

## Septic System Density and Infectious Diarrhea in a Defined Population of Children

Mark A. Borchardt,<sup>1</sup> Po-Huang Chyou,<sup>1</sup> Edna O. DeVries,<sup>2</sup> and Edward A. Belongia<sup>1</sup>

<sup>1</sup>Marshfield Medical Research Foundation and <sup>2</sup>Department of Pediatrics, Marshfield Clinic, Marshfield, Wisconsin, USA

One-quarter of U.S. households use a septic system for wastewater disposal. In this study we investigated whether septic system density was associated with endemic diarrheal illness in children. Cases—children 1 to < 19 years old seeking medical care for acute diarrhea—and controls resided in the Marshfield Epidemiologic Study Area, a population-based cohort in central Wisconsin. Enrollment was from February 1997 through September 1998. Study participants completed a structured interview, and septic system density was determined from county sanitary permits. Household wells were sampled for bacterial pathogens and indicators of water sanitary quality. Risk factors were assessed for cases grouped by diarrhea etiology. In multivariate analyses, viral diarrhea was associated with the number of holding tank septic systems in the 640-acre section surrounding the case residence [adjusted odds ratio (AOR), 1.08; 95% confidence interval (CI), 1.02–1.15;  $p = 0.008$ ], and bacterial diarrhea was associated with the number of holding tanks per 40-acre quarter-quarter section (AOR, 1.22; 95% CI, 1.02–1.46;  $p = 0.026$ ). Diarrhea of unknown etiology was independently associated with drinking from a household well contaminated with fecal enterococci (AOR, 6.18; 95% CI, 1.22–31.46;  $p = 0.028$ ). Septic system densities were associated with endemic diarrheal illness in central Wisconsin. The association should be investigated in other regions, and standards for septic systems should be evaluated to ensure that the public health is protected. *Key words:* communicable diseases, diarrhea, drinking water, sanitation, water microbiology. *Environ Health Perspect* 111:742–748 (2003). doi:10.1289/ehp.5914 available via <http://dx.doi.org/> [Online 17 January 2003]

Acute infectious diarrhea remains a common illness in the United States, particularly among young children, the age group most susceptible to gastrointestinal infections. Children < 5 years old experience 1.3–2.3 mild diarrhea episodes per year, and approximately 220,000 children < 6 years old are hospitalized annually with severe diarrhea (Glass et al. 1991). Diarrheal illness accounts for 10% of all hospitalizations in this age group (Glass et al. 1991).

One major reservoir of human enteropathogens in the environment is private onsite wastewater treatment systems (i.e., septic systems). Septic systems process wastewater from approximately 25 million rural and suburban households, or one-quarter of all households in the nation (U.S. Bureau of the Census 1993). More than a trillion gallons of wastewater pass through these systems each year, according to the U.S. Environmental Protection Agency (U.S. EPA 1977). Effluent is released directly into the land subsurface, where enteric microorganisms are removed by soil filtration and adsorption. However, depending on environmental conditions, the effectiveness of this process may be limited. Laboratory and field studies have documented that bacteria and especially viruses can be transported rapidly through the soil profile and contaminate groundwater, where they can move horizontally hundreds of meters and survive up to several months (Bitton and Harvey 1992; Gerba and Bitton 1984; Hagedorn 1984; Jansons et al. 1989;

Scandura and Sobsey 1997; Vaughn et al. 1983; Woessner et al. 2001; Yates and Yates 1988). Enteropathogens can also be released unintentionally on top of the land surface when a septic system malfunctions because of age or neglect.

Septic systems remain a common method of wastewater disposal as the U.S. population continues to expand into rural and suburban areas not served by municipal sewers. Septic systems have been implicated in disease outbreaks (Beller et al. 1997; Craun 1979, 1981, 1984; McGinnis and DeWalle 1983; Vogt 1961; Yates 1985), but their role as a transmission source of endemic diarrhea is unknown. People living in rural central Wisconsin are potentially exposed to enteropathogens from a type of septic system called a holding tank. Holding tanks are used where the soil is unsuitable for disposing effluent from a septic drain field. Unlike a conventional septic system, a holding tank is a sealed concrete vault that prevents the release of wastewater and stores it until it is removed by a licensed waste hauler. However, in central Wisconsin, improper discharge of wastewater from holding tanks by homeowners has been reported to be common (Popelka 1994). To assess the role of septic systems, particularly holding tanks, as risk factors for acute infectious diarrhea, we conducted a case-control study of children living in a defined population of central Wisconsin. Because groundwater may be one transmission route for septic system pathogens, we also investigated case and control household wells

for pathogen occurrence and assessed indicators of water sanitary quality as diarrhea risk factors.

