Epidemiologic evaluation of diarrhea in dogs in an animal shelter

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Objectives—To determine associations among infectious pathogens and diarrheal disease in dogs in an animal shelter and demonstrate the use of geographic information systems (GISs) for tracking spatial distributions of diarrheal disease within shelters.

Sample Population—Feces from 120 dogs.

Procedure—Fresh fecal specimens were screened for bacteria and bacterial toxins via bacteriologic culture and ELISA, parvovirus via ELISA, canine coronavirus via nested polymerase chain reaction assay, protozoal cysts and oocysts via a direct fluorescent antibody technique, and parasite ova and larvae via microscopic examination of direct wet mounts and zinc sulfate centrifugation flotation.

Results—*Salmonella enterica* and *Brachyspira* spp were not common, whereas other pathogens such as canine coronavirus and *Helicobacter* spp were common among the dogs that were surveyed. Only intestinal parasites and *Campylobacter jejuni* infection were significant risk factors for diarrhea by univariate odds ratio analysis. *Giardia lamblia* was significantly underestimated by fecal flotation, compared with a direct fluorescent antibody technique. Spatial analysis of case specimens by use of GIS indicated that diarrhea was widespread throughout the entire shelter, and spatial statistical analysis revealed no evidence of spatial clustering of case specimens.

Conclusions and Clinical Relevance—This study provided an epidemiologic overview of diarrhea and interacting diarrhea-associated pathogens in a densely housed, highly predisposed shelter population of dogs. Several of the approaches used in this study, such as use of a spatial representation of case specimens and considering multiple etiologies simultaneously, were novel and illustrate an integrated approach to epidemiologic investigations in shelter populations. (*Am J Vet Res* 2005;66:1018–1024)

Diarrhea in dogs housed in animal shelters may be influenced by numerous factors, including stress, change in diet, primary gastrointestinal pathogens, opportunistic infections, and predisposing disease conditions.[1] Animal health management in shelters poses considerable challenges attributable to dense animal housing with frequent animal turnover. In addition, shelter resources are often limited, and infectious diseases, particularly respiratory infections and diarrhea, are highly prevalent.[1]

Primary and opportunistic gastrointestinal pathogens affecting domestic dogs include bacteria, viruses, protozoa, and helminths. Many gastrointestinal pathogens in dogs pose a zoonotic risk to humans, including *Campylobacter* spp, *Salmonella enterica*, *Trichuris vulpis* (whipworm), *Strongyloides stercoralis*, *Clostridium difficile*, *Cryptosporidium* spp, and *Escherichia coli* strain O157H7. Therefore, managing diarrhea in dogs in shelters should decrease risk of exposure to gastrointestinal pathogens for shelter animals, personnel, and people adopting dogs from shelters.

Surveillance for specific pathogens is an underutilized tool because of lack of information and resources to monitor and reduce the spread of infectious diseases in shelters. Routine monitoring for all suspected pathogens is not feasible. Therefore, methods of disease prevention are often nonspecific, including prophylactic disinfection of the environment and isolation, treatment, or culling of apparently sick animals. The development of effective strategies for prevention of diarrhea in shelters depends on identification of the most important causal and predisposing factors. However, results from any single or small number of diagnostic tests could be misleading because they may imply causation inaccurately. If sufficient information concerning diarrhea in shelters were available, diagnostic profiles could be developed with diagnostic tests, treatments, and preventive measures designed to target the most important pathogens and could lead to cost-effective management and prevention programs.

The purposes of the study reported here were to determine associations among infectious pathogens and diarrheal disease in dogs in an animal shelter and demonstrate the use of **geographic information systems (GIS)** for tracking spatial distributions of diarrheal disease within shelters.

Materials and Methods

Animals and study site—Fresh fecal specimens were collected from 120 dogs housed at a large municipal animal shelter in northern California from June to August 2002. Most dogs at the shelter were cohoused with dogs of similar age, size, sex, and adoption readiness. Dogs in the rabies quarantine area (kennel B; not sampled) and aged dogs, puppies, and dogs under veterinary care (kennel 5) were housed singly. Shelter runs were 1.8 × 3.6 m, with half of the run in a building and the other half outside. The floor and walls to

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1 m high were cement; above that was a stainless steel chain-link fence. Mean yearly impound rate at the shelter was approximately 25,000 dogs and cats, and the shelter remained near capacity (approx 400 dogs) throughout the study period (and typically throughout the year). A common drain ran in front of all cages down a hall past all downstream cages. Runs were evacuated daily and cleaned with water from a hose followed by application of 5.25% sodium hypochlorite (full-strength household bleach) diluted 1:10.

