Evaluation of a routine diagnostic fecal panel for dogs with diarrhea

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Objectives—To assess the diagnostic yield of a routine fecal panel and determine whether *Clostridium perfringens* or *C difficile* toxin production is associated with acute hemorrhagic diarrheal syndrome (AHDS) in dogs.

Design—Case-control study.

Animals—260 dogs with diarrhea and 177 dogs with normal feces.

Procedure—Medical records were reviewed for results of culture for *C difficile, Campylobacter* spp, and *Salmonella* spp; *C perfringens* fecal enterotoxin (CPE) assay via ELISA or reverse passive latex agglutination (RPLA) assay; fecal endospore enumeration; *C difficile* toxin A assay; and parasite evaluation.

Results—Prevalence of CPE in dogs with diarrhea was 22/154 (14.3%) via ELISA and 47/104 (45.2%) via RPLA assay, versus 9/74 (12%) via ELISA and 26/103 (25%) via RPLA assay in control dogs. Prevalence of *C difficile* was 47/260 (18%) in dogs with diarrhea and 41/74 (55%) in control dogs. Prevalence of *C difficile* toxin A was 26/254 (10.2%) in dogs with diarrhea and 0/74 in control dogs. Diagnosis of AHDS was made in 27 dogs; 8 had positive results for CPE, 7 had positive results for toxin A, and 1 had positive results for both toxins. *Campylobacter* spp were isolated from 13 of 260 (5%) dogs with diarrhea and 21 of 74 (28.4%) control dogs. *Salmonella* spp were isolated from 3 (1.2%) dogs with diarrhea.

Conclusions and Clinical Relevance—Diagnostic value of a fecal panel in dogs with diarrhea appears to be low. (*J Am Vet Med Assoc* 2002;221:52–59)

Diarrhea in dogs is a common cause for examination by veterinarians, and enteric bacterial pathogens are often considered as potential causes of acute and chronic disease. Initial diagnostic evaluation of all but the most severely affected dogs is usually conservative, since many episodes are either self-limiting or respond well to dietary manipulation. Nonresponsive or severely affected dogs usually require further evaluation that will range from noninvasive and relatively inexpensive assays such as fecal flotation and smear, to more expensive and invasive procedures such as endoscopy and intestinal biopsy. Bacteriologic culture of feces for putative bacterial pathogens is frequently considered a reasonable test to perform prior to the more expensive and invasive investigations. However, the value of any diagnostic test should be judged mainly by a combination of its specificity, sensitivity, predictive value, and cost efficiency, with invasiveness and risk to the patient as independent considerations. The value of indiscriminate bacteriologic culture of feces in human patients has been examined and found to be generally low.[1-3] Although similar studies have not been performed in canine patients, some authors warn against routine bacteriologic culture of feces because of suspicion of the same relative yield.[4,5]

The organisms most commonly considered to be causes of bacterial-associated diarrhea in dogs are *Salmonella* spp, *Campylobacter* spp, *Clostridium perfringens*, and *C difficile*.[6,7] The most common type of disease associated with *Clostridium* spp in dogs has typically been described as either acute or chronic colitis.[6-9] There have also been reports of apparent associations between an acute hemorrhagic enteritis and the presence of *Clostridium* species.[10] Recently, Sasaki et al[11] reported a case of acute hemorrhagic enteritis and death in a dog in which the principle enteric lesion was superficial hemorrhagic mucosal necrosis limited to the jejunum and ileum. Although *C perfringens* was isolated from the jejunal contents and was seen histologically in the necrotic regions of the intestinal villi, the prevalence of this organism in clinically normal dogs and its postmortem proliferation preclude firm conclusions regarding its role in this and other cases, as judged on the basis of culture results alone.[5] In addition, identification of the principle enterotoxin produced by *C perfringens* was not performed in either of these dogs. Therefore, uncertainty remains regarding the true nature of the enteric disease attributable to toxin production by either organism in dogs.

Interpretation of routine bacteriologic culture of feces for these organisms is problematic, because all of these organisms are opportunistic pathogens and have been frequently isolated from the feces of clinically normal dogs. In addition, the clinical signs and nature of diarrhea in dogs with these bacteria are extremely variable, further contributing to the confusion surrounding the role of these organisms in clinically affected dogs. Given these considerations, it seemed appropriate to determine the diagnostic yield of these assays as an indicator of their true value. Furthermore, continued evaluation of the dogs from which these organisms are isolated may provide a basis for selecting those dogs that would ultimately benefit from bacteriologic assays.