### Methods

**Study population.** The study population included children living in the Marshfield Epidemiologic Study Area (MESA), a dynamic cohort of all persons living in 14 contiguous zip codes around Marshfield, Wisconsin (Figure 1) (DeStefano et al. 1996). Nearly all MESA residents receive their medical care from Marshfield Clinic and its regional network, and their medical records are computerized and linked to the MESA residency database. The MESA population denominator is continuously updated. As of 1 February 1997, the MESA population was 58,466, including 15,681 children 1–18 years old. Approximately half the residents lived in a municipality, and the remainder lived in the surrounding rural area without municipal sewer or water. Approximately half of MESA residents were enrolled in a health maintenance organization at the time of the study.

The research protocol was reviewed and approved by the Institutional Review Board of Marshfield Clinic, and informed consent was obtained from the parents of all participants. The specific hypothesis concerning septic systems was not disclosed to participants or their parents.

**Case ascertainment and enrollment.** The enrollment period extended from February 1997 through September 1998. Children with acute diarrhea were identified by health care providers when a child sought medical treatment, and by research coordinators daily reviewing appointment records and diagnosis codes [based on *International Classification of Diseases*, 9th revision (ICD-9 1995)]. Parents of potentially eligible children were contacted during their clinic visit or as soon as possible thereafter.

Address correspondence to M. Borchardt, Marshfield Medical Research Foundation, 1000 North Oak Ave., Marshfield, WI 54449 USA. Telephone: (715) 389-3758. Fax: (715) 389-3808. E-mail: borchardt.mark@mrmf.mfldclin.edu

We thank the staff of Planning and Zoning offices of Clark, Marathon, and Wood counties, Wisconsin.

This project was supported by the Wisconsin Department of Commerce and Marshfield Clinic. The study team included D. Kempf, J. Treutel, K. Konitzer, L. Wittman, N. Herr, L. Ziegelbauer, R. Vierkant, P. Bertz, S. Spencer, A. Witte, and A. Stargardt.

The authors declare they have no conflict of interest.

Received 26 July 2002; accepted 16 January 2003.

Children living in MESA were eligible to participate if they were between 1 and < 19 years old on the date of enrollment and they had three or more loose, watery stools in a 24-hr period. Children with < 24 hr of diarrhea or diarrhea lasting more than 21 days at the time of enrollment were not eligible. We excluded children with immunosuppressive conditions, chronic or recurrent diarrhea (based on parent report), or antibiotic use during the 48 hr before onset. Only the first case in each household was enrolled.

**Determination of etiologic agents.** Stool specimens from case children were collected within a few days after enrollment (median elapsed time, 1 day; 95% of specimens collected within 5 days) and hand-delivered to Marshfield Clinic. Cultures were performed to identify *Salmonella*, *Shigella*, *Escherichia coli* O157:H7, *Campylobacter* species, and *Yersinia enterocolitica* using standard media and biochemical screens (Murray et al. 1995). *Cryptosporidium* oocysts and *Giardia* cysts were identified using the Merifluor direct immunofluorescence assay (Meridian Diagnostics, Cincinnati, OH). Rotavirus and adenovirus 40/41 antigens in stool were detected by enzyme immunoassays (Premier Rotaclone and Premier Adenoclone, type 40/41; Meridian Diagnostics). Tests for caliciviruses were unavailable.

**Selection of controls.** Controls were randomly selected every 2 weeks from the MESA population to maintain a 1:2 ratio of cases to controls. They were frequency matched to cases enrolled in the preceding 2-week period based on sex and age group (1–4 years, 5–11 years, and 12–18 years). Frequency matching was employed to ensure that the age and sex distributions of cases and controls were similar, but the analysis of risk factors was performed without individual matching. Cases and controls were enrolled and frequency matched in 2-week time blocks to ensure the same seasonal distribution of enrollment. Selected controls were contacted by letter and then telephoned to confirm eligibility and request their participation. Controls were excluded if they had diarrhea (same definition as cases) within 30 days before their interview.

**Septic system risk factors.** The septic system density surrounding each case and control residence was determined for three geographic scales corresponding to conventional land survey units: section (640 acres, 259 hectares), quarter section (160 acres, 64.75 hectares), and quarter-quarter section (40 acres, 16.19 hectares). These land survey units are square and defined by fixed lines established under the federal Public Land Survey System (U.S. Bureau of Land Management 1973). The choice of these three land survey units as the denominators for septic system density was made *a priori* before statistical analysis. Septic system data were obtained from public records

of property taxes and sanitary permits. Case and control addresses were merged with property tax records to find the corresponding parcel identification number (PIN). The PIN specified actual property location by section, quarter section, and quarter-quarter section. Case and control PINs were merged with property tax records to identify all property in the same land survey units. Property listings with improved valuation < \$10,000 were excluded because these were unlikely to include houses or other buildings with septic systems. PINs were linked with sanitary permits, which have been required for installation or renovation of septic systems since approximately 1980. Permits were excluded if the system was closed or an inspection had not been performed (i.e., system had not been used). If there were multiple permits for renovations at the same property, only the most recent permit was included.