Experimental design and sample collection—A 1:1 matched case-control study design was used, with matching based on spatial location. Specifically, the first nondiarrheic fecal sample encountered near (but not within) the cage containing a case specimen was collected immediately as a control specimen. Because of shelter housing policies (ie, cages and kennels usually housed dogs of similar sex and age), matching on spatial location also implied similarity with respect to sex and age. Fecal specimens were scored based on a modification of a fecal scoring system for dogs and cats[a] with the modified version including only 5 possible scores: 1, liquid or watery feces with no form (corresponding to a fecal score in cats[a] of 0; for a score of 1, the fecal scoring system for cats was used because the chart had photographs of diarrhea samples that were more severe than those on the chart for the fecal scoring system for dogs); 2, very soft, unformed feces (corresponding to a fecal score in dogs[a] of 0); 3, very soft, moderately formed feces (corresponding to a fecal score in dogs[a] of 25); 4, loose but formed feces (corresponding to a fecal score in dogs[a] of 50); and 5, well-formed to hard feces (corresponding to a fecal score in dogs[a] of 75 to 100). A fecal score of 1 to 3 was used to define a case specimen, and a fecal score of 5 was used to define an acceptable control specimen. To minimize the possibility of ambiguous case and control designations, fecal specimens corresponding to scores of 4 were not included in this study. To obtain fresh fecal samples, runs were cleaned of all fecal material by shelter personnel and then observed for recent defecation. All diarrhea specimens encountered by a designated observer (CR) within 1 hour of cage cleaning were collected from the entire shelter (with the exception of the rabies quarantine area). Samples were collected into clean fecal cups, transported within 6 hours of collection to the University of California Center for Companion Animal Health laboratory, and processed immediately after arrival. At the time of collection, dog identification number, date of the sample, body condition score, date of entry into the shelter, housing location, age, breed, and sex were recorded, if known. On arrival at the laboratory, samples were separated into 4 aliquots for further testing including centrifugation flotation and ELISA for parasites, bacteriologic culture (aerobic, microaerophilic, and anaerobic), bacterial toxin and enterotoxin testing, and viral testing via ELISA and **polymerase chain reaction (PCR)** assay.

Direct wet mounts were prepared from fresh feces and evaluated microscopically for *Giardia lamblia* trophozoites. Additionally, zinc sulfate centrifugation flotation preparations were performed, and samples were examined microscopically at a magnification of 10X with confirmation at a magnification of 40X for ova, larvae, cysts, and oocysts. Fresh fecal specimens also were examined via **direct fluorescent antibody**[b] **(DFA)** for *G lamblia* cysts and *Cryptosporidium parvum* oocysts.

After arrival at the laboratory, an aliquot of feces was immediately inoculated onto 4 types of media for bacteriologic culture including a **MacConkey (MAC)** plate,[c] a **sorbitol MAC (SMAC)** plate,[d] selenite F broth,[e] and a **cefoperazone vancomycin amphotericin (CVA)** plate.[f] The MAC and SMAC plates and selenite F broth were incubated aerobically at 37°C for 24 hours. After 24 hours, an aliquot from the selenite broth was obtained via an inoculation loop and plated for isolation on 5% sheep blood agar plates and incubated aerobically for an additional 24 hours at 37°C. Genus and species of isolated white colonies from the MAC and SMAC plates were identified by use of a defined panel of standard biochemical tests.[2] The CVA plates were incubated in a microaerophilic chamber system[g] at 42°C for 3 to 5 days. Bacterial colonies on the CVA plates were gram-stained and subcultured on 5% sheep blood agar for further isolation and characterization. Isolates with catalase and oxidase activity were further tested with growth at 25°C and 42°C, susceptibility to cephalothin and nalidixic acid, hippurate and urea hydrolysis, and utilization of nitrate, by use of previously published biochemical tests.[2] Isolates without or with weak catalase activity were further evaluated by use of species-specific PCR assays as described.[3] Determination of the presence of *Clostridium perfringens* enterotoxin was performed by use of a commercially available ELISA kit,[h] and determination of *C difficile* toxin A was performed by use of an ELISA.[i,j]

An aliquot of each fecal sample was weighed and mixed with phosphate-buffered saline in a one-to-one ratio (wt:vol) and then stored at –80°C. Nucleic acids were extracted from frozen feces by the Boom silica method.[4] The sample was treated for 30 minutes with ribonuclease, and PCR assays were performed by use of published protocols[5-7] for *Brachyspira* spp, *Helicobacter* spp, and *Tritrichomonas* spp.

