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Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area

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Abstract

Background: Cryptosporidiosis, caused by *Cryptosporidium*, is an enteric illness that has received much attention as an infection of immunocompromised persons as well as in community outbreaks (frequently waterborne). There are, however, no studies of the risk factors for sporadic community-acquired cryptosporidiosis in the immunocompetent US population. We undertook a case-control study in the San Francisco Bay Area as part of a national study sponsored by the Centers for Disease Control and Prevention to ascertain the major routes of transmission for endemic cryptosporidiosis, with an emphasis on evaluating risk from drinking water.

Methods: Cases were recruited from a population-based, active surveillance system and age-matched controls were recruited using sequential random-digit dialing. Cases (n = 26) and controls (n = 62) were interviewed by telephone using a standardized questionnaire that included information about the following exposures: drinking water, recreational water, food items, travel, animal contact, and person-to-person fecal contact, and (for adults) sexual practices.

Results: In multivariate conditional logistic regression analyses no significant association with drinking water was detected. The major risk factor for cryptosporidiosis in the San Francisco Bay Area was travel to another country (matched odds ratio [95% confidence interval]: 24.1 [2.6, 220]).

Conclusion: The results of this study do not support the hypothesis that drinking water is an independent risk factor for cryptosporidiosis among the immunocompetent population. These findings should be used to design larger studies of endemic cryptosporidiosis to elucidate the precise mechanisms of transmission, whether waterborne or other.

Background

Cryptosporidiosis is caused by a coccidian parasite, *Cryptosporidium*, which is transmitted in its infective form, the oocyst, by the fecal-oral route [1]. It causes self-limited, watery diarrhea in immunocompetent individuals but can be severe and life threatening in people with immunosuppressive conditions [2]. Because of the severe nature of the disease in the immunocompromised, most epidemiologic

studies have focussed on people with HIV/AIDS and other immunosuppressed states [3–9]. What is known about transmission patterns in the general US population is based on studies undertaken during outbreaks of the disease, usually waterborne [10–16]. Even though cryptosporidiosis is a reportable disease, limited data are available on the routes of endemic transmission and on accurate incidence rates of cryptosporidiosis, especially in the immunocompetent population. The few studies that have utilized information on cases detected via surveillance [17–19] have presented only a partial picture of the risk of cryptosporidiosis because of incomplete case ascertainment. The principal reason for this is that the disease is under-reported by physicians and laboratories and it is frequently under-diagnosed [20]. Even when people seek care for diarrhea, fecal specimen tests for *Cryptosporidium* are not part of the routine ova and parasites testing protocols. In our study area only two of the 40 laboratories reporting to the active surveillance system conduct *Cryptosporidium* analysis along with routine ova and parasite exams on stool specimens.

It is possible that sporadic cryptosporidiosis in the community is transmitted in a different manner from what is observed in outbreak settings. Large outbreaks have been associated primarily with water, including drinking water, from a variety of sources [10,11,16,21–24] and from recreational water contact [15,25–27]. Other routes of transmission have also been identified. Foodborne outbreaks of cryptosporidiosis in which the vehicle of transmission was identified are few and only one outbreak in Maine was definitively associated with contaminated fresh-pressed apple cider [28]. Other foodborne outbreaks due to cryptosporidiosis have implicated food handlers [29] and social events [30,31]. Person-to-person outbreaks have been better documented, such as those in hospitals [32–37] and day care centers [38,39]. It is becoming increasingly evident that cryptosporidiosis is one of the multitude of enteric pathogens that is endemic in hospital and day care settings [40–43]. Other routes of exposure to *Cryptosporidium* may be responsible for sporadic disease in the general population such as specific sexual contact with an infected individual [44–46], travel to endemic countries [47–50], and contact with animals, both domestic and livestock [1,2,51,52]. Recent studies in Australia have demonstrated that exposure to persons with diarrhea and swimming in public pools rather than consumption of untreated tap water are the sources of community-acquired cryptosporidiosis in that country [53]. The relative contribution for each of these modes of transmission to the total burden of sporadic cryptosporidiosis among immunocompetent persons continues to be unknown in the U.S. Better definition of the importance of the factors associated with endemic cryptosporidiosis would assist public health and regulatory authorities in making decisions regarding policies and programs for the control and prevention of cryptosporidiosis.

Cases of cryptosporidiosis are identified through the California Emerging Infections Program (CEIP) active surveillance system which covers selected Northern California counties. During the first year of active surveillance, between June 1996 and May 1997, 143 cases were identified. During the subsequent years through May 2001, the number of cases of cryptosporidiosis detected were 155, 112, 99, and 66. The source of infection remains unconfirmed for sporadic community-acquired disease in this region. We therefore undertook an incidence density case-control study using prospectively enrolled immunocompetent cases of cryptosporidiosis (identified through the population-based, active surveillance system) and controls (recruited from the general population) to determine the major endemic routes of transmission of cryptosporidiosis in the San Francisco Bay Area. The primary research question was whether municipal drinking water was the major source of cryptosporidiosis, or whether there were other exposures more strongly associated with this disease.

Methods

Study population

In 1997, the Centers for Disease Control and Prevention (CDC) established an active surveillance system in seven sites around the country as part of the Emerging Infections Program (EIP). The California EIP (CEIP) surveillance for cryptosporidiosis covers eight Northern California counties in the San Francisco Bay Area (San Francisco, Alameda, Contra Costa, San Mateo, Santa Clara, Solano, Sonoma, Marin) serving an estimated population of over six million people. The California active surveillance program, jointly funded by CDC and the local water utilities, identifies cases by contacting hospitals and other testing laboratories. It is known that passive reporting is incomplete because these testing sites often do not report all positive test results. Moreover, the active surveillance team asks for additional patient information which is not present on routine passive reporting forms. Case ascertainment is therefore more complete for diagnosed cases because of active detection.

Informed consent was obtained from all human adult participants and from parents or legal guardians of minors. This study received full human subjects approval for each year during the study period from four institutional review boards: the University of California, Berkeley; the California Department of Health Services; the CDC; and Public Health Foundation Enterprises, Inc.

Selection of cases and controls

Cases were recruited from those individuals identified through surveillance who had a stool test that was positive for *Cryptosporidium*. Cases who were reported to the CEIP from July 1999 through July 2001 were invited to participate in the case-control study and were screened for eligibility before administration of verbal consent and the questionnaire over the telephone. Of a total of 171 new cryptosporidiosis cases reported to the surveillance system, 26 (15%) were eligible and recruited for the study along with 62 age-matched controls. Major reasons for exclusion of cases included the presence of an immunocompromising condition (46%), participants who were not reachable after 15 telephone attempts (15%), and participants who refused interview (11%). All cases and controls were remunerated for their participation: $25 for a completed interview and an additional $10 for controls who provided a stool specimen used to rule out asymptomatic cryptosporidiosis.

Controls were category age-matched to cases in all instances. The study attempted to recruit an average of two to three controls for each case out of six possible types: sexual/household, non-sexual/household, sexual/neighborhood, non-sexual neighborhood, sexual/different water district, and non-sexual different water district. These represent all possible combinations of sexual contact (Yes/No) and location (household/ neighborhood/different water district). For all the non-sexual contacts recruitment was done using sequential random digit dialing (described below). For sexual contacts we asked the case to refer their sexual partners to us, if they wished to participate. For household controls of either type, sexual or non-sexual contact was asked at the end of the interview. We were unable to study each control group separately except for non-sexual/neighborhood controls because of very small numbers. The results are presented for all controls together as well as for the largest single group: non-sexual/neighborhood controls.

Progressive and sequential random digit dialing anchored on the telephone number of the case is a CDC-designed sampling scheme that we were required to use as part of the national study for the enrollment of neighborhood and different water district age-matched controls. When searching for an eligible control, for the first 100 calls the number to call was determined by progressively adding 1 to the last digit of the telephone number of the case, followed by subtracting 1 from the last digit of the telephone number of the case for the second 100 telephone calls. Household controls were recruited via the case after the case interview was completed. If an age-matched household control was available and willing to participate, we also determined whether they were sexual contacts of cases. A random number generating algorithm was used to identify controls who lived in other water districts than the cases. Sequential random digit dialing using the random number was then used to identify controls in the selected water district.

Participation rates for controls could not be calculated because the recruitment procedure involved population-based, random digit dialing for which we did not have a roster for the population controls, and therefore no denominator data.

Exposures

Our questionnaire was based on a standard CDC foodborne pathogen questionnaire that was adapted specifically for cryptosporidiosis. Most exposure questions referred to the two-week period prior to the onset date for the case. The following exposures, known to be transmission routes and risk factors for other similar enteric pathogens, were studied: drinking water quality (i.e., sources and post-tap treatment methods), and quantity (measured in glasses per day) at home and outside the home; travel; recreational water exposure including types of swimming locations and entering hot tubs; person-to-person fecal exposures, specifically, contact with child-care centers, diapered, and ill individuals; consumption of "risky" food items (risky foods refers to a list of standard food items asked in the CDC foodborne questionnaires, e.g. salads, cold cuts/meats, raw vegetables/fruits, raw oysters/shellfish, cider/juice), consumption of unpasteurized foods and handling of raw foods; zoonotic contact including farm animals and pets; and, for adults over 18 years of age, details on specific sexual practices. Sexual contact was retained as a separate category apart from other person-to-person fecal exposures since, unlike exposure to diapers and individuals in diapers, it may or may not involve exposure to feces. Additional information gathered included demographic characteristics and health status indicators.

Although all questions asked of the subjects refer to the two weeks prior to development of cryptosporidiosis and the statement is reiterated at several points during the questionnaire, it is likely that people do not recall exact consumption but tend to describe their usual patterns when asked about routine habits such as consumption of drinking water. For those who were traveling during the risk period this may or may not be the case.

For analytic purposes, composite variables for the exposure classification of cases and controls were created based on the biologic construct for each major mode of transmission. For drinking water, the potential of consuming oocyst-contaminated water was considered. Three categories were identified: those who drank exclusively boiled water, those who drank bottled or filtered water, and those who drank any amount of tap water without further

Table 1: Baseline demographic characteristics of cases and controls

Characteristic	*Cases*		*Controls*	
	N	**%**	***N***	**%**
Gender				
Male	13	50.0	33	53.2
Female	13	50.0	29	46.8
Race[a]				
White	17	65.4	43	69.3
Black	3	11.5	4	6.5
Native American	0	0.0	1	1.6
Asian/Pacific Islander	1	3.9	6	9.7
Other	4	15.4	8	12.9
Ethnicity[a]				
Hispanic	7	26.9	14	22.6
Non-Hispanic	18	69.2	48	77.4
County of residence[b]				
Alameda	2	7.7	9	14.5
Contra Costa	1	3.9	6	9.7
Marin	1	3.9	2	3.3
San Francisco	10	38.5	17	27.4
San Mateo	5	19.2	7	11.3
Santa Clara	6	23.1	18	28.0
Solano	1	3.9	1	1.6
Sonoma	0	0	2	3.2
Age group				
1–5 years	5	19.2	10	16.1
6–11 years	0	0.0	0	0.0
12–17 years	3	11.5	8	12.9
18–25 years	3	11.5	8	12.9
26–44 years	12	46.2	29	46.8
45–64 years	2	7.7	5	8.1
65+ years	1	3.9	2	3.2
Chronic medical condition				
Yes	9	34.6	16	24.2
No	15	61.5	46	74.2
Missing	1	3.9	1	1.6
TOTAL	26[c]		62	

[a]One case refused to provide information on race or ethnicity. [b]Distribution of this variable cannot be meaningfully evaluated because of matching across counties. [c]No matched controls were recruited for one case; only 25 were analyzed further

treatment. There were three sources of foodborne cryptosporidiosis: consumption of "risky" foods, consumption of unpasteurized food products, and handling of raw or uncooked items. All three sub-categories were evaluated in the analysis. Recreational water exposure included swimming, entering hot tubs or hot springs. The three person-to-person fecal exposures were: contact with child-care settings, contact with individuals with diarrhea, and exposure to diapers or diapered individuals. Animal contact referred to any type of contact with any animal. There were two travel questions which were not combined because they represent different levels of potential exposure to *Cryptosporidium*: traveling more than 100 miles from home and traveling to another country. Of the detailed sexual practices questions, only two were asked of all adults: whether they had had any sexual relations in the two-week risk period prior to the onset date of the index case, and the number of sexual partners in the last six months. The remaining questions on specific sexual practices were contingent on the respondent answering "yes" to the first question: there were insufficient data to address the role of these behaviors. A dichotomous sexual activity variable was constructed based on any sexual relations in the two-week risk period and more than one sexual partner in the last six months.

Analyses

Because of the large number of specific exposure variables and the small sample size, we realized that we did not have the power to study each specific exposure for which

we had collected data. Therefore, after a preliminary conditional logistic regression analysis of the univariate odds ratios, we decided to create one composite variable for each of the major transmission route categories to allow a more meaningful analysis. For univariate analyses 102 variables were evaluated. For the multivariate analyses, 13 composite variables were created *a priori* based on transmission route and then evaluated. The collapsed variables, described in the Exposures section, were based on the clinical relevance of the exposures as routes of transmission for *Cryptosporidium* oocysts. The reduction of analytic variables by combining several sub-categories was determined prior to conducting further multivariate analyses. Because drinking water is known to be the major epidemic route of transmission of *Cryptosporidium*, this study aimed at understanding its importance in non-outbreak situations. Therefore, in all multivariate models the drinking water variable was retained regardless of its statistical significance. Multivariate conditional logistic regression models were constructed using variables that were significant in the composite univariate analyses. All analyses were performed using Stata 7.0 (Stata Corporation, College Station, TX).

Results

Univariate analyses

The study was only able to enroll non-sexual contacts for each category of controls. Two household sexual contact controls were enrolled but the numbers were too small to be useful in sub-analyses. All 13 of the controls who agreed to have their stool tested for Cryptosporidium had negative test results. The baseline and demographic characteristics of cases and controls are presented in Table 1. The distribution of gender, race, Hispanic ethnicity, and chronic medical conditions for controls was similar to that of cases. Because controls were matched across counties for different water district controls, it is not possible to compare the distribution of county of residence between cases and controls; this is shown for descriptive purposes only. Since chronic medical condition was not found to be a significant factor in the univariate analysis, we did not pursue this further in multivariate modeling.

We compared demographic data obtained through routine surveillance of non-enrolled immunocompetent cryptosporidiosis cases to the cases enrolled in the study. Non-enrolled cases were mostly male (84.1%) and 91.3% were between the ages of 18 and 64 years. The race and ethnicity distributions of those enrolled and not enrolled were very similar, specifically non-enrolled cases were 70.6% white and 25.5% Hispanic. Of the 69 immunocompetent cases who did not participate in the case-control study and for whom we had data, broad risk factor information was only available for 17. Therefore, no risk factor comparisons were made with the enrolled cases.

Univariate odds ratios were determined for each of the individual questions on the questionnaire using conditional logistic regression (data not shown). Protective factors included filtering drinking water at home (OR [95% CI]: 0.28 [0.09, 0.88]); drinking three or more glasses of water at home (OR [95% CI]: 0.07 [0.01, 0.57]); handling raw or uncooked meat (OR [95% CI]: 0.10 [0.02, 0.48]) and raw or uncooked vegetables (OR [95% CI]: 0.26 [0.08, 0.86]); and suburban as opposed to urban area of residence (OR [95% CI]: 0.10 [0.01, 0.76]). The adverse risk factors in the univariate analyses were: consuming ice outside the home (OR [95% CI]: 3.35 [1.22, 9.24]) or meals outside the home (OR [95% CI]: 1.04 [1.00, 1.09] per meal consumed); living in an urban area (OR [95%CI]: 13.4 [1.7, 104]); and traveling over 100 miles from home (OR [95%CI]: 4.44 [1.53, 12.8]) or to another country (OR [95%CI]: 25.7 [3.28, 201]). Of borderline significance was increased sexual activity, measured as number of sexual partners in the last 6 months (OR [95%CI]: 1.82 [0.99, 3.32] increase per sexual partner).

Frequencies and univariate odds ratios with 95% confidence intervals and *p*-values for the association with cryptosporidiosis of the composite variables were calculated for each major transmission category and are presented in Table 2. Handling raw or uncooked foods (i.e., meat, fruit, or vegetables), was significantly protective (OR [95% CI]: 0.23 [0.06, 0.85]) whereas travel displayed a significantly higher risk, either over 100 miles from home (OR [95% CI]: 4.44 [1.53, 12.8]) or to another country (OR [95% CI]: 25.7 [3.28, 201]).

The association of cryptosporidiosis with drinking water was studied by comparing those who only drank boiled water to those who drank tap water with no further treatment. The univariate odds ratio for drinking tap water with no further treatment was not significant (OR [95% CI]: 0.86 [0.14, 5.43]). Unfortunately this "pure" (i.e. drinking only boiled water) subgroup consists of just over half of the total study population: 16 (61.5% of the total) cases and 35 (56.5% of the total) controls. In a more complete analysis the remaining "intermediate" risk group was also compared. This consisted of those who either filter their drinking water or drink bottled water. The results were similar to the previous analysis: for filtered or bottled water: OR [95% CI]: 0.74 [0.11, 5.02]; and for untreated tap water: OR [95% CI]: 0.92 [0.16, 5.30]. The test for linear trend was not significant.

Multivariate analyses

Multivariate conditional logistic regression models were constructed using the composite covariates that were significant in the univariate analysis. Specifically, we constructed models for handling raw or uncooked food items and travel (either to another country or more than 100

Table 2: Univariate analyses of composite variables constructed for each major route of transmission

EXPOSURE	***Cases***	***Controls***	***Univariate***	95% CI	P-value
	N (%)	***N (%)***	***OR***		
Drinking water					
Level of risk[a]					
Boil water	2 (*7.7*)	4 (*6.5*)	1.00		
Filter or bottle water	10 (*38.5*)	27 (*43.6*)	0.74	0.11, 5.02	0.754
Tap, no further treatment	14 (*53.9*)	31 (*50.0*)	0.92	0.16, 5.30	0.929
Recreational water					
Swimming, hot tub/spring	8 (*30.8*)	18 (*29.0*)	1.02	0.28, 3.75	0.973
Food sources[b]					
Unsafe foods consumed	22 (*84.6*)	59 (*95.2*)	0.38	0.08, 1.79	0.223
Handle raw foods	**5 (*19.2*)**	**28 (*45.2*)**	**0.23**	**0.06, 0.85**	**0.028**
All combined	22 (*84.6*)	60 (*96.8*)	0.25	0.44, 1.44	0.121
Travel					
>100 miles from home[c]	**17 (*65.4*)**	**18 (*29.0*)**	**4.44**	**1.53, 12.8**	**0.006**
To another country	**13 (*50.0*)**	**3 (*4.84*)**	**25.7**	**3.28, 201**	**0.002**
Person-to-person (fecal)					
Day care/camp contact	6 (*23.1*)	19 (*30.7*)	0.76	0.27, 2.14	0.604
Contact with diapers	12 (*46.2*)	31 (*50.0*)	1.03	0.38, 2.78	0.959
Contact with people with diarrhea	6 (*23.1*)	13 (*21.0*)	1.07	0.28, 4.09	0.927
All combined	15 (*57.7*)	39 (*62.9*)	0.76	0.28, 2.09	0.599
Animal contact	14 (*53.9*)	45 (*72.6*)	0.48	0.16,1.45	0.194
Sexual activity[d]	9(*52.9*)	20 (*45.5*)	1.59	0.44, 5.74	0.476

[a]Tests for trend: linear *P*-value = 0.674; non-parametric extension of Wilcoxon rank sum *P*-value = 0.660. [b]Unpasteurized food consumption could not be analyzed because of insufficient data. [c]This includes the subset who traveled to another country. [d]Any sexual relations in 2-week risk period or >1 sexual partner in last 6 months (asked of all adults over 18 years of age)

miles from home). The drinking water variable was included in these analyses since the association of drinking water with cryptosporidiosis was the primary hypothesis under examination in this study. Separate models were analyzed for both the entire study population and for the subset with neighborhood controls only. The neighborhood controls provide a comparison to the cases that is unbiased by the source of drinking water since neighborhoods are supplied by the same water utility. The results of the initial models are shown in Table 3. The magnitude of the adjusted odds ratio for potential exposure to *Cryptosporidium* oocysts via unboiled drinking water ranged from 1.58 to 2.62 for neighborhood controls and from 2.00 to 3.87 for all controls. Travel to another country was significantly associated with cryptosporidiosis in the analysis using all controls (OR [95% CI]: 20.9 [1.55, 279]) and borderline significant for the subset with neighborhood controls (OR [95% CI]: 12.3 [0.93, 162]). Multivariate models were also built with the "pure" drinking water exposure, which compared boiled to tap water drinkers (data not shown) but the only exposure associated with cryptosporidiosis was travel to another country (OR [95% CI]: 18.7 [1.16, 303]) for all controls.

Final multivariate models evaluating the association of drinking water and travel to another country with cryptosporidiosis are presented in Table 4 for all controls and for the subset using only neighborhood controls. The odds ratios estimated for drinking water were not significant: among those who drank filtered or bottled water the point estimates were 2.29 (all controls) and 2.11 (neighborhood controls only) that among boiled water drinkers, and this estimate was 3.56 (all controls) and 2.91 (neighborhood controls) among those who drank tap water without further treatment or processing. There was no evidence suggesting that local drinking water is an important source of cryptosporidiosis in this population. The risk of cryptosporidiosis for those who undertook foreign travel was significant with a point estimate of 24.1 [95% CI: 2.64, 220] for neighborhood controls and 34.7 [95% CI: 3.58, 327] for all controls. There were insufficient data with which to study interactions between drinking water risk and foreign travel. The only subgroup that could be analyzed was tap water ("yes") versus filter or bottle ("no") among the subset who did not travel out of the country, which demonstrated a non-significant odds ratio of 1.45 [95% CI: 0.37, 5.64].

Table 3: Multivariate model with water and univariately significant composite variables

EXPOSURE	**ALL CONTROLS**			**NEIGHBORHOOD CONTROLS**		
	Odds Ratio	***95% CI***	***P-value***	***Odds Ratio***	***95% CI***	***P-value***
Drinking water						
Boil water	1.00			1.00		
Filter or bottled	2.00	0.09, 46.8	0.666	1.58	0.05, 51.7	0.796
Tap water[a]	3.87	0.20, 74.0	0.369	2.62	0.11, 57.5	0.541
Handle raw foods[a]	0.60	0.13, 2.85	0.526	0.44	0.07, 2.88	0.392
Travel 100 miles from home	1.48	0.35, 6.29	0.599	1.33	0.28, 6.29	0.716
Travel to another country	**20.9**	**1.55, 279**	**0.022**	12.3	0.93, 162	0.057

[a]Without further treatment or processing. [b]Includes handling raw meat, fruit, vegetables

Table 4: Multivariate model with drinking water and travel to another country

EXPOSURE	**ALL CONTROLS**			**NEIGHBORHOOD CONTROLS**		
	Odds Ratio	***95% CI***	***P-value***	***Odds Ratio***	***95% CI***	***P-value***
Drinking water						
Boil water	1.00			1.00		
Filter or bottled	2.29	0.21, 60.6	0.589	2.11	0.08, 54.7	0.652
Tap water[a]	3.56	0.11, 46.5	0.381	2.91	0.15, 54.9	0.477
Travel to another country	**34.7**	**3.58, 327**	**0.002**	**24.1**	**2.64, 220**	**0.005**

[a]Without further treatment or processing

Discussion

In the US the potential for drinking water to be associated with cryptosporidiosis among immunocompetent persons is relevant since most major known outbreaks of cryptosporidiosis involved transmission through contaminated water [10,11,15,16,21–27]. Additionally, other modes of transmission are of interest because of published reports implicating them (such as food, travel, institution-associated, sexual behavior, etc.) [1,2,28–52]. This study was undertaken as the first population-based study in the United States of risk factors for endemic cryptosporidiosis in immunocompetent persons.

The major finding in this study was that travel to another country is a very strong and significant risk factor for cryptosporidiosis transmitted in non-outbreak settings in the San Francisco Bay Area. This is consistent with studies of other enteric pathogens [47,48]. In fact, intestinal protozoa are the most common infecting organisms identified in travelers with chronic diarrhea; the precise etiology of traveler's diarrhea is unclear but it is believed that duration of stay, hygiene, and level of socioeconomic development in the host country are associated with acquisition of infection [48]. The current study was unable to evaluate any of these factors because of limited sample size, but it was observed that 7 of the 13 cases (50.0%) who traveled outside the United States traveled to Central America or Mexico, compared to one out of three controls (33.3%). This finding is very similar to a laboratory-based survey conducted in the mid-1980s in Canada where 52% of the cases of cryptosporidiosis had traveled to Mexico [54]. There is, however, a possibility that ascertainment bias could be the reason that foreign travel was found to be a significant risk factor in our study. If patients presenting with diarrhea who have traveled recently are more likely to have a fecal sample taken, then this will appear as a risk factor. Since we have no information on the likelihood of diagnosis given travel history in the clinical setting in the Bay Area we were unable to evaluate whether this was indeed the case.

Another important finding of this study is that consumption of tap water without further treatment or processing was not associated with cryptosporidiosis when compared to drinking boiled water. Our data were too few to allow further evaluation of the fact that there was a consistent finding of an increased point estimate for tap water in the adjusted analyses. The adjusted analyses involved the

composite variables described in the Methods section with the purpose of constructing the most parsimonious model. The reversion of the odds ratio for water from a protective effect in the univariate analysis to an elevated risk in the multivariate is probably associated with its interaction with travel. Because of the small numbers in this study, this interaction could not be addressed – either by looking at interactions in the model or via a stratified analysis. We do, however, feel that the results in this paper will greatly benefit the design and planning of future studies of cryptosporidiosis particularly with respect to understanding the relationship between water consumption and travel.

A study conducted in South Australia in 1993 using a similar approach found that only water-related risks, i.e. rain and spring water consumption, were significantly associated with cryptosporidiosis [55]. But more powerful, recent studies in the same country found no elevated risk for plain tap water consumption [53]. Given our difficulties in recruitment and sometimes conflicting data, such studies must be done in multiple communities simultaneously to obtain an accurate picture of the local situation. The larger CDC study, which will pool data from all seven EIP sites around the country, may be able to better address the issue of drinking water-related risk factors because of the resulting larger sample size. However, even with a larger dataset, the multi-site study will not be able to clarify the risk of cryptosporidiosis from drinking water specifically for residents of the San Francisco Bay Area.

Our planned sample size, determined by available funding, was for 100 cases and 200 controls. Such a sample size would allow the detection of a difference of 15% in exposure to tap water between cases and controls with 80% power and a 0.05 level of statistical significance. We assumed (based on consumer survey data from the Water Quality Association http://www.wqa.org/) that 70% of the controls used tap water without further treatment. Based on the difference in exposure to tap water that we actually observed (4%), a study would require approximately 2500 cases and 2500 controls to detect a statistically significant difference in the use of tap water. Wide confidence intervals for some of the other point estimates indicate that clinically important findings cannot be conclusively ruled out for residents of the San Francisco Bay Area.

An intriguing observation in this study is that exposures that would generally be considered to increase the risk of cryptosporidiosis, such as contact with fecal matter, exposure to raw or uncooked food items, and animal contact, did not have elevated risks. One theoretical explanation for this was offered by Casemore [56] who expressed the opinion that long-term, low-level exposures to oocysts in raw vegetables and in unpasteurized products may confer protection by boosting the immune system. The recent Australian studies also found significant protective effects of contact with pets and consumption of uncooked vegetables [53]. Indeed, it is likely that acquisition of specific antibodies and an effective protective cellular immune response requires repeated exposure [48,57]. Although the exposures in this study were assessed for the two-week risk period prior to the onset date of the case, it is probable that some of these exposures are no different in the long and the short term, which may account for the protective effect. Serologic evaluation of cases and controls for immunity to cryptosporidiosis prior to enrollment may be a worthwhile addition to such case-control studies. Serologic definitions of *Cryptosporidium* exposure were considered as a potential outcome for the current study, especially for controls, but were not feasible due to resource and logistic constraints. Because symptomatic cryptosporidiosis cases represent only a small fraction of those who may be infected with *Cryptosporidium*, the conclusions drawn from a study such as ours may not apply to those asymptomatically infected.

Conclusions

Future studies that aim to address the drinking water issue in greater detail must bear in mind the appropriate metric for measuring individual exposure to potentially oocyst-contaminated drinking water. A continuous measure that explicitly differentiates between the amount of tap and of other types of water consumed would more likely suitably address the issue. In addition, studies that aim to assess the impact of travel to endemic countries will also need to conceptualize the myriad causal pathways and the possible role of each route of transmission, e.g. waterborne, foodborne, person-to-person, comprising the complex web of factors that are responsible for traveler's diarrhea. More detailed studies will be required to elucidate the specific behaviors and practices that expose travelers to *Cryptosporidium* in endemic countries, and possibly the determinants of protective immunity in these individuals. Studies are required that stratify subjects on the basis of their travel history prior to analyzing other cryptosporidiosis exposures so that it is possible to distinguish between the specific travel and non-travel associated risk factors. In particular, the differentiation must be made between the quantity of different types of water consumed in the United States and that consumed in other, less developed countries.

Competing interests

None declared.

Authors' contributions

AK, DV, and JC designed and coordinated the study. AK, JN, and GR implemented the subject recruitment, data

collection (interviews) and collation. AK coordinated the study and carried out the statistical data analysis under the guidance of JC. DV and JC conceived of the study.

All authors read and approved the final manuscript.

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