Septic systems were classified as holding tanks, nonholding tanks  $\leq$  20 years old, and nonholding tanks > 20 years old. Nonholding tanks included conventional septic drain fields, Wisconsin mound systems, privies, and a few experimental sand filters. Twenty years is the approximate functional life span of a septic drain field. Properties without a sanitary permit (i.e., septic systems installed before the permit requirement implemented in 1980) were categorized as nonholding tank septic systems > 20 years old, because holding tanks were uncommon before 1980. Systems installed after the date of enrollment for each participant were excluded. Case and control subjects who resided in a village or city with municipal sewer were assigned a septic system density of zero at all three geographic scales.

**Household water quality.** Household water quality was determined for all case and control households with private wells, usually within 1 week after enrollment. Four-liter samples were aseptically collected by a study technician and filtered, and cultures were performed to identify *Salmonella*, *Shigella*, *E. coli* O157:H7 (Greenberg et al. 1992), and *Y. enterocolitica* (Schiemann 1982). A separate 1-L sample was analyzed for *Campylobacter* (Korhonen and Martikainen 1990). Water samples were also analyzed for standard indicator organisms of sanitary quality. Total coliforms were measured by two chromogenic substrate assays performed in parallel, Colilert (IDEXX, Portland, ME) and Colisure (Millipore Corp., Bedford, MA). Both assays also detect *E. coli*, and a sample was classified as positive for total coliform or *E. coli* if either assay was positive. Fecal enterococci were detected by a separate chromogenic substrate assay, Enterolert (IDEXX).

**Telephone interview.** Parents of case and control children completed a structured telephone interview with questions pertaining to demographic information and disease

symptoms and 68 questions covering potential risk factors or confounders for acute diarrhea (Appendix). Environmental and dietary exposures were ascertained for the 5-day period before onset of symptoms (case children) or the 5 days before the interview (control children). Source of drinking water (municipal, private well, or bottled water) and method of wastewater disposal (municipal sewer or septic system) were also determined in the interview. The median elapsed time between the clinical encounter and telephone interview was 2 days; controls were interviewed at the time of the initial phone call seeking their participation.

**Statistical analysis.** The Wilcoxon rank sum test was used to test for median differences in continuous variables between cases and controls. Univariate odd ratios and *p*-values were calculated using unconditional logistic regression (Breslow and Day 1980). Independent variables with a *p*-value < 0.15 were eligible to be selected in a stepwise (both forward selection and backward elimination) multiple logistic regression model. Regression models were created using all cases and controls and for specific subgroups based on the results of stool tests: bacterial, viral, and *Cryptosporidium* infections and diarrhea of unknown etiology. Each subgroup of cases was compared with the entire group of control children to identify significant associations. Because the study protocol used frequency matching, these analyses were performed without individual matching of cases and controls. Variables with a *p*-value < 0.05 were retained in the final multivariate logistic regression models. Interaction effects were evaluated for the retained risk factors. All regression models were assessed for goodness of fit. Adjusted population attributable risks (PARs) were estimated for risk factors that were significantly associated with each pathogen subgroup based on stepwise regression modeling, and 95% confidence intervals (CI) for covariate-adjusted PARs were obtained by bootstrapping (Bruzzi et al. 1985; Efron and Tibshirani 1993;



**Figure 1.** Location of MESA (shaded region), where the study was conducted.

Kahn and Sempos 1989). All statistical analyses were performed using SAS version 6.12 (SAS Institute, Cary, NC) (SAS 1990).

## Results

A total of 188 eligible case children were identified during the study period, and 160 (85%) agreed to participate. Seven (4%) case children were excluded because they failed to complete the questionnaire ( $n = 2$ ), were taking immunosuppressive medications ( $n = 2$ ), or lived in households where a sibling had been previously enrolled ( $n = 3$ ). Four hundred eight control households were contacted, and 316 (78%) agreed to participate. Of these, 42 children (13%) were excluded because they were immunosuppressed ( $n = 2$ ), had diarrhea during the past 30 days ( $n = 33$ ), or were enrolled as a case child or lived in the same household with a case child ( $n = 7$ ).