The objectives of the study reported here were to assess the diagnostic yield of a routine fecal panel in dogs and test the hypothesis that *Clostridium perfringens* or *C difficile* toxin production is associated with

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an **acute hemorrhagic diarrheal syndrome (AHDS)** in dogs.

Materials and Methods

Case and control selection—Five groups of dogs were used in this study. Group-1 dogs (n = 260) were patients with diarrhea, group-2 dogs (50) were outpatients with normal feces, and group-3 dogs (24) were hospitalized patients with normal feces. Two groups of dogs with normal feces representing outpatients (group 4; n = 52) and hospitalized patients (group 5; 50) were used only as controls for evaluation of the **reverse passive latex agglutination (RPLA)** assay used to detect ***C perfringens* enterotoxin (CPE)** in a subset of group-1 dogs (104). In groups 2 and 3, CPE was detected only by use of ELISA. The dogs with diarrhea (group 1) represented 260 consecutive submissions for fecal analysis at the University of California, Davis, **Veterinary Medical Teaching Hospital (VMTH)** between September 1994 and May 1999. Fecal samples from groups 2 and 3 were collected from February 1999 to August 1999, whereas fecal samples from groups 4 and 5 were collected from September 1996 to February 1997. Hospitalized patients with normal feces represented a heterogeneous group of surgical and medical patients with periods of hospitalization ranging from 24 hours to several weeks. Outpatients with normal feces included dogs referred for routine procedures such as vaccination and consultations not requiring admission to the hospital.

Collection of data—Medical records for dogs with diarrhea were reviewed for signalment (breed, age, weight, sex), staple diet, month of fecal analysis, primary complaints, concurrent diseases, primary diagnosis, onset of diarrhea (prior to or after referral), duration of diarrhea (prior to fecal analysis), nature of feces, recent history of antimicrobial exposure (including timing of administration relative to onset of diarrhea), fecal flotation results, and fecal panel results. Medical records for hospitalized dogs with normal feces were reviewed for the same data except that pertaining to diarrhea. Owners of outpatient dogs were questioned regarding history of antimicrobial administration and diet, and signalment and fecal analysis data were likewise recorded. The anatomic location of the enteric lesion in dogs with diarrhea was classified as large intestine, small intestine, diffuse, or uncharacterized, according to the description of clinical signs and fecal appearance. Features attributed to large intestinal disease included tenesmus, dyschezia, defecation frequency > 5 episodes/24 h, increased fecal mucus, hematochezia, and small fecal volumes. Features attributed to small intestinal disease included large-volume diarrhea without tenesmus or dyschezia, < 5 defecations/24 h, and melena. Antimicrobial treatment was recorded if the treatment occurred within 4 weeks of the commencement of diarrhea or within 1 month of fecal analysis if it was administered after the onset of diarrhea. Staple diets were grouped into 4 categories: commercial, home-prepared, mixed, and unknown.

Acute hemorrhagic diarrheal syndrome was diagnosed if hemorrhagic diarrhea had been present for < 7 days. Dogs were excluded if there was any potential concurrent disease or historical finding that could reasonably be expected to cause AHDS or if there were signs of colitis only. Exclusion diagnoses included but were not limited to leptospirosis, nonsteroidal anti-inflammatory or corticosteroid toxicosis, hypoadrenocorticism, doxorubicin treatment, inflammatory bowel disease (of any infiltrative type), severe hepatitis or hepatic neoplasia, acute renal failure, pancreatitis, anticoagulant toxicosis, gastrointestinal neoplasia, and enteric infection with parvovirus, *Salmonella* spp, *Campylobacter* spp, *Ancylostoma caninum* or *Trichuris vulpis*.

Fecal analysis—Fecal analysis consisted of culture for *C difficile, Campylobacter* spp, and *Salmonella* spp; *C perfringens* fecal enterotoxin assay and fecal endospore enumeration; *C difficile* toxin A assay; and fecal smear and flotation for parasite evaluation. Fresh feces were obtained from all dogs within 1 hour of defecation and were processed within 1 hour of collection. In the rare instance that a fecal specimen could not be fully processed after normal hospital hours, it was refrigerated, and an aliquot was placed in the appropriate buffer solution for evaluation of toxins within 1 hour of collection for analysis the following morning.