Fresh fecal samples were tested for parvovirus antigen by use of an ELISA snap test.[k] For canine coronavirus, cDNA was produced from nucleic acid extracts by use of random hexamers and superscript II reverse transcriptase[l]; then PCR assays were performed as previously reported.[8]

Statistical analyses—Data were maintained in a spreadsheet[m] and analyzed with a statistical package.[n] Statistical analyses to assess the association of diarrhea with exposure to microbial pathogens were performed by calculating matched odds ratios and confidence intervals. Pathogen exposures were included in the logistic regression as risk factors when significant univariate associations with diarrhea were detected (**Table 1**) or to address possible interactions with other risk factors. Logistic regression was performed stepwise in both directions (ie, adding and removing parameters) by use of a general linear modeling function, with a binomial family. The optimal model was chosen to minimize the Akaike Information Criterion. For all statistical analyses,

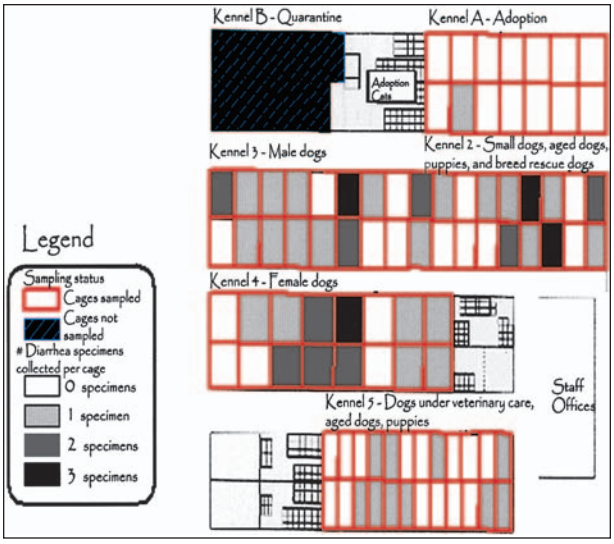

Figure 1—Spatial diagram of diarrhea case specimens collected from dogs housed at a large municipal animal shelter from June to August 2002.

values of $\alpha = 0.05$ were considered significant. Because spatial location was used as a matching criterion, it could not be tested as a risk factor in the epidemiologic portion of the study. Instead, spatial issues were addressed through investigating the pattern of diarrhea occurrence by use of a GIS and spatial statistics.

For spatial analysis, individual cages were displayed by use of a GIS[o] over an image of the shelter floorplan (**Figure 1**). Cages were digitized as polygons, and the spatial location of each case specimen was recorded and linked to the spatial map. Diarrheic case specimens were mapped based on the cage from which they were collected. Statistical analysis of spatial data consisted of assaying for deviation from complete spatial randomness.[9] By calculating the Ripley K-function[10] with edge effects adjustment[11] by use of the function "Kest" in the R library "Spatstat" and comparing that function with the function generated by a random (Poisson) distribution of points, we could detect clusters in the distribution of diarrheic case specimens. If data were clustered, K(r) would be expected to be $> \pi r^2$, where r is the distance between 2 arbitrarily chosen points. If data were randomly distributed in space, the expected K(r) would be $< \pi r^2$.

Results

Fecal specimens collected from 60 case dogs and 60 control dogs were evaluated from 1 animal shelter. Twenty-seven of the 60 (45%) case dogs collected were male, 27 (45%) were female, and 6 (10%) dogs' sexes were not reported. Similarly, 27 (45%) control dogs

Table 1—Exposure and 95% confidence intervals (CIs) among dogs with (case dogs) and without (controls dogs) diarrhea for all pathogens investigated.

	Case dogs				Control dogs			
Risk factors	**No. positive**	**No. tested**	**Exposure (%)**	**95% CI**	**No. positive**	**No. tested**	**Exposure (%)**	**95% CI**
Parasites								
Fecal macroparasites*	19	57	33.3	17.35–33.95	9	56	16.1	8.05–28.83
Ancylostoma caninum†	2	57	3.5	0.61–13.16	1	56	1.8	0.09–10.81
Trichuris vulpis†	5	57	8.8	3.27–20.04	3	56	5.4	1.39–15.80
Isospora spp	6	57	10.5	4.35–22.19	0	56	0	0.00–8.00
Toxocara canis†	8	57	14.0	6.68–26.35	5	56	8.9	3.33–20.37
Giardia lamblia via fecal flotation†	2	57	3.5	0.61–13.16	1	56	1.8	0.09–10.81
G lamblia via DFA†	18	49	36.7	23.79–51.75	18	49	36.7	23.79–51.75
Tritrichomonas spp via PCR assay	0	60	0	0.00–7.50	2	60	3.3	0.58–12.55
Cryptosporidium parvum via DFA†	4	49	8.2	2.65–20.48	4	48	8.3	2.70–20.87
Bacteria								
Clostridium perfringens via enterotoxin ELISA†	3	49	6.1	1.59–17.87	6	49	12.2	5.08–25.46
Clostridium difficile toxin A via ELISA†	16	48	33.3	20.81–48.51	24	46	52.2	37.13–66.86
Escherichia coli 0157H7†	3	60	5.0	1.30–14.82	1	60	1.7	0.09–10.14
Vibrio parahaemolyticus via PCR assay	1	60	1.7	0.09–10.14	0	60	0	0.00–7.50
Brachyspira spp via bacteriologic culture	3	60	5.0	1.30–14.82	2	60	3.3	0.58–12.55
Campylobacter jejuni via bacteriologic culture*†	11	60	18.3	9.93–30.85	2	60	3.3	0.58–12.55
Campylobacter coli via bacteriologic culture	9	60	15.0	7.50–27.08	6	60	10.0	4.13–21.17
Campylobacter upsaliensis via bacteriologic culture	7	60	11.7	5.21–23.18	6	60	10.0	4.13–21.17
Campylobacter sputorum via bacteriologic culture	3	60	5.0	1.30–14.82	1	60	1.7	0.09–10.14
Campylobacter lari via bacteriologic culture	1	60	1.7	0.09–10.14	1	60	1.7	0.09–10.14
Campylobacter fetus via bacteriologic culture	1	60	1.7	0.09–10.14	0	60	0	0.00–7.50
Helicobacter spp via PCR assay	57	60	95.0	85.18–98.70	53	60	88.3	76.82–94.79
Salmonella spp via bacteriologic culture	0	60	0	0.00–7.50	0	60	0	0.00–7.50
Aeromonas spp via bacteriologic culture	0	53	0	0.00–8.42	0	53	0	2.45–19.07
Plesiomonas spp via bacteriologic culture	0	60	0.0	0.00–7.50	0	60	0.0	0.00–7.50
Viruses								
Parvovirus via ELISA	1	59	1.7	0.09–10.30	0	59	0.0	0.00–7.62
Canine coronavirus via nested PCR assay	44	60	73.3	60.11–83.55	35	59	59.3	45.76–71.67

*Significantly ($P < 0.05$) different between case and control dogs. Statistical analyses revealed significantly higher exposure among case dogs than control dogs. †Potentially zoonotic. The number of samples tested for each pathogen varied because of limited sample or test availability at various times throughout the study.

DFA = Direct fluorescent antibody. PCR = Polymerase chain reaction.

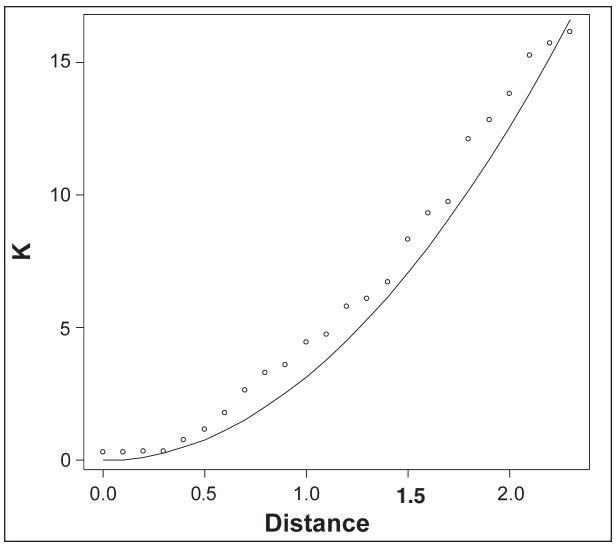

Figure 2—Estimate of the Ripley's K statistic at various distances (measured in relative map units) for diarrhea case specimens collected from dogs housed at a large municipal animal shelter from June to August 2002.The solid line represents a theoretically random (Poisson) distribution; the dots represent the distribution of diarrhea case specimens. Spatial analysis revealed that the distribution of diarrhea case specimens was not different from random, and there was no evidence of spatial clustering in the data.

were male, 27 (45%) were female, and 6 (10%) dogs' sexes were not reported. Case and control matched pairs were of the same sex 71% of the time, of different sex 11% of the time, and indeterminable (because of lack of reporting among case or control dogs or both) 16% of the time.

Nineteen of 57 (33.3%) case dogs and 9 of 56 (16.1%) control dogs had evidence of intestinal parasites as determined by fecal flotation, including hookworms (*Ancylostoma* spp), whipworms (*T vulpis*), *Isospora* spp, roundworms (*Toxocara canis*), and *G lamblia* (Table 1). The most common intestinal parasite among case (14%) and control (8.9%) dogs was *T canis*. Although the prevalence of parasites was consistently higher among case dogs than control dogs, the differences between case and control dogs were not significant by univariate analysis for any single parasite. However, a significant ($P < 0.05$) difference was detected when fecal parasites were compared as a group between case and control dogs (odds ratio = 3; 95% confidence interval = 1.04 to 10.5).

The most common bacterial pathogens detected were *Helicobacter* spp (95.0% of case dogs and 88.3% of control dogs) and *C difficile* (33.3% of case dogs and 52.2% of control dogs as detected by toxin ELISA). *Campylobacter jejuni* was detected among 11 of 60 (18.3%) dogs with diarrhea and only 2 of 60 (3.3%) dogs without diarrhea. With the exception of *C jejuni,* none of the bacteria were significantly different in case versus control dogs. The difference in exposure to *C jejuni* between case and control dogs was significant (OR = 10; 95% CI, 1.42 to 433.4; $P < 0.05$). Other notable but rare bacterial pathogens detected included *E coli* O157H7 in 3 case dogs (5.0%) and 1 control dog (1.7%), *Vibrio parahaemolyticus* in 1 (1.7%) case dog, *Brachyspira* spp in 3 (5.0%) case and 2 (3.3%) control dogs, and *Campylobacter lari* in 1 (1.7%) case and 1 (1.7%) control dog.

Canine coronavirus (CCV) and parvovirus were tested by PCR assay and ELISA, respectively. Canine coronavirus was detected in 44 of 60 (73.3%) case dogs and 35 of 59 (59.3%) control dogs. Although the prevalence of coronavirus was higher among case than control dogs, the difference was not significant. Parvovirus was detected in only 1 (1.7%) case dog and no control dogs.

On the basis of significant univariate risk factors for diarrhea, the generalized linear regression model of diarrhea included intestinal parasites grouped together and *C jejuni*, with canine coronavirus added because of biologically plausible interactions (a suspected underlying or predisposing infection), and all possible interactions among these 3 factors. Final results of the stepwise logistic regression model indicated that the Akaike Information Criterion was minimized when the 3 risk factors were included individually but all interaction terms excluded, indicating that interactions were not associated the case status.

Case and control dogs were observed throughout the sampled shelter buildings (Figure 1). A high prevalence of diarrhea was particularly evident on the north side of the female dog cages in kennel 4, intermixed among male dog cages in kennel 3 and among aged dogs, puppies, and dogs under veterinary care (typically for illnesses not associated with diarrhea) in kennel 5. There was a low occurrence of diarrhea among adoption-ready dogs in kennel A. Statistical analysis for spatial clustering by use of a Ripley's K statistic revealed that the distribution of diarrhea case specimens was not significantly different from random **(Figure 2)**.

Discussion

The study reported here provides an epidemiologic overview of diarrhea and interacting diarrhea-associated pathogens in a densely housed, highly predisposed shelter population of dogs. To the authors' knowledge, no other study has used a case-control study design to assess interactions among such an array of possible primary and secondary pathogens with diarrhea in dogs in a shelter environment. Results of this study indicated that several pathogens associated with diarrhea in dogs such as *S enterica* and *Brachyspira* spp were uncommon, whereas others such as canine coronavirus and *Helicobacter* spp were nearly ubiquitous among the population surveyed.

Although no individual parasite detected by fecal flotation was significantly associated with diarrhea by univariate analysis, prevalence of fecal parasites as a group (including hookworms [*Ancylostoma* spp], whipworms [*T vulpis*], *Isospora* spp, roundworms [*T canis*], and *G lamblia*) was significantly higher in case dogs than in control dogs. Dogs with and without diarrhea shed fecal parasites into the environment, supporting the notion that the absence of clinical signs such as diarrhea does not preclude parasite infection. Approximately 33% of case dogs and 16% of control dogs had evidence of fecal parasites on fecal flotation, underscoring the importance of empiric deworming in these dogs. Furthermore, several of the parasites

detected, including *Ancylostoma caninum*, *T vulpis*, *Cryptosporidium* spp, and *G lamblia,* have zoonotic potential, and potential adopters should be informed of the zoonotic risks. Many fecal parasite infections do not cause diarrhea directly, but are usually secondary to other gastrointestinal disorders or stresses.[1] Exceptions include *T vulpis* and *G lamblia,* which can cause mild to severe diarrhea, most commonly in young or immunocompromised dogs.[1] In the study reported here, it is likely that the association between fecal parasites and diarrhea was an incidental finding or was related to underlying immunocompromise, debilitation, or stress in these dogs. However, a causal association between fecal parasite infection and diarrhea in these dogs was not ruled out.

Giardia lamblia is well described as an important pathogen in dogs, humans, and other species. Results of 1 study[12] indicate that a high prevalence of *G lamblia* was detected via ELISA in dogs with diarrhea (49%) and healthy dogs (40%), which is in agreement with results of our study when the DFA technique was used (36.7% for case and control dogs), but much higher than results obtained by use of the centrifugation flotation technique (3.5% of case dogs and 1.8% of control dogs). Results of that study also indicate a much lower level of detection with fecal flotation, compared with DFA. These findings underscore the limitations of relying on results of fecal flotation for diagnosing *G lamblia* infection, and shelters relying only on fecal flotation for detection of protozoa associated with diarrhea may substantially underestimate the magnitude of the problem.

Campylobacter spp, *C perfringens*, *C difficile*, and *Salmonella* spp are well-documented causes of bacteria-associated diarrhea in dogs.[13] In our study, results of bacteriologic culture for *Salmonella* spp were negative in all dogs evaluated (n = 120), whereas several *Campylobacter* spp, including *Campylobacter coli*, *C lari*, *Campylobacter upsaliensis*, *Campylobacter sputorum*, *C fetus*, and *C jejuni* were cultured from feces of case and control dogs. *Clostridium* spp exposures were detected with moderate frequency among the dogs surveyed; *C difficile* toxin A was detected in 33.3% of case and 52% of control dogs, whereas *C perfringens* enterotoxin was detected in 6.1% of case and 12.2% of control dogs. Although the differences between case and control dogs with respect to *Clostridium* spp exposures were not significant, it is interesting to note that exposures to these pathogens in control dogs were as high as or higher than in case dogs. Of all the bacterial pathogens isolated, only *C jejuni* was significantly more prevalent in case dogs than control dogs. This finding is in agreement with results of other studies,[14,15] which document *C jejuni* more frequently in dogs with diarrhea than in dogs without diarrhea.

Diarrhea associated with *C jejuni* infection varies and is often mild; however, dogs can occasionally have liquid feces containing mucus and blood.[1] *Campylobacter jejuni* is widely recognized as a cause of enteritis in humans, and a small proportion of the cases of human infections with *C jejuni* have been associated with exposure to infected dogs and cats with diarrhea.[16] Other studies[17] have also documented diarrhea in shelters caused by *C coli*. In our study, various *Campylobacter* spp, such as *C upsaliensis*, *C lari*, *C fetus*, and *C sputorum*, also infected shelter dogs; however, their contribution to clinical signs was not clear. *Campylobacter upsaliensis* has been implicated in diarrheal outbreaks in children in daycare centers,[18] and zoonotic transmission from dogs has been reported.[19] Age is a risk factor for *C jejuni*-associated diarrhea, and puppies are at a greater risk for infection and disease.[17] Infection can be detected via bacteriologic culture of feces by use of campylobacter-selective agars in a microaerophilic atmosphere; however, these specialized techniques are inefficient in shelters unless coupled with an effective strategy for preventing additional spread. Surveillance for *Campylobacter* spp can be an excellent tool for evaluating shelter practices because the bacteria are common, zoonotic, and spread by direct exposure to infected feces. Practices that manage *Campylobacter* spread will likely be effective for managing many other shelter pathogens as well. Because of zoonotic potential, it may be advisable to screen dogs with and without diarrhea for *C jejuni* before adoption from the shelter.

Although no *Helicobacter* spp bacteria were obtained on fecal culture, results of PCR assay for members of the genus were positive in 95.0% of case and 88.3% of control dogs tested. Helicobacters in dogs include gastric inhabitants such as *Helicobacter bizzozeronii* and a group of poorly understood intestinal and hepatic colonizers such as *Helicobacter cinaedi*, *Helicobacter fennelliae*, and *Helicobacter canis*. Although these bacteria have been detected in samples from dogs, their roles in the cause of diarrhea are not clear, particularly because similar infections caused by *H cinaedi* and *H fennelliae* are known to be most problematic in immunocompromised humans with proctitis and colitis.[20,21] *Helicobacter canis*, which has been detected in feces of healthy dogs and dogs with diarrhea[22] and a child with enteritis,[23] also was detected during an assessment of an outbreak of severe diarrhea, enterocolitis, and mild portal hepatitis in a Bengal cattery.[24] Assessment for intestinal helicobacters was included in the study reported here because the bacteria may be zoonotic and because of the possibility that they may contribute in synergy with other intestinal pathogens to diarrhea in shelter dogs. Unfortunately, DNA from gastric helicobacters (which are common in dogs) likely contaminated the fecal DNA extractions, yielding high PCR estimates, consistent with results of other studies.[25,26] Further characterization (eg, by DNA sequencing) was not performed for these amplicons because no isolates were obtained via bacteriologic culture. Nevertheless, the results support the theory that *Helicobacter* spp can exist as nonpathogenic inhabitants within the gastrointestinal tract of dogs.[27,28]

Canine coronavirus is a common pathogen in dogs, but often it is not evaluated in cases of clinical diarrhea, and its actual role in diarrhea in densely housed dogs is poorly appreciated. Studies often focus on either viral or bacterial pathogens associated with diarrhea, but not both, possibly because techniques required for detection of pathogens that vary phylogenetically are different. Serologic tests for CCV would be

difficult to interpret in shelters because of cross-reaction with vaccine-induced titers and because of the need to detect active shedding. Results of several studies[12,29] indicate that the prevalence of CCV ranged from 15% to 26% in family pets, 30% in kenneled dogs, and 55% in dogs evaluated at various veterinary clinics in Japan. Persistent shedding for as much as 37 days has been reported from clinically affected and healthy dogs.[30] Clinical disease associated with CCV infection tends to be mild or absent, consisting of self-limiting vomiting and diarrhea, although it is probable that the lesions in the intestinal villi predispose to or act synergistically with other gastrointestinal pathogens. In our study, dogs with and without diarrhea had high rates of CCV infection (73.3% in case and 59.3% in control dogs), and CCV was not a significant risk factor for diarrhea. Results of 1 study[12] indicate that the rates of CCV infection in dogs with diarrhea and healthy dogs were 57% and 40%, respectively. Analysis of CCV in the study reported here indicated that infection was widespread among the shelter dogs, as expected for such an infectious virus.

The low prevalence of parvovirus at this shelter was not unexpected because severely ill dogs typically were identified by staff and removed from the general population. Infected dogs occasionally are introduced into this shelter, and mildly ill dogs could go unnoticed, increasing the risk of spread to other dogs. The high prevalence of extremely contagious pathogens such as CCV indicates an almost unhindered spread of infection throughout the shelter population. These results suggest that management of such highly infectious diseases should be directed towards vaccination, quarantine if possible, and reduction of transmission, although vaccines are not available for many of the pathogens important in shelters.

Tracking the spatial distribution of diarrhea case specimens by use of GIS, as used in this study, may serve as a useful tool for management by identifying disease patterns, which can reveal clues about shelter-specific diarrhea problems and management options. For example, in this study, spatial analysis revealed that diarrhea was widespread throughout the entire shelter and that the spatial pattern of diarrhea was not different from random (ie, there was no evidence of clustering among case specimens). This lack of clustering could be explained by a small sample size or could represent the true pattern of diarrhea case specimens in this shelter. A random distribution of diarrhea case specimens does not support a contagion model for the spread of diarrhea in this shelter; conversely, it suggests that noncontagious causes of diarrhea, such as stress or diet change, may have played an important role in causing diarrhea in this shelter.

This study had several limitations. First, the shelter policy of cohousing dogs limited the ability to identify individual dogs with diarrhea and thus restricted the spatial analysis to the cage level. Also, specific characteristics of individual case and control dogs, such as body condition and time in the shelter, could not be accurately determined in most cases, precluding an analysis of these characteristics as risk factors. It would be valuable to repeat this study in 1 or several shelters in which dogs with diarrhea could be tracked individually and statistical independence among sampled dogs could be better insured. Second, use of a matched case-control study design limited the ability to report overall prevalences of disease and exposures in the population surveyed. Matching in this study was performed because of the expectation that infectious agents and diarrhea case specimens may cluster around a source dog, thus making spatial location a potential confounder, obscuring the associations among infectious pathogens and diarrhea. In fact, the lack of spatial clustering of diarrhea case specimens implies that noninfectious factors may be important causes of diarrhea in shelters, and repeating this study with a cohort or cross-sectional study design based on randomization of case and control dogs may be useful in testing for noninfectious risk factors such as stress, change in diet, and spatial location. Finally, this study incorporated data from only 1 animal shelter. Each shelter may have different local patterns of diarrhea occurrence and spread. Future research in this area should incorporate both large-scale, multiple-shelter analyses to determine regional trends and small-scale, individual-shelter analyses to develop management policies tailored to shelter-specific problems.

Although a diverse array of pathogens and opportunists were evaluated in the study reported here, there were several that were not included and could have contributed to problems in this shelter, particularly rotaviruses and canine distemper virus. In addition, diarrhea in individual dogs may have been induced by more uncommon pathogens such as *Prototheca* spp, *Histoplasma capsulatum*, or *Neorickettsia helminthoeca*, which were not examined in this study. Nevertheless, some of the approaches in this study, such as use of a spatial representation of case specimens and an evaluation of multiple infectious etiologies simultaneously, were novel and will facilitate individual-dog and population management in this shelter and others in the future. This study also illustrates an integrated approach to the investigation of the causes of diarrhea, which may be applied in other vulnerable populations of dogs.

a. Purina Fecal Scoring System for Dogs and Cats, Nestle-Purina Pet Food Co, St Louis, Mo.
b. Direct fluorescent antibodies, Merifluor IFA, Cincinnati, Ohio.
c. MacConkey plates, School of Veterinary Medicine Media Kitchen, University of California, Davis, Calif.
d. Sorbitol MacConkey plates, School of Veterinary Medicine Media Kitchen, University of California, Davis, Calif.
e. Selenite F broth, School of Veterinary Medicine Media Kitchen, University of California, Davis, Calif.
f. Cefoperazone vancomycin amphotericin plates, Remel Labs, Lenexa, Kan.
g. CampyPlus, Becton-Dickinson, Franklin Lakes, NJ.
h. ELISA test kit for *Clostridium perfringens* enterotoxin, Techlab, Blacksburg, Va.
i. ImmunoCard Toxin A test, Meridian Diagnostic Inc, Cincinnati, Ohio.
j. Triage Micro Biosite assay, Biosite Diagnostics, San Diego, Calif.
k. ELISA snap test for parvovirus, IDEXX Laboratories, Westbrook, Me.
l. Superscript II reverse transcriptase, Gibco BRL, Gaithersburg, Md.
m. Excel 2002, Microsoft Corp, Redmond, Wash.

n. R, The R-Development Core Team. Available at: www.r-project.org. Accessed September 15, 2004.
o. ArcMap, version 8.0, ESRI, Redlands, Calif.

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