Case ascertainment was evaluated by reviewing an age-stratified sample of 196 medical encounters for children living in MESA who had any of 39 ICD-9 diagnosis codes corresponding to acute diarrhea or gastroenteritis during the enrollment period. Of the 196 encounters, 89 (45%) appeared to meet the eligibility criteria based on the clinical note, and 31 (35%; 95% CI, 25–46) of these were enrolled in the study. The distributions of age, sex, and zip code (Marshfield vs. other) were similar for enrolled and nonenrolled children.

**Demographic and clinical characteristics.** The median age of the 153 case children was

2.2 years, compared with the median age of 3.7 years among 274 controls ( $p = 0.03$ ). Eighty-six case children (56%) and 153 controls (56%) were male. Similar proportions of case and control subjects lived in rural households with private wells and septic systems (Table 1). The median duration of diarrhea was 7 days (range, 1–16 days), and the median maximum number of loose stools was 6 per 24 hr (range, 3–30 per 24 hr). Fever and vomiting were common during the acute illness. Of 153 case children, 130 (85%) submitted a stool specimen. The most commonly identified pathogens were *Cryptosporidium*, *Campylobacter* species, and rotavirus. No pathogen was identified in more than half the specimens (Table 1). None of the specimens was positive for *Shigella*, *Yersinia*, or *Giardia*.

**Household water quality.** Tap water was analyzed for 191 (90%) of 212 case and control households with private wells. Forty-four wells (23%) were positive for total coliform, seven (4%) were positive for fecal enterococci, two (1%) were positive for *E. coli*, and one well had a putatively pathogenic bacterium, *Yersinia intermedia*. None of the wells sampled was positive for *Campylobacter*, *Salmonella*, *Shigella*, or *E. coli* O157:H7.

**Risk factor analysis.** For all cases analyzed as a single group, diarrheal illness was not associated with septic system density, source of drinking water, or sanitary quality of the household well. Because transmission factors may vary for different pathogens, subanalyses

were conducted for different etiologic groups. Cases of *E. coli* O157:H7, *Salmonella*, and *Campylobacter* infection were grouped together as bacterial infections ( $n = 20$ ); rotavirus and adenovirus 40/41 cases were classified as viral ( $n = 18$ ), and *Cryptosporidium* cases were analyzed as a separate subgroup ( $n = 16$ ). Cases with no identified pathogen were classified as diarrhea of unknown etiology ( $n = 76$ ). Cases within each etiologic subgroup were enrolled throughout the study period without any evidence of significant seasonal clustering.

In univariate analyses, diarrheal illnesses of viral and bacterial etiologies were associated with septic system densities of several classifications based on type, age, and geographic scale (Table 2). The *Cryptosporidium* and unknown etiologic groups were not associated with septic system densities. Diarrheal illnesses of viral, bacterial, and unknown etiologies were marginally associated with drinking water source, and the unknown etiologic group was also associated with drinking from a private well positive for fecal enterococci (Table 3). *Cryptosporidium* infections were not associated with any risk factors related to drinking water. Viral, bacterial, and unknown etiology groups were also associated with a number of behavioral, dietary, and lifestyle variables ( $p < 0.15$ ) in the univariate analysis (data not shown). These variables were considered potential independent risk factors for diarrheal illness or potential confounders with the septic system risk factors and, as such, were included in the stepwise multiple logistic regression modeling to identify independent significant predictors for each etiologic group.

In multivariate analysis, viral diarrhea was independently associated with the number of holding tanks in the same section (640-acre block) as the residence (Table 4). The median holding tank density for controls and viral diarrhea cases residing in rural areas without municipal sewer was 3.0 and 7.0 holding tanks per section, respectively (range, 0–50 holding tanks per section for each group). Viral diarrhea was also independently associated with younger age and living in a household where another person had diarrhea during the previous 4 weeks (Table 4).

Bacterial diarrhea was independently associated with the number of holding tanks in the quarter-quarter section (40-acre block) of the residence (Table 4). The median holding tank density in areas without municipal sewer was 1 (range, 0–15) per quarter-quarter section for case children with bacterial diarrhea, and 0 (range, 0–15) per quarter-quarter section for controls. Bacterial diarrhea was also independently associated with a child entering a calf hutch or pen. No interactions between holding tank density and other risk factors were detected for either bacterial or viral etiologic groups.

**Table 1.** Characteristics of the study population.

Characteristics	Cases ( $n = 153$ )		Controls ( $n = 274$ )	
	No.	Percent <sup>a</sup>	No.	Percent
Male	86	56	153	56
Age group				
12–23 months	67	44	58	21
24–59 months	51	33	143	52
5–11 years	17	11	37	14
12–18 years	18	12	36	13
Attended group child care	66/122	54	97/203	48
Drinking water source				
Municipal water	75	49	135	49
Private well	71	46	134	49
Bottled water	7	5	5	2
Household wastewater system				
Septic system	68	44	121	44
Municipal sewer	85	56	153	56
Symptoms				
Fever	80/148	54		
Vomiting	77/152	51		
Abdominal pain	84/126	67		
Bloody stool	19/148	13		
Diarrhea etiology <sup>b</sup>				
<i>Cryptosporidium parvum</i>	16	12		
<i>Campylobacter</i>	11	8		
Rotavirus	11	8		
<i>Salmonella</i> spp.	7	5		
Adenovirus 40/41	7	5		
<i>E. coli</i> O157:H7	2	2		
No pathogen identified	76	58		

<sup>a</sup>Percentages were calculated with  $n$  as denominator unless noted. <sup>b</sup>Etiology was reported for 130 case children who submitted stool samples.



To avoid potential confounding due to differences between city and noncity populations, we conducted an additional analysis of risk factors for viral and bacterial diarrhea after excluding cases and controls with municipal sewer. In the original multivariate models these participants were assigned a septic system density of zero. In the new analysis, viral diarrhea was marginally associated with holding tank density per section [7 cases, 111 controls; adjusted odds ratio (AOR), 1.32; 95% CI, 0.99–1.75;  $p = 0.06$ ] and inversely associated with age (AOR, 0.019; 95% CI, 0.0006–0.644;  $p = 0.03$ ), but it was no longer associated with recent diarrhea in a household member (AOR, 10.64; 95% CI, 0.31–367.77;  $p = 0.19$ ). Bacterial diarrhea was marginally associated with the number of holding tanks per quarter-quarter section (13 cases, 111 controls; AOR, 1.12; 95% CI, 0.99–1.46;  $p = 0.07$ ) but remained associated with entering a calf hutch or pen (AOR, 10.6; 95% CI, 2.75–40.81;  $p < 0.001$ ).

Multivariate analysis demonstrated that drinking water source was not independently associated with diarrhea of unknown etiology (data not shown). The analysis was then restricted to the subset of case and control households with a private well (30 cases, 121 controls) to assess whether drinking from a private well positive for fecal enterococci was independently associated with this etiologic group. The subanalysis showed that among households with a private well, two risk factors were independently associated with diarrhea of unknown etiology: Household member had diarrhea during the previous 4 weeks and private well was positive for fecal enterococci (Table 4).

The fecal enterococci test was the only microbial indicator of water sanitary quality that was significantly associated with diarrheal illness. A positive total coliform test, the most frequently used indicator of water sanitary quality, was not associated with diarrheal disease as a single group, nor was it associated with any of the etiologic subgroups in univariate analyses. Only two wells were positive for *E. coli*, too few to determine associations of this indicator with disease outcome.

**PAR estimates.** The adjusted PAR was estimated for the variables that were significantly positively associated with each etiologic subgroup, assuming that there was a causal relationship between the risk factor and diarrhea event (Table 5). To calculate PAR, it was necessary to categorize holding tank density as a dichotomous exposure variable. For viral diarrhea, the exposure threshold was  $\geq 6$  holding tanks per section ( $\geq 90$ th percentile of the holding tank density distribution of the controls), resulting in an AOR of 3.76 ( $p = 0.029$ ). For bacterial diarrhea, the threshold was  $\geq 1$  holding tank per quarter-quarter section ( $\geq 75$ th percentile of the

density distribution of the controls), resulting in an AOR of 2.17 ( $p = 0.145$ ). On the basis of these thresholds and after adjusting for other risk factors, it was estimated that 20% of viral diarrhea and 19% of bacterial diarrhea were attributable to a holding tank density. Among the subset of children who drank from a private well, it was estimated that 11% of diarrhea of unknown etiology was attributable to wells positive for fecal enterococci.

### Discussion

This observational study identified septic system density as a risk factor for sporadic cases of viral and bacterial diarrhea in central Wisconsin children. The risk of viral diarrhea illness increased by 8% for every additional holding tank per section (640 acres), and the risk of developing bacterial diarrhea increased by 22% for every additional holding tank per quarter-quarter section (40 acres). Density is a continuous variable; therefore, the AORs are expressed per unit change in density. Holding tank density was highly correlated with the density of other septic system types, and the relative contribution of other septic systems versus holding tanks could not be assessed.

Thus, the observed associations may be due in part to the parallel effect of other septic systems. The associations identified in the univariate analyses between viral or bacterial diarrhea and other types of septic systems suggest that this parallel effect is possible. Septic systems are a recognized source of enteropathogens, but we believe this is the first study to assess residential proximity to septic systems as a risk factor for enteric infections.