***Clostridium perfringens* assays**—Fecal specimens (50 µl or a 3-mm-diameter sample) were tested by use of a qualitative *C perfringens* enterotoxin ELISA.[a] Test specimens were added to 200 µl of the supplied diluent (buffered protein solution with 0.02% thimerosal) and vortexed for 10 seconds. A 100-µl aliquot of diluted specimen was tested for CPE according to the manufacturer's instructions.[12]

For the RPLA, 2 g of fresh feces were mixed with 2 ml of phosphate-buffered saline solution (pH 7.3) and centrifuged at 13,000 × g for 10 minutes. The supernatant was filtered through a 0.45-µm membrane filter and tested for CPE by use of a qualitative test kit[b] according to the manufacturer's instructions.[12] Results were considered positive when a 1-well or greater difference in agglutination was observed when serial dilutions of the sample and positive control were compared with the negative control.

For fecal endospore enumeration, 1 g of fresh feces was added to 2 ml of sterile saline (0.9% NaCl) solution and vortexed until completely homogenized. By use of a loop, a 10-µl aliquot was spread on a 15 × 15-mm area of a glass slide and allowed to air dry. The smear was methanol-fixed and gram-stained. Ten random monolayered oil-immersion 100× fields were examined for *C perfringens* endospores, defined as spores contained within large, relatively short, fat, box-car shaped, gram-positive rods.[13] If spores were seen in 1 to 3 fields, the sample was considered to contain few endospores, whereas smears with 4 to 7 and 8 to 10 fields that contained endospores were considered to contain moderate and large numbers of endospores, respectively.

***Clostridium difficile* assays**—Toxin A was assayed by use of 1 of 2 commercial ELISA kits.[c,d] Fecal specimens (25 µl[c] or 500 µl[d]) were tested within 1 hour of defecation, according to manufacturer's instructions.

For bacteriologic culture, fresh feces were spread with a sterile swab onto prereduced **cycloserine-cefoxitin-fructose agar (CCFA)**.[e] Plates were incubated anaerobically at 37 C for 24 to 48 hours and examined for nonswarming yellow colonies with a ground glass appearance. Yellow colonies were subcultured to prereduced **blood agar (BA)** and egg-yolk agar and incubated for 24 hours at 37 C. Colonies on BA were used for gram staining and detection of L-proline-aminopeptidase.[f] A presumptive identification of *C difficile* was made on the basis of lack of aerotolerance, colonial morphologic features and color on both CCFA and BA, gram stain revealing large straight gram-positive bacilli, and fluorescence, odor, and detection of L-proline-aminopeptidase activity. Assays for *C difficile* were performed in all 260 dogs with diarrhea and on the same control populations used for the *C perfringens* ELISA assessment (groups 2 and 3).

***Campylobacter* spp assay**—Fresh feces were plated with a sterile swab onto a selective *Campylobacter* agar that contained cefoperazone, vancomycin, and amphotericin B[g] and were streaked for isolation. Plates were incubated at 42 C by use of a microaerophilic gas generating system.[h] Plates were examined for growth at 48 to 72 hours. Suspect colonies were gram-stained and subcultured to 5% sheep BA. Tests for catalase, oxidase, indoxyl acetate, nitrate, hippurate hydroly-

sis, and susceptibility to 30-µg disks of cephalothin and nalidixic acid were performed.

***Salmonella* spp assay**—Fresh feces were plated with a sterile swab onto MacConkey agar[i] and streaked for isolation. One gram of feces was inoculated into 4% selenite broth for salmonellae enrichment. Incubated selenite broth was subcultured to **xylose-lysine-tergitol 4 (XLT4)** agar. All cultures were incubated without CO_2 for 24 to 48 hours at 37 C, except selenite broth, which was incubated for 24 hours. Lactose-negative colonies from MacConkey agar and H_2S-positive colonies from XLT4 agar were subcultured to biochemical media according to identification schema used by our diagnostic microbiology laboratory.

Parasite examination—Fresh feces were examined for parasite ova or cysts by use of a zinc sulfate double centrifugation flotation technique, as described.[14,15]

Statistical analyses—An exact χ^2 test of association was used to compare categoric variables (signalment, exposure to antimicrobials, presence of fecal parasites, month of diagnosis, presence of diarrhea, presence of AHDS) with outcomes (negative vs positive) in each of the following 5 organism and assay-specific subpopulations tested: *C perfringens* enterotoxin ELISA, *C perfringens* RPLA, *C difficