Cryptosporidium infection, onsite wastewater systems and private wells in the arid Southwest

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ABSTRACT

Few prior studies have examined the potential health risks from transmission of enteric parasites via aquifers contaminated by wastewater from onsite systems. A cross-sectional study of 600 residents in households served with either onsite wastewater systems and private wells or city sewer/water systems in three different sites in central New Mexico compared serological responses to *Cryptosporidium*, a common waterborne infections agent. Study participants completed a short self-administered questionnaire with questions on demographic characteristics, characteristics of the onsite wastewater system and private well, and common risk factors associated with cryptosporidiosis. A sample of household tap water was collected, as well as a blood sample from each study participant to measure IgG responses to antigen groups for *Cryptosporidium*. Logistic regression analysis showed a significant association between having an onsite wastewater system and private well and the 27-kDa marker for *Cryptosporidium* in the River Valley site after adjusting for covariates (OR = 1.98; 95% CI = 1.11–3.55). This study, together with one prior study, suggests that the presence of onsite wastewater systems and private wells might be associated with an increased risk of *Cryptosporidium* infection.

Key words | drinking water quality, groundwater, wastewater, waterborne infectious diseases, water epidemiology

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INTRODUCTION

Recently, there has been concern that onsite wastewater systems, especially in areas that are densely populated or have porous soils, may increase the risk of enteric disease transmission (Carroll *et al.* 2004, 2006; Zarate-Bermudez 2009). Surveillance of waterborne disease outbreaks in the United States has suggested that outbreaks of gastrointestinal (GI) illnesses are associated with failed onsite wastewater systems (Craun & Calderon 1996). Borchardt *et al.* (2003) found that septic system densities were associated with endemic diarrheal risk in children in Wisconsin. Denno *et al.* (2009) also found that *Salmonella* infection was also associated with use of private wells and residential onsite wastewater systems. The United States Environmental Protection Agency (US EPA) has estimated that annually there are 168,000 viral illnesses and 34,000 bacterial illnesses due to contamination of public ground water systems (US EPA 2000).

Approximately 20% of US households rely on onsite wastewater systems for treatment and disposal of domestic sewage (US EPA 2008). In rapidly growing rural areas of the USA, onsite wastewater systems are the most common method of sewage treatment and disposal. Onsite wastewater systems include any system for the collection, storage, treatment, neutralization or stabilization of sewage that occurs on a property. A common type of onsite wastewater system is a septic system that is designed to remove solids in a septic tank and disperse the wastewater in a leach field that relies on soils to perform secondary

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treatment of liquid waste. Under optimum conditions, an onsite wastewater system will remove 99.9% of viruses and other wastewater constituents (Van Cuyk *et al.* 2004). However, a US EPA review of studies found failure rates ranging from 10 to 20%, resulting in groundwater contamination (US EPA 2002). Furthermore, detailed information on soil characteristics in areas with onsite systems is generally unavailable.

Data from the United States Geological Survey estimate that groundwater supplies almost 50% of the American population with drinking water; groundwater was the primary source of the water for 98% of people using private wells (Hutson *et al.* 2004). The growing use of onsite wastewater systems and the reliance on groundwater sources for drinking water has raised concerns among local and state public health agencies, the US EPA, and the Centers for Disease Control and Prevention over the potential health effects of these trends and the risks to sensitive populations such as children and those with compromised immune systems (ASTHO 2009).

The increased population growth outside urban areas has increased the pressure on rural and semi-urban areas to absorb more housing. Because of greater distances between houses in rural areas and the costs of building new centralized wastewater treatment plants, developers and home-owners often rely on relatively inexpensive onsite wastewater systems. Very often the homes served by onsite wastewater systems are also served by private wells drilled on the same building lot. These private wells are not regulated by the US EPA under the Safe Drinking Water Act. State and local regulations may regulate well construction, but not water quality. State and local regulations for locating wells near sources of contamination may specify minimum distances (at least 50 feet) between the well and onsite wastewater system. This is seldom adequate and can be especially inadequate in certain soils that facilitate the rapid movement of pathogens from the onsite wastewater system to the well.

Unfortunately, soil characteristics that might predict protection from pathogens near drinking water wells remain either unknown or unexamined during the well drilling process. Well water testing generally only examines bacterial indicators of contamination. Since the indicator bacteria are much more fragile than parasites, the absence of indicator bacteria may provide little useful information about the risks of parasite contamination.

Reviews of waterborne outbreaks in the United States have suggested that groundwater contamination is a very common mode of parasite and virus transmission (Barwick *et al.* 2000; Craun & Calderon 2000; Frost *et al.* 2002; Lee *et al.* 2002; Craun *et al.* 2006; Liang *et al.* 2006; Yoder *et al.* 2008). However, outbreaks account for only a very small fraction of all infections and illnesses. For many infectious agents, infections do not always result in disease, disease clusters, or severe disease. More commonly, infectious agents may result in only a few infections at any one time, and, therefore, are never recognized as an epidemic. This is likely to be especially common in sparsely populated rural areas where few infections occur.

For viral gastrointestinal illnesses, enteric viruses may be transmitted by either direct transmission, such as food or water contamination, or via person-to-person transmission. Since a large fraction of viral gastrointestinal agents are transmitted person-to-person (Morens *et al.* 1979), it is often difficult to identify the food or waterborne sources for these viral agents. In contrast, the parasite *Cryptosporidium* does not appear to be commonly transmitted person-to-person. One study of a 1993 Milwaukee, Wisconsin *Cryptosporidium* outbreak found that only 5% of *Cryptosporidium* infections among adults may have been due to person-to-person transmission (MacKenzie *et al.* 1995). A second study estimates secondary transmission rates were slightly higher at 10% (95% CI = 6–21%) (Eisenberg *et al.* 2005). Therefore, it is possible to use serological prevalence of *Cryptosporidium* antibody to identify prior food, waterborne, or other routes of *Cryptosporidium* infections. We believe that *Cryptosporidium* antibody levels, an indicator of prior infection, may effectively identify populations exposed to prior oocyst contamination of food or water, especially when the level of oocyst contamination may be low.

No prior studies have compared the risks of prior *Cryptosporidium* infection among individuals having both an onsite wastewater system and a private well compared to users of municipal drinking water systems. In this study, we examined whether living in a household with an onsite wastewater system and private well was associated with increased serological responses to *Cryptosporidium*. An elevated serological response to *Cryptosporidium* was used as an indicator of exposure to and/or infection by this common waterborne pathogen. A serological test for this pathogen is readily available and has already been validated (Frost & Craun 1998; Frost *et al.* 1998a, b, 2000, 2002). We also analyzed the drinking water for common indicators of contamination (fecal and total coliform, enterococcus, and coliphage). We hypothesized that study participants who were living in households with onsite wastewater systems and private wells would be more likely to have elevated serological responses compared to those living in households using city sewer and water systems because of increased exposure to *Cryptosporidium* oocysts.

METHODS

The study design was a cross-sectional study of 600 residents in households served with either onsite wastewater systems and private wells or city sewer/water systems in three different geographic sites in the greater Albuquerque, New Mexico area. The three included: (1) a semi-rural area on the river valley floor of the Rio Grande in north and north-central Albuquerque (River Valley), (2) a more urban area on an alluvial plain in northeast Albuquerque (Alluvial Plain), and (3) a semi-rural area with limestone soils in the Sandia and Manzano Mountains foothills east of Albuquerque (East Mountains). Households were selected to participate based on whether they used an onsite wastewater system and a private well (exposed group) or city sewer/water systems (unexposed group). The River Valley and Alluvial Plain sites included a mix of households using these two types of systems. For these two sites, the exposed and unexposed groups lived in the same geographic areas. Unfortunately, there were no municipal sewer and water systems in the East Mountains site. The River Valley unexposed households were used as the comparison group for the East Mountains site because of their comparability in the rural nature of their neighborhoods that included relatively undeveloped lots.

Study participants were recruited using neighborhood association meetings, flyers, advertisements in local papers, mailings to residential addresses in the study area, and telephone calls. Addresses and phone numbers for residences in the study areas were purchased from a commercial vendor. Individuals who expressed interest in participating in the study were screened for eligibility. Criteria for eligibility to participate included being at least 18 years of age, living in the site area for at least 1 year, drinking tap water which was not filtered or had not been treated by reverse osmosis, and being in overall self-reported good health. Consent was obtained from individuals eligible to participate in the study using the protocol approved by the University of New Mexico Human Research Review Committee. Arrangements were also made to collect blood sera, administer a brief questionnaire, and collect a water sample if the participant lived in a household using an onsite wastewater system and private well. For the River Valley site, questionnaire data, water and blood samples were collected over a 1 year time period from November 2004 to October 2005. For the East Mountains site, this information was collected from May to October 2006, and from August 2006 to February 2007 for the Alluvial Fan site.