Because this was a case-control study, the observed disease associations do not necessarily represent causal relationships. Septic system density may be a surrogate for rural residential density, and unmeasured confounders unique to the rural environment may explain the observed associations between diarrheal illness and holding tanks. For example, residents of higher-density neighborhoods (e.g., subdivisions) in rural areas may have higher levels of education and/or income, and this may increase the likelihood of seeking medical care for diarrheal illness. Socioeconomic data were not collected in the present study, so these factors were not assessed for potential confounding. Population density was unlikely to be a confounder in this study because case and control households in municipalities, where

**Table 2.** Univariate associations between septic system densities and viral or bacterial diarrhea in children.

Septic system type and density scale <sup>a</sup>	Bacterial diarrhea (n = 20) <sup>b</sup>			Viral diarrhea (n = 18) <sup>b</sup>		
	OR <sup>c</sup>	95% CI	p-Value	OR <sup>c</sup>	95% CI	p-Value
All systems						
Section	1.022	0.984–1.062	0.258	1.033*	0.998–1.069*	0.067*
Quarter section	1.062*	0.986–1.144*	0.113*	1.078*	1.007–1.154*	0.030*
Quarter-quarter section	1.153*	0.992–1.341*	0.064*	1.177*	1.015–1.365*	0.031*
Holding tanks						
Section	1.030	0.979–1.084	0.253	1.049*	1.003–1.098*	0.038*
Quarter section	1.062	0.967–1.166	0.211	1.076*	0.983–1.177*	0.113*
Quarter-quarter section	1.148*	0.968–1.362*	0.112*	1.146*	0.963–1.365*	0.126*
All nonholding tanks <sup>d</sup>						
Section	1.033	0.947–1.128	0.464	1.036	0.951–1.128	0.422
Quarter section	1.222*	0.959–1.558*	0.101*	1.218*	1.013–1.464*	0.036*
Quarter-quarter section	1.272	0.827–1.957	0.274	1.390*	0.960–2.013*	0.081*
Nonholding tanks $\leq 20$ years old						
Section	0.691	0.253–1.886	0.471	1.502*	0.998–2.262*	0.051*
Quarter section	0.854	0.151–4.819	0.859	2.528*	1.125–5.684*	0.025*
Quarter-quarter section	0 <sup>e</sup>	— <sup>e</sup>	0.980	4.292*	1.426–12.911*	0.009*
Nonholding tanks $> 20$ years old						
Section	1.043	0.952–1.142	0.370	1.025	0.933–1.127	0.605
Quarter section	1.249*	0.972–1.606*	0.082*	1.199*	0.991–1.451*	0.061*
Quarter-quarter section	1.328	0.869–2.031	0.190	1.259	0.812–1.950	0.304

OR, odds ratio.

<sup>a</sup>The land survey units of section, quarter section, and quarter-quarter section correspond to 640, 160, and 40 acres, respectively. <sup>b</sup>274 controls. <sup>c</sup>Septic system density was analyzed as a continuous variable. OR was calculated per additional septic system per land survey unit. <sup>d</sup>Nonholding tanks include conventional septic drain fields, Wisconsin mound systems, privies, and experimental sand filter systems. <sup>e</sup>Unable to perform complete maximum likelihood iteration. \*Variable met statistical significance criterion for inclusion in multivariate model.

**Table 3.** Univariate associations between diarrhea etiology and drinking water-related factors found eligible for stepwise multiple regression modeling (i.e., factors with  $p < 0.15$ ).

Etiology	Factor	OR	95% CI	p-Value
Viral <sup>a</sup> (n = 18)	Household uses Marshfield municipal water	2.08	0.80–5.42	0.134
Bacterial <sup>a</sup> (n = 20)	Household uses private well	2.44	0.91–6.67	0.076
Unknown <sup>a</sup> (n = 76)	Household uses Marshfield municipal water	1.60	0.95–2.68	0.078
	Private well positive for fecal enterococci <sup>b</sup>	6.05	1.28–28.68	0.023

OR, odds ratio.

<sup>a</sup>274 controls. <sup>b</sup>Analysis restricted to cases (n = 30) and controls (n = 121) living in a household with a private well.

population density is greatest, had a septic system density of zero. When municipal households were excluded from the analysis, holding tank density remained a predictor of viral and bacterial diarrhea, although statistical significance was reduced, probably because of smaller sample size.

The associations between viral or bacterial diarrhea and septic system density are biologically plausible. Holding tanks constitute approximately one-third of all private septic systems in the study area. Properly managed holding tanks do not release effluent to the environment, but county sanitarians in central Wisconsin estimate that as many as 40% of all holding tanks have some illegal surface discharge (Popelka 1994). When water use was estimated for all households with holding tanks in Wood County, Wisconsin, and compared with the volume of wastewater reportedly pumped in the year 2000, 40 million gallons of wastewater were unaccounted for and presumably released untreated to the environment (G. Popelka. Personal communication). The region used in this study overlaps approximately one-half of Wood County.

Conventional septic systems could also be a transmission source of enteric pathogens. Properly functioning septic drain fields may allow viruses to reach groundwater (Alhajjar et al. 1988; DeBorde et al. 1998), and when a drain field fails, it discharges to the land surface, allowing people to be potentially exposed to untreated fecal wastes. There are more than 700,000 septic systems in Wisconsin, and 133,000 (19%) are conventional drain fields that were constructed before 1970 and are

likely failing because of age and design limitations (WDC 1998).

Enteric bacteria and viruses in groundwater can be transported long distances and survive for months (Bitton and Harvey 1992; Gerba and Bitton 1984; Hagedorn 1984; Jansons et al. 1989; Scandura and Sobsey 1997; Vaughn et al. 1983; Woessner et al. 2001; Yates and Yates 1988). Bacteria are significantly larger and tend to move shorter distances than do viruses. This is consistent with the finding that bacterial diarrhea was associated with holding tank density expressed at the smallest scale investigated (40 acres), whereas viral diarrhea was associated at the largest scale (640 acres). Diarrhea of unknown etiology was not associated with septic system density, which is difficult to explain if this etiologic subgroup contained mostly viruses. However, different virus types can vary widely in their abilities to survive and be transported in the environment, depending on their size, isoelectric point, and other physical characteristics (Bitton and Harvey 1992; Dowd et al. 1998; Gerba and Bitton 1984; Yates and Yates 1988), so there is no reason necessarily to expect the viral and unknown etiologic subgroups to be similarly associated with septic system density.

Consumption of well water was not a likely transmission route of bacterial infection from nearby septic systems in this study, because bacterial pathogens were not isolated from the wells of case households, although contamination may have been sporadic. We did not test well water for the presence of viral pathogens, so the potential role of groundwater consumption as a source of viral diarrhea is unknown.

Another potential transmission route was via direct or indirect exposure to septic system effluent released to the land surface in the vicinity of case households. We have observed houses in central Wisconsin where untreated holding tank effluent is piped to a nearby open ditch. Children could have possibly contacted effluent indirectly through toys, pets, or vectors, especially given the low infectious dose of many enteric pathogens. Further research is needed to assess these potential sources of transmission.

The total coliform test is the standard indicator for gauging the risk of disease transmission from drinking water. This indicator has limitations because coliform bacteria may originate from nonfecal sources, and the test does not correlate with all waterborne diseases (Craun et al. 1997; Payment et al. 1993). In this study, children who drank from private wells that were coliform positive were not at increased risk for diarrheal disease. However, children who drank from private wells that were positive for fecal enterococci had 6-fold greater odds of becoming ill with diarrhea of unknown etiology. The etiologic agents in this subgroup likely included human caliciviruses, because these viruses have been responsible for several groundwater-related outbreaks (Beller et al. 1997; Lawson et al. 1991; McNulty et al. 1993; Taylor et al. 1981), and most nonbacterial gastroenteritis outbreaks in the United States are due to caliciviruses (Fankhauser et al. 1998). Other studies have also shown that fecal enterococci in drinking water or recreational water is associated with gastrointestinal illnesses (Dufour 1984; Fleisher et al. 1993; Moe et al. 1991).

The PAR estimates suggest that eliminating the holding tank risk factor would prevent some sporadic diarrhea in central Wisconsin. This risk factor may account for up to one-fifth of viral and one-fifth of bacterial diarrheal illnesses. Drinking from a well positive for fecal enterococci may account for 11% of diarrhea of unknown etiology. Although the PAR for drinking contaminated groundwater estimated in this study was based on a small number of cases, it does provide an initial estimate of the potential burden of endemic diarrheal disease attributable to private wells. More than 15 million households in the United States use a private well for drinking water (U.S. Bureau of the Census 1993), and approximately half the drinking water disease outbreaks in the United States each year are due to contaminated groundwater (Barwick et al. 2000; Craun 1992; Herwaldt et al. 1991; Kramer et al. 1996; Levy et al. 1998; Moore et al. 1993). However, the fraction of endemic diarrhea attributable to groundwater is unknown. In the only other estimate of attributable risk for drinking water, Payment et al. (1991) found that 35% of gastrointestinal illnesses among

**Table 4.** Multivariate independent risk factors for diarrhea based on etiology.<sup>a</sup>

Etiology	Risk factor	AOR	95% CI	p-Value
Viral (n = 18) <sup>b</sup>	Number of holding tanks in same 640-acre section (per additional tank)	1.08	1.02–1.15	0.008
	Household member had diarrhea in past 4 weeks	5.04	1.70–14.95	0.004
	Age (per year)	0.66	0.47–0.92	0.015
Bacterial (n = 20) <sup>b</sup>	Number of holding tanks in same 40-acre quarter-quarter section (per additional tank)	1.22	1.02–1.46	0.026
	Entered calf hutch or pen	12.74	4.67–34.72	< 0.001
	Private well positive for fecal enterococci	6.18	1.22–31.46	0.028
Unknown (n = 30) <sup>c</sup>	Household member had diarrhea in past 4 weeks	4.06	1.66–9.94	0.002

<sup>a</sup>AORs were determined using stepwise multiple logistic regression models. Variables with a univariate p-value < 0.15 were eligible for inclusion in each model, and variables with a p-value < 0.05 were retained in the final model. <sup>b</sup>274 controls. <sup>c</sup>121 controls; analysis restricted to cases and controls living in a household with a private well.

**Table 5.** Adjusted PAR for risk factors independently associated with diarrhea etiologic subgroups.

Etiology	Risk factor	Percent cases exposed	Adjusted PAR (%)	95% CI
Viral (n = 18) <sup>a</sup>	Number of holding tanks in same 640-acre section (≥ 6 vs. 0–5)	28	20	2–42
	Household member had diarrhea in past 4 weeks	39	31	10–53
Bacterial (n = 20) <sup>a</sup>	Number of holding tanks in same 40-acre quarter-quarter section (≥ 1 vs. 0)	35	19	0–39
	Entered calf hutch or pen	55	50	28–72
	Private well positive for fecal enterococci	13	11	2–23
Unknown (n = 30) <sup>b</sup>	Household member had diarrhea in past 4 weeks	43	33	14–50

<sup>a</sup>274 controls. <sup>b</sup>121 controls; analysis restricted to cases and controls living in a household with a private well.

residents of a suburb of Montreal, Canada, were attributable to municipal treated water derived from a river.

Several methodologic limitations should be considered when interpreting the results of this study. The study was conducted in a rural area where holding tanks comprise a large proportion of septic systems. The results may not be generalizable to areas with different proportions of septic system types. A large number of variables were examined in this study, increasing the potential for spurious associations. Because of the nature of the geographic data, all residences within the same land survey unit were classified as having the same septic system density, although for those households located near the outer perimeter of a land survey unit the actual density may have differed. Selection bias may have occurred by enrolling only those children with diarrhea who were seeking medical treatment. Finally, the subgroup analyses were based on relatively few cases, suggesting that the reported AORs and PARs could be overestimated.

Many regions of the United States have higher septic system densities than does central Wisconsin. In this study, the highest septic system density was 56 per square mile. Of 472

census tracts in 16 counties surrounding Atlanta, Georgia, 98 tracts have septic system densities > 100 per square mile, and in Suffolk County, New York, 6 census tracts exceed 2,000 septic systems per square mile (U.S. Bureau of the Census 1993). The U.S. EPA has suggested that densities of conventional septic drain fields > 40 per square mile (i.e., section) may result in groundwater contamination (U.S. EPA 1977). As of 1999, 31 states were reviewing the adequacy of their septic system codes (NSFC 1999). In addition, the U.S. EPA is preparing to promulgate the Groundwater Rule, a set of measures intended to reduce disease transmission from the more than 158,000 public groundwater systems in the nation (U.S. EPA 2000). The results of the present study support the public health importance of these activities and demonstrate a need for further research regarding septic systems and groundwater as sources of endemic diarrhea in rural and suburban populations.

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### Appendix. Categories of behavioral, dietary, and lifestyle factors investigated as potential independent risk factors for acute diarrhea or confounders with septic system density.

Person-to-person transmission  
Daycare attendance  
Recent diarrhea in daycare children  
Recent diarrhea in family members  
Travel  
Travel outside the United States  
Destination  
Dietary history  
Raw milk  
Other dairy products and eggs  
Meat and poultry  
Salad items and fruits  
Home garden produce  
Undercooked foods  
Meal locations  
Pets  
Contact with dogs, cats, or reptiles  
Recent diarrhea in pets  
Farm activities  
Farm resident or farm visitor  
Type of farm  
Kinds of livestock and poultry  
Number of animals  
Contact with young animals  
Direct or indirect manure exposures  
Recent diarrhea in livestock  
Recreational water activities  
Swim in lake, pond, or river  
Swallow untreated water  
Drinking water and sewage disposal  
Drinking water source  
Bottled water consumption  
Quantity of water consumed  
Sewage system type

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