003). Soil characteristics can affect the retention time and flow rates as well as pathogen removal/inactivation efficiency (Ray *et al.* 2003). Information about the epidemic and endemic health risks that may be associated with bank filtration is limited and difficult to interpret. We are not aware of studies comparing risks of infection from waterborne pathogens in bank filtration communities with communities using groundwater or conventionally filtered surface water. Although indicators of the effectiveness of disinfection (e.g. concentration and contact time for pathogen inactivation, analysis of indicator organisms) and filtration (e.g. turbidity and particulate analysis) are widely used and understood, the value of the same indicators for bank filtration performance remains unclear (Ray *et al.* 2003).

Since most bank-filtered water will be disinfected, bacteria and viruses in source waters are of limited concern. These pathogens are generally susceptible to the commonly used water disinfectants. However, for waterborne protozoa such as *Cryptosporidium* and *Giardia* that are frequently detected in streams, lakes and reservoirs, the commonly used drinking water disinfectants are ineffective at concentrations and contact times normally applied (CDC 1997). Ineffective removal of *Cryptosporidium* and *Giardia* by bank filtration could result in increased health risks.

Since *Cryptosporidium* infection elicits a serological response in most infected humans (Moss *et al.* 1998; Frost 1998*a*, *b*; Frost *et al.* 2000*a*), surveys of the prevalence of responses can be used to estimate prior *Cryptosporidium* infection in populations (Frost & Craun 1998; Frost *et al.* 2000*a*). Studies have tracked serological responses in people intentionally or unintentionally exposed to *Cryptosporidium* oocysts (Moss *et al.* 1994, 1998; Frost & Craun 1998; Frost *et al.* 1998*a*; Muller *et al.* 2001). Most recent serological studies have focused on responses to a 15/17-kDa and a 27-kDa antigen group. Serological responses to these two markers appear to be specific for *Cryptosporidium* infection (Moss *et al.* 1994, 1998; Frost *et al.* 2000*a*, *b*; Muller *et al.* 2001). Infection usually elicits a serological response to these antigen groups that peaks 4–6 weeks after infection (Moss *et al.* 1998; Muller *et al.* 2001). The 15/17-kDa marker declines to baseline levels observed prior to the infection in 4–6 months after infection while the 27-kDa marker remains elevated for 6–12 months (Muller *et al.* 2001).

We conducted a study in Hungary to compare serological responses to *Cryptosporidium* antigens among females residing in areas with riverbank filtration systems, conventionally filtered/disinfected surface water systems, and groundwater systems. These serological responses should provide evidence of prior *Cryptosporidium* infection. Because the populations studied were demographically similar, we also felt confident that the serological responses to *Cryptosporidium* would reflect waterborne exposures even though we were unable to obtain information from each of the study participants about other possible risk factors.

METHODS

Description of the study sites and populations

Sera collected for routine screening from 153 pregnant women with a mean age of 27 years were obtained in April and May 2001 from three counties in Hungary and analysed for evidence of previous exposure to *Cryptosporidium parvum*. The counties were selected primarily based on their public drinking water systems. We selected the study population not only to reflect different water sources but also for similar socio-economic characteristics. The populations live in semi-rural areas served by public water systems with limited opportunities for exposure to farm animals.

Sera samples were collected from 48 women residing in Csongrad County. These women are supplied with high quality drinking water from deep wells (150–300 m deep) drilled into a confined aquifer. Samples were also collected from 50 women residing in four communities in Pest County. These women receive drinking water from four riverbank filtration systems developed near the Danube River upstream from Budapest. The bank-infiltration wells are located 30–100 m from the River Danube in alluvial fragmentary deposits from the late Pleistocene period. The deposits are approximately 5–10 m in depth, and a constant water layer of 4–6 m is maintained in the deposits. Most wells are tube-wells, but in some locations, the wells are extended horizontally under the Danube. The bank filtration wells are replenished with water from the Danube River after natural filtration through a sandy-gravel soil. The residence time of the infiltrating water is about 4–12 days.

At low water levels of the Danube, about 20–40% of the water pumped from the bank-filtration wells may be groundwater that also collects in the deposits.

Finally, sera samples were collected from 55 women residing in Borsod-Abauj-Zemplen County. This group of women received drinking water from several systems whose sources included surface water streams and impoundments and wells drilled into a karst, unconfined aquifer. We identified the specific areas and water systems for each of the participants. We identified one donor from Borsod-Abauj-Zemplen County who lives in an area served by deep wells from a confined aquifer. For our analysis, we classified this individual with users of water from deep wells.

The remaining sera samples from Borsod-Abauj-Zemplen County residents came from three subgroups of women: 31 women who live in areas that primarily receive drinking water from conventionally filtered and disinfected surface sources (a small impounded mountain stream) and the Bodva River; 15 women who live in areas where drinking water is primarily from karst wells; and three women who live in an area that receives a mix of groundwater from an unconfined, karst aquifer and conventionally treated surface water. These later three women were grouped with the 31 women who receive filtered surface water. Wells drilled in a karst aquifer are highly likely to be subject to surface water contamination and thus, may be contaminated with oocysts. Water from the karst wells is not filtered, and any disinfection applied would not be effective in inactivating *Cryptosporidium* oocysts. Although surface water sources may be contaminated with oocysts, conventional filtration can, to some degree, remove the oocysts. The removal efficiency depends upon the pretreatment and operation of the filtration plant.

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 (Frost *et al.* 1998*a*, *b*). 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). 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 use of the computer to measure detection and the intensity of responses minimizes the risk of introducing operator errors or day-to-day variation in the classification of a serological response.

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 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 for all blots. An extensive quality control effort was conducted analysing replicate samples (Frost *et al.* 2002). These studies found a correlation between the intensity of serological response for replicate analyses ranged from 0.92 for the 15/17-kDa marker to 0.84 for the 27-kDa marker. Analysis was done using logistic regression (SAS Version 8). Additional analyses for comparison of results among the women who resided in the various areas were carried out using a Tobit model (Tobin 1958). The Tobit model assumes an underlying normal distribution of the responses about the mean intensity, but allows a point mass at zero. This accounts for the absence of negative serological responses. We have used this model in prior serological publications (Frost *et al.* 2001, 2002) and it is commonly used for the analysis of truncated cost (Tollestrup *et al.* 2001) and laboratory outcomes data (Chi *et al.* 2002).

RESULTS

A total of 50 sera were obtained from users of riverbank filtered water, 49 from users of well water from a confined aquifer, 20 from users of water from karst wells drilled in aquifers that are not confined and 34 from users of surface filtered and disinfected drinking water. For all 153 women,

the mean responses to the 15/17-kDa and 27-kDa marker were 17% and 29% of the positive control response. There were no statistically significant differences in the mean responses for users of karst well water and filtered surface water ($p > 0.50$) and, therefore, these two groups were combined for all additional analyses.

The mean intensity of serological responses by source was tested using the Tobit model (Table 1). Our analysis indicates that women residing in areas where groundwater is obtained from a confined aquifer had a significantly lower mean serological response to both markers than women residing in the areas that use filtered/disinfected surface-derived drinking water or karst well water ($p < 0.01$). The mean intensity of response to each marker was also lower in women drinking bank-filtered water compared with women using filtered/disinfected surface-derived drinking water or karst well water ($p = 0.29$ for the 15/17-kDa marker and $p = 0.07$ for the 27-kDa marker). The mean intensity of response for both antigen groups for women using bank-filtered water was almost one-third of the mean response observed for women using conventionally filtered and disinfected surface water. Additional analyses also suggested that the intensity of the response (>30% of the positive control) for both the 15/17-kDa and 27-kDa antigen groups was less common for women using bank-filtered water than for women using conventionally filtered and disinfected surface water. Because the donors were all pregnant women, the range in ages was very limited and, therefore, the age adjustment made very little difference in statistical testing.

DISCUSSION

Although our study was exploratory and the results should be confirmed in an analytical epidemiological study, the results are very noteworthy because they suggest that bank-filtration as practised in Pest County is effective in reducing drinking water exposures for *Cryptosporidium*. Any interpretation, however, should be cautious. Our study considered individual responses to *Cryptosporidium* infection, but an ecological measure of exposure was used. Two concerns are the possible use of bottled water and swimming exposures for our population. Although the sale of bottled water has increased in Hungary during the past several years and restaurant patrons frequently consume bottled water when dining, we believe that the study population consumed primarily tap water at home. Exposures from swimming in *Cryptosporidium* contaminated lakes should not have confounded our results, since any such exposures would have been limited and taken place over 7 months before collection of the sera. Any exposure misclassification from the use of ecological measures should be non-differential. We believe that the results reflect drinking water exposures, and had we been able to assess individual tap water exposures, the results would have been more convincing in terms of the effectiveness of bank-filtration.

In this study approximately 43% of the participants had a detectable response to the 15/17-kDa antigen group and 77% had a detectable response to the 27-kDa antigen group. Approximately 50% of the participants who used conventionally filtered surface water and 18% who used groundwater from

Table 1 | Mean *Cryptosporidium* antigen responses for women residing in each water system area (*p*-values computed from the Tobit Analysis, adjusted for age of the donor)

Water system	Number of women	Mean response 15/17-kDa (% positive control)	*p*-value (direction)*	Mean response 27-kDa (% positive control)	*p*-value (direction)*
Riverbank filtration	50	0.15	0.29 (−)	0.29	0.07 (−)
Deep wells (confined aquifer)	49	0.11	0.002 (−)	0.16	0.0001 (−)
Karst wells and surface water	54	0.24		0.41	
Karst wells (unconfined aquifer)	20	0.27		0.39	
Surface water filtered/disinfected	34	0.22		0.42	

* +, mean response is greater than response in filtered/disinfected surface water; −, lower mean response. Tests were based on a comparison with users of either karst wells or filtered and disinfected surface water.

a confined aquifer had detectable responses to the 15/17-kDa antigen group. We detected responses to the 27-kDa antigen group in 88% of surface water users and 61% of groundwater users. These responses are similar to responses found in North American populations. In one US study we found that 46% of conventionally filtered surface water users and 26% of groundwater users had detectable responses to the 15/17-kDa antigen group (Frost *et al.* 2002). We also detected responses to the 27-kDa antigen group in 54% of surface water users versus 39% of groundwater users (Frost *et al.* 2002). In a Canadian study we found that 45% of users of surface-derived drinking water had a response to the 15/17-kDa antigen group and 45% had a response to the 27-kDa antigen group (Frost *et al.* 2000*a*). However, in a Canadian city affected by a cryptosporidiosis outbreak, 69% had a response to the 15/17-kDa antigen group and 88% had a response to the 27-kDa antigen group (Frost *et al.* 2000*a*). In a southern European population not affected by a cryptosporidiosis outbreak 83% of people tested had a response to the 15/17-kDa antigen group and 62% had a response to the 27-kDa antigen group (Frost *et al.* 2000*b*).

Historically, drinking water quality is monitored by the presence or absence of indicator organisms (e.g. coliforms, fecal coliform bacteria). Standard filtration performance can be studied by testing filter efficacy under laboratory, pilot plant and field conditions. Turbidity levels or particle counts are established indicators of the efficacy of various filtration technologies to remove pathogens. However, for bank filtration, each application has unique characteristics that may affect particle removal and pathogen disinfection. In theory, by frequently testing the drinking water for the presence of pathogens, water officials should be able to determine the safety of the water. But methods to detect *Cryptosporidium* oocysts are particularly unreliable (Allen *et al.* 2000). In addition, the tests are not able to distinguish viable from non-viable oocysts or strains that may be infective for humans. So, if an oocyst is recovered from filtered water, the public health significance of the finding is not clear.

Limitations of the *Cryptosporidium* tests for monitoring water contamination indicate that failure to detect *Cryptosporidium* oocysts in drinking water supplies does not ensure the absence of oocysts in the water (Allen *et al.* 2000). Testing for various water quality indicators should help to determine the presence of waterborne pathogens. However, the data are not yet available to determine which easily measured water quality indicators can best assess either the presence of a pathogen or an increased health risk. Even if a suitable indicator(s) is found, frequent or continuous monitoring is required to detect breaches in the integrity of bank filtration. An alternative to oocyst recovery from water or the measurement of one or more water quality indicators is a serological survey, such as we conducted in Hungary. Detection of serological responses to *Cryptosporidium* antigens among users of the drinking water is a reliable measure of prior infection (Moss *et al.* 1998; Muller *et al.* 2001; Frost *et al.* 2002). Sera samples are relatively inexpensive to collect and the analysis is relatively inexpensive and sensitive.

To test this approach, we have conducted two paired city studies in the United States that compared serological responses among residents of a city that uses filtered and disinfected surface water with similar residents of a nearby city that uses high quality groundwater as a drinking water source (Frost *et al.* 2001, 2002). Both studies show that users of the surface-derived drinking water have higher levels of serological responses to *Cryptosporidium* antigens than users of well water. We observed these differences in the frequency and intensity of serological responses even when oocysts were not detected in the conventionally filtered surface-derived drinking water. We believe that increased serological responses to *Cryptosporidium* antigens may be a more sensitive indicator of drinking water oocyst contamination than the detection of oocysts or routine measurement of water quality parameters. Serological studies may effectively assess risks of waterborne infection associated with certain water sources and types of treatment.

CONCLUSIONS

Applications to state drinking water programmes in the United States for using bank filtration as a primary or secondary method for removing drinking water pathogens have recently increased. However, the efficacy of bank filtration to remove or inactivate pathogens has not been rigorously investigated. Since bank filtered waters have low levels of turbidity and are usually disinfected, the primary pathogen of concern in finished water is *Cryptosporidium*. The results of this study suggest that bank filtration may be as effective, if not more effective, in removing oocysts from drinking water than

convention filtration. Unfortunately, these conclusions are based on relatively small sample sizes and an analysis of bank filtration systems from one geographic location.

Further research is needed on the efficacy of riverbank filtration to remove pathogens. In particular, serological studies of populations using riverbank filtration in a variety of different settings with different source water quality and different soil characteristics should be performed. It would be most helpful to identify soil characteristics predictive of either good or poor pathogen removal for riverbank filtration applications. We hope to expand our study to additional populations in Hungary and other central European countries and collect sequential sera samples over time from volunteers. This type study can help assess potential confounding and other risk factors as well as changes in serological responses over time. We are currently studying a population in the United States and hope to collaborate on serological studies with investigators in other European countries where bank filtration is applied.

ACKNOWLEDGEMENTS

The authors wish to thank Dr Jászai Sándorné, a hydrogeologist with the Danubian Waterworks (DMRV) for her assistance on this project and the National Institute of Environmental Health, Budapest, for collecting the sera samples.

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