A short self-administered questionnaire was completed at the time of the blood draw. Information on the questionnaire included: demographic characteristics, length of time at the residence, age of wastewater system and frequency of pumping, depth of the well, type of water supply, amount of water consumed in the past 24 hours, past diagnoses of cryptosporidiosis, diarrhea (three or more loose bowel movements a day) that lasted 4 or more days in the past 2 months, and number of episodes of gastrointestinal illness (diarrhea, vomiting, nausea) in the past year. The questionnaire also collected information on common risk factors associated with cryptosporidiosis such as having young children, having children in day care, changing diapers, caring for someone with diarrhea, visiting someone in hospital, handling pets, young pets, and livestock, engaging in water activities, having plumbing work done in the home and traveling outside the USA. The information on these risk factors was used to identify confounding variables in the regression models.

Water samples

Field staff collected a sample of the participant's home tap water in accordance with the US EPA Method 1601 for coliphage and *Standard Methods for the Examination of Water and Wastewater* protocols for the bacterial indicators. Two sterile water bottles, a 500 mL and a 1 L bottle, were used for each sample. The water was collected from either the kitchen faucet (preferable) or an outside hose bib. The water was allowed to run for 3–5 minutes at a rate that flushed the lines but did not cause splash back onto the faucet. After the flush time had elapsed, the bottles were filled to within 1 inch of the top. The bottle and cap connection were sealed using parafilm, stored in gallon zip-lock bags, and shipped in a cooler to Dr Marylynn Yates' laboratory at the University of California, Riverside for analysis.

The water samples were tested for coliphage and other indicators of fecal contamination. Somatic coliphage analyses were performed using the two-step enrichment procedure, US EPA Method 1601. The water samples were also tested for total coliforms (Standard Methods: Standard Total Coliform Membrane Filter Procedure. Sect. 9222.B), fecal coliforms (Standard Methods: Fecal Coliform Membrane Filter Procedure. Sect. 9222.D), and enterococci (Standard Methods: Fecal *Streptococcus* and *Enterococcus* Groups Membrane Filter Techniques Sect. 9230.C).

Once the water results were available, each household was sent a letter stating whether their total coliform results showed a presence or absence of bacteria. If total coliform bacteria were present, the letter also included information on how to chlorinate the well and telephone numbers for the county environmental health office.

Blood samples

Blood samples were collected using vacutainers to collect a 5 mL volume of whole blood. Blood was allowed to clot at room temperature for 30 minutes and was centrifuged according to American Society for Clinical Pathology protocols. The serum was transferred to a cryotube for frozen storage and shipment. Sera samples were not labeled with names of individual study participants.

Sera were analyzed by immunoblot to measure IgG serological response to the 15/17-kDa and 27-kDa antigen groups. This method, which uses the miniblot format, has been described in detail elsewhere (Frost *et al.* 1998a, b). The intensities of the serological responses to the 15/17-kDa and 27-kDa antigen groups on the immunoblots were digitally analyzed by an IS-2000 Digital Imaging System (Alpha Innotech). Serological responses to the two antigen groups were 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 quality control procedures, involving digitizing response intensities, were standardized to the responses of a reference sample. They also maintained relatively low costs to accurately identify responses and estimate the intensity of each response.

The IgG result for each sample specimen was standardized by computing the ratio of the response intensity for the sample specimen to the response intensity for a positive control serum contained on each blot. A strong serological response was defined as having a ratio of at least 0.20 (20% of the response of the standard positive control). The IgG positive control sera were 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 sera were used on all blots. For comparison purposes as a quality control measure, we had laboratory results from hundreds of individuals from many countries (USA, Canada, Australia, New Zealand, South Africa, Hungary, Italy, Russia, and UK) (Frost *et al.* 2000, 2002, 2004, 2005a, b; Duncanson *et al.* 2003; Egorov *et al.* 2004; Kozisek *et al.* 2008).

Data analysis

Descriptive statistical analyses used the Pearson chi-square test to assess associations between the exposure (onsite wastewater and water systems), categorical outcome (serological response), and covariates. The Mann–Whitney U test was used to examine differences among continuous serological results (intensity of response) and other variables not having a normal distribution. Multivariate logistic regression models examined the association between having an onsite wastewater system/private well and having a strong response to the 15/17-kDa or the 27-kDa markers defined as at least 20% of a positive control and controlled for potential confounding factors that might explain elevated or reduced risks of prior infection. The comparison city system group for the East Mountains site was that from the River Valley since both areas were more rural and the lifestyle factors were more similar than the more urban Alluvial Fan site. Variables were manually selected for the final models, and the models always included the exposure (having an onsite wastewater system/private well). Risk factor and behavior variables, as well as the two illness measures (diarrhea lasting more than 4 days in the past 2 months and having at least one gastrointestinal illness in the past year), were entered into the full model if they were found to be associated with the outcome in the univariate analyses (Pearson χ^2 test p-value ≤ 0.25). Covariates were removed from the model if they were non-significant at the 0.1 alpha level and did not change the estimate of the main effect by more than 20%. Each of the three geographic sites was modeled separately.

RESULTS

The demographic characteristics of the study participants from the five groups are shown in Table 1. A significantly higher percentage of participants with onsite wastewater systems/private wells living in either the River Valley site or the East Mountains site were men compared to those using city sewer/water systems ($p = 0.008$ and $p = 0.010$, respectively). A significantly higher percentage of participants with onsite wastewater systems/private wells living in the Alluvial Fan site were over the age of 40 years compared to those using city sewer/water systems ($p = 0.001$).

Table 1 | Demographic characteristics of study participants by geographic area and type of onsite wastewater system/private well

	City systems		Onsite wastewater system/private well		
	River Valley $N = 99$	Alluvial Fan $N = 134$	River Valley $N = 101$	Alluvial Fan $N = 134$	East Mountains $N = 130$
Gender (%):					
Male	23.2	35.1	40.6[a]	46.3	50.8
Female	76.8	64.9	59.4	53.7	49.2
Age group (%):					
18–39 yrs	20.4	23.9	10.9	8.3[b]	16.2
40–49 yrs	22.4	20.1	24.8	36.4	25.4
50–59 yrs	24.5	27.6	32.7	29.5	30.8
60+ yrs	32.7	28.4	31.7	25.8	27.7
Race/ethnicity (%):					
NHW[c]	62.6	80.6	73.3	84.3	83.8
Other	36.4	19.4	25.7	15.7	16.2
Education					
Non-college grad	39.4	29.9	35.6	20.1	34.6[d]
College grad	60.6	70.1	64.4	79.9	65.4

[a]Difference between city and onsite system participants, chi-square p-value $=0.008$.
[b]Difference between city and onsite system participants, chi-square p-value $=0.001$.
[c]Non-Hispanic white.
[d]Difference between city and onsite system participants, chi-square p-value $=0.010$.

Information on risk factors for cryptosporidiosis and gastrointestinal illness was also collected from the questionnaire (Table 2). The percent of participants reporting that they had a specific risk factor varied considerably. For example, only 1.0–4% of participants reported drinking untreated water away from home, whereas 85.8–93.8% of participants reported handling pets. There were significant differences among the five groups in the percent of participants reporting handling young pets ($p = 0.001$), handling livestock ($p = 0.001$), swimming or wading in a lake or stream ($p = 0.011$) and traveling outside the USA in the past 12 months ($p < 0.001$).

The percent of participants reporting that they had had diarrhea lasting 4 or more days in the past 2 months ranged from 4.5% in Alluvial Fan participants on city systems to 11.9% in River Valley participants with onsite wastewater systems/private wells (Table 2). Approximately one-half of all participants reported that they had had at least one gastrointestinal illness in the past year, ranging from 46.9% in East Mountain participants to 59.6% of River Valley participants on city systems. The differences in percentages of diarrhea and gastrointestinal illness did not differ among the five sites.

The results of the water analyses are reported in Table 3 as the percent of samples testing positive for total coliforms, fecal coliforms, enterococci, and coliphage. One water sample was collected from each household during the study time period. The range in colony counts is also shown in Table 4. A total of 83 samples tested positive for total coliforms, 22 tested positive for fecal coliforms, 11 tested positive for enterococci, and 1 tested positive for coliphage. Two samples, one from the River Valley and one from the East Mountains, tested positive for total coliforms, fecal coliforms, and enterococci. The sample testing positive for coliphage also tested positive for total coliforms. The percentages of samples testing positive on any of the four tests were significantly different in the three areas: 16.8% (River

Table 2 | Risk factors associated with cryptosporidiosis and gastrointestinal illness in study participants

Risk factor	City systems River Valley $N = 99$	Alluvial Fan $N = 134$	Onsite wastewater system/Private well: River Valley $N = 101$	Alluvial Fan $N = 134$	East Mountains $N = 130$
Children under 5 yrs in household	9.1%	17.9%	7.9%	11.9%	14.6%
Child attends daycare	9.1%	12.7%	10.9%	9.0%	10.8%
Handled child with diapers	21.2%	31.3%	18.8%	23.1%	20.2%
Cared for someone with diarrhea	14.1%	23.1%	16.8%	20.9%	19.7%
Visited anyone in hospital	43.4%	53.0%	48.5%	51.1%	46.9%
Handled pets	90.9%	85.8%	91.1%	88.8%	93.8%
Handled young pets	45.5%	23.9%	44.6%	34.3%	30.0%
Handled livestock, wild animals, or zoo animals	26.3%	10.4%	42.6%	17.2%	28.5%
Drank untreated water from lakes or streams	1.0%	1.5%	4.0%	2.2%	2.3%
Swam or waded in a lake or stream	22.2%	20.1%	34.7%	26.9%	36.9%
Swam or waded in a pool, hot tub, or water park	58.6%	52.2%	45.5%	55.2%	60.8%
Had plumbing done in the home	23.2%	27.6%	29.7%	19.4%	19.2%
Traveled outside the USA	14.1%	18.7%	30.7%	38.1%	21.5%
Had diarrhea lasting 4+ days in past 2 months	10.1%	4.5%	11.9%	7.5%	8.5%
Had at least 1 episode GI illness in past yr	59.6%	57.5%	57.4%	51.5%	46.9%

Table 3 | Results from household water samples for the three geographic areas with onsite wastewater systems/private wells

Type of test	Percent positive results: River Valley $N = 101$	Alluvial Fan $N = 133$	East Mountains $N = 128$
Total coliforms	16.8%	24.8%	25.8%
Colony counts	1–35	1–TNTC[a]	1–TNTC
Fecal coliforms	2.7%[b]	4.5%	10.9%
Colony counts	1	1–16	1–68
Enterococci	2.0%	3.0%	3.9%
Colony counts	1–3	1–39	1–TNTC
Coliphage presence	0.0%	0.0%	0.8%
Any agent	16.8%	27.8%	31.5%

[a]Too numerous to count.
[b]Only 69 wells tested for fecal coliforms.

Table 4 | Percent of participants with a strong response (≥20% of a positive control) to the serological marker for each site by type of wastewater and water system

Serological marker	River Valley City	River Valley Onsite	Alluvial Fan City	Alluvial Fan Onsite	East Mountains Onsite[a]
	$N = 99$	$N = 134$	$N = 101$	$N = 134$	$N = 130$
15/17-kDa	37.4%	43.6%	35.8%	26.9%	39.2%
27-kDa	47.5%	61.4%[b]	57.5%	59.7%	43.8%

Percent of respondents with a strong response to the marker.

[a]Compared to River Valley city system participants.
[b]Chi-square test, $p = 0.048$, for comparison city and onsite.

Valley), 27.8% (Alluvial Fan), and 31.5% (East Mountains) ($p = 0.036$).

The results from the serological analyses for *Cryptosporidium* are shown in Table 4 as the percent of participants with a strong response (an intensity of ≥20% of a positive control) to the two markers (15/17-kDa and 27-kDa). Participants using onsite wastewater systems/private wells in the River Valley and Alluvial Fan sites were compared to those using city sewer/water systems in the River Valley and Alluvial Fan sites, respectively. In the East Mountains, the participants were compared to participants from the River Valley using the city sewer/water systems because of the comparability of the rural nature of the neighborhoods.

There was a significantly higher percentage of participants using onsite wastewater systems/private wells in the River Valley who had a strong response to the 27-kDa antigen than participants using city sewer/water systems ($p = 0.048$). However, there were no significant differences in the percent of participants with a strong response to the 15/17-kDa antigen between participants using onsite wastewater systems/private wells and those using city sewer/water systems in any of the groups. It should be noted that response to the 15/17-kDa antigen is shorter lived and returns to background levels in several months following an infection, whereas the response to the 27-kDa antigen remains elevated for 9 months or longer (Muller *et al.* 2001). Therefore, using the 27-kDa increases the likelihood that differences will be detected between the two exposure groups.

The relationship between well contamination (measured by any positive test for an indicator microorganism) and a strong serological response to the 15/17-kDa and 27-kDa markers were also examined (Table 5). There was no significant association between having a contaminated well and showing a strong response to either of the markers.

Logistic regression models were completed to determine whether having an onsite wastewater system/private well was associated with a stronger serological response for each of the antigen groups after controlling for other confounding risk factor variables (Table 6). The final models for each site are shown for each of the two markers. A significant association was observed between using onsite wastewater systems/private wells and the 27-kDa marker in the River Valley. Participants using onsite wastewater systems/private wells were almost two times more likely (OR = 1.98; 95% CI = 1.11–3.55) to have a strong serological response to the 27-kDa marker compared to those using the city sewer and water systems after controlling for handling livestock, the only risk factor variable entering the model.

This positive association between having onsite wastewater/private well systems and a strong response to the 27-kDa marker was also observed for the Alluvial Fan site. The odds ratio for participants using onsite wastewater systems/private wells was 1.28 after controlling for several risk factors, but it was not significantly elevated (95% CI = 0.74–2.21). In this group, several risk factors were significantly associated with an increased adjusted odds ratio: having plumbing done in the home (OR = 2.11; 95% CI = 1.10–4.03), handling pets (OR = 2.83; 95% CI = 1.24–6.49), and being 60 years of age or older (OR = 4.20; 95% CI = 1.79–9.89). In the East Mountains, this increase in the odds ratio was not observed for the overall effect, but participants in the East Mountains 60 years of age or older were over three times more likely to have a strong response (OR = 3.69; 95% CI = 1.61–8.46).

For the 15/17-kDa marker, there was no significant association between using onsite wastewater systems/private wells and having a strong response in any of the three groups. However, the overall adjusted odds ratio in the River Valley group was elevated (OR = 1.34; 95% CI = 0.74–2.44). In the River Valley, the adjusted odds ratio for handling a child with diapers was significantly increased (OR = 2.24; 95% CI = 1.07–4.69) and being of a race/ethnicity other than non-Hispanic white significantly increased the adjusted odds ratio (OR = 1.92; 95% CI = 1.01–3.66). Participants residing in the Alluvial Fan had significantly

Table 5 | Relationship between well contamination and serological response to *Cryptosporidium* antigen for participants with onsite wastewater systems/private wells

	No. of private wells tested	Participants with a strong response to 15/17-kDa % (N)	*p*-value	Participants with a strong response to 27-kDa % (N)	*p*-value
River Valley					
Uncontaminated	84	40.5% (34)	0.164	61.9% (52)	0.812
Contaminated	17	58.8% (10)		58.8% (10)	
Alluvial Fan					
Uncontaminated	96	30.2% (29)	0.189	60.4% (58)	0.700
Contaminated	37	18.9% (7)		56.8% (21)	
East Mountains					
Uncontaminated	87	39.1% (34)	0.922	47.1% (41)	0.200
Contaminated	40	40.0% (16)		35.0% (14)	

Table 6 | Results of logistic regression analysis examining the effect of having an onsite wastewater system/private well on serological responses to *Cryptosporidium* antigens

		15/17-kDa marker		27-kDa marker	
Geographic area	Variables in model	Odds ratio	95% CI	Odds ratio	95% CI
River Valley	Onsite wastewater system/private well	1.34	0.74–2.44	1.98	1.11–3.55
	Handled livestock			0.53	0.29–0.97
	Handled a child with diapers	2.24	1.07–4.69		
	Age: 18–39 yrs	1.00			
	40–49 yrs	1.95	0.70–5.37		
	50–59 yrs	1.68	0.61–4.60		
	60+ yrs	4.00	1.29–9.00		
	Other race/ethnicity than NHW	1.92	1.01–3.66		
Alluvial Fan	Onsite wastewater system/private well	0.64	0.35–1.17	1.28	0.74–2.21
	Child attends daycare	0.34	0.09–1.35		
	Plumbing work done in home	2.26	1.16–4.39	2.11	1.10–4.03
	Handled pets (cats, dogs)			2.83	1.24–6.49
	Age: 18–39 yrs	1.00		1.00	
	40–49 yrs	1.05	0.35–3.17	0.89	0.40–2.01
	50–59 yrs	1.85	0.64–5.30	1.80	0.80–4.03
	60+ yrs	7.31	2.60–20.58	4.20	1.79–9.89
	College graduate	0.49	0.25–0.94	0.37	0.19–0.72
East Mountains	Onsite wastewater system/private well	1.07	0.62–1.84	0.85	0.49–1.46
	Handled pets (cats, dogs)	0.23	0.08–0.68		
	Age: 18–39 yrs			1.00	
	40–49 yrs			1.76	0.74–4.18
	50–59 yrs			1.92	0.83–4.43
	60+ yrs			3.69	1.61–8.46

elevated adjusted odds ratios if they were 60 years of age or older (OR = 7.31; 95% CI = 2.60–20.58); adjusted odds ratios were significantly reduced for participants who were college graduates (OR = 0.49; 95% CI = 0.25–0.94). In participants from the East Mountains, the odds ratio was significantly reduced if they had handled pets (cats, dogs) (OR = 0.23; 95% CI = 0.08–0.68).

Neither of the two illness variables (diarrhea and gastrointestinal illness) was included in the final models for the two serological markers. For River Valley participants, they were not included in the model because they did not reach the required cutoff level in the univariate analyses of their association with the outcome variables. The initial models for the Alluvial Fan site for the 27-kDa marker included both variables, but they both dropped out of the final model. For the East Mountains site, the variable for diarrhea was included in the initial model for the 15/17-kDa marker and the variable for gastrointestinal illness was included in the initial model for the 27-kDa marker. Again, both dropped out of the final analysis.

DISCUSSION

According to the American Water Works Association Research Foundation (AWWA) an American family of four uses 400 gallons of water each day (AWWA 1999). Approximately 70% of that is used indoors, 26.7% of which is used by the toilet. Approximately one-fifth of US homes use septic systems (US EPA 2008). The ability of a viable pathogen to survive from septic tank discharge to entering the groundwater depends on a number of factors, including pathogen-specific survival time in the soil. On the soil surface, elevated temperatures significantly reduce oocyst survival (Jenkins *et al.* 2002). However, if released below surface levels, such as in a septic tank drain field, where the oocysts experience reduced temperatures, *Cryptosporidium* oocysts are believed to survive 60–180 days after leaving the septic tank (Salvato 1992).

Detecting parasites in the water is both difficult and expensive. Furthermore, it is subject to very wide confidence intervals. Previous studies have suggested that groundwater contamination may be a route for diarrheal diseases from bacteria, viruses, and parasites (Barwick *et al.* 2000; Craun & Calderon 2000; Frost *et al.* 2002; Lee *et al.* 2002; Craun *et al.* 2006; Liang *et al.* 2006; Yoder *et al.* 2008). However, these studies have only focused on detectable outbreaks and have not examined infection risks from residential onsite wastewater systems combined with private wells in the absence of an outbreak. In this study, instead of detecting parasites in drinking water, we examined human sera for evidence of prior infection. A significant association was observed in the River Valley participants between having onsite wastewater systems/private wells and having a strong response to the 27-kDa *Cryptosporidium* antigen. The odds ratio was increased approximately two-fold after controlling for confounding variables. Although this association was only observed in one of the three study sites, this is one of the first studies to suggest a relationship between having onsite wastewater systems/private wells and increased serological response to *Cryptosporidium* antigens. Together with a previous study in Iowa (Frost *et al.* 2002), these two studies provide suggestive evidence that inadequately treated sewage from onsite wastewater systems may have contaminated private wells with *Cryptosporidium* oocysts and infected users of private well water. This is not unexpected since there are no data available to show onsite wastewater systems are capable of inactivating *Cryptosporidium* oocysts.

Unfortunately, few studies have determined the occurrence of parasites in at-risk groundwater that is not suspected of causing illness. Based on outbreak data, groundwater contamination is a very common mode of *Cryptosporidium* transmission (Craun & Calderon 2000). We believe that a low probability of outbreak detection should be expected, especially for populations using private drinking water wells. Small outbreaks and outbreaks associated with private wells are not likely to be recognized and investigated (Lee *et al.* 2002) and once protective immunity is developed in the small number of exposed people, the outbreak will not continue. However, visitors to the home who have not developed protective immunity may be at risk for both infection and illness. It would be important to consider monitoring performance of onsite wastewater systems as a public health measure at the local or state level.

The absence of detectable disease outbreaks does not indicate that having both an onsite wastewater system and private well can be a health problem. Even in large cities, small to moderate sized outbreaks can remain undetected. Furthermore, long-term residents of rural areas who drink contaminated groundwater are likely to develop a protective immunity after years of exposure to pathogens in their drinking water. In our study, two measures of past illness were collected. There was no significant association between either of these two measures and a strong response for both serological markers, suggesting that the participants in the study had developed a protective immunity. However, new residents and individuals who develop auto-immune diseases may be the ones at greatest risk of illness. The magnitude of the illness risk depends upon the size of the susceptible population. Thus, if a large fraction of residents in a geographic area have developed protective immunity, it is unlikely that a detectable disease cluster will be identified.

It is difficult to detect viruses in either infected humans or in drinking water near the time of an outbreak. Disease surveillance systems are relatively insensitive to detecting mild cases of gastroenteritis. We do not know how many waterborne outbreaks go unrecognized and the extent to which viral outbreaks may be underestimated in the USA (Frost *et al.* 1996).

Coliphage testing is one analysis recommended by the US EPA as an indicator of groundwater contamination by viruses. Unfortunately, there have been few studies actually linking coliphage occurrence with increased risk of virus infection or even water contamination. The findings of this study are not encouraging since only one sample tested positive for coliphage when using an experienced laboratory with state-of-the-art techniques.

This study also found a high percentage of wells with positive tests for total coliforms, fecal coliforms, or enterococci in all three sites. Overall, a total of 26% (94 of 361) of wells tested had indicators of bacterial contamination, with the highest level being in the East Mountains site, an area with limestone soils. Evidence of fecal coliforms was also found in 7.7% of these. This elevated prevalence of indicators of contamination has been observed elsewhere (Tuthill *et al*. 1998; Swistock & Sharpe 2005; Locas *et al*. 2008). It is of interest to note that no association was observed between well contamination and a strong response to either serological marker for *Cryptosporidium*.

The results of this study may be limited since the three geographic sites were all located in the arid Southwest where rainfall is concentrated during the monsoon season in the summer. However, each site was selected to represent a different geologic stratum (river valley, alluvial fan, and limestone formations). Information on soil tests that were completed for approval of the onsite wastewater systems by the county was also not available from the county regulatory agencies.

The cross-sectional design of the study was not able to take into account the temporal association between exposure and outcome. Information on type of wastewater treatment system, source of water, and bacterial and viral contaminants in the water was collected at the same time as the blood samples. Thus, it was not possible to track the participants' serological responses forward in time. However, the two *Cryptosporidium* antigens are indicative of past exposure history, rather than current history. The 15/17-kDa marker remains elevated for a relatively short period of time (several months), whereas the 27-kDa marker remains elevated for 9 months or longer. The fact that we observed a significantly increased odds ratio for the 27-kDa marker may be a reflection of a chronic, long-term exposure to contaminated well water. Our information on well water contamination was also based on one point-in-time sample. Sequential sampling of 60 wells has shown that seasonal contamination occurs frequently (unpublished data). The use of the long-term elevated 27-kDa marker is appropriate for this situation.

The study may also be subject to selection bias since a convenience sample of volunteers was recruited into the study. Volunteers often differ from the sampling population in underlying characteristics related to the exposure and outcome. Although we recruited most study participants by 'cold calling' lists of telephone numbers in the study areas, rather than from flyers and newspaper advertisements, the possibility of a biased sample still exists. For example, potential participants who were worried about the quality of their well water or who wanted their water tested because they thought it might be contaminated might have been more likely to volunteer for this study. It is not clear how this may affect the estimates of the odds ratio. Recall bias may be an issue since past history of illness and risk factors for cryptosporidiosis were collected using a questionnaire. However, this type of information is usually easily remembered and identified.

CONCLUSIONS

This study, together with one prior study, suggests that the presence of onsite wastewater systems and private wells might be associated with an increased risk of *Cryptosporidium* infection. If a relatively large parasite can be transmitted from inadequately treated sewage from an onsite wastewater system to a private well, then other smaller enteric pathogens may also be transmitted. Borchardt *et al*. (2003) observed a relationship between the density of onsite septic systems and the risk of viral and bacterial diarrhea in children residing in an area that primarily uses septic systems for disposal of fecal waste. However, they did not find this same relationship with respect to *Cryptosporidium* infections. Continuing surveillance of waterborne disease outbreaks in the USA has also shown that outbreaks of gastrointestinal illnesses are associated with failed onsite wastewater systems (Craun & Calderon 1996). Thus, there is some evidence to suggest that there may be a potential for increasing risk of enteric disease illness as more households in the USA use onsite wastewater systems and private wells and the density of these systems increases.

Since the economic cost of installing an onsite wastewater system is a relatively high one-time expense, it may be beneficial to better understand the efficacy of these systems to insure that the systems are properly designed and maintained to minimize adverse health effects from groundwater contamination. This is an emerging public health problem that requires additional studies to identify the environmental, behavioral, and health factors associated with increasing the risk of enteric illnesses from onsite wastewater systems and private wells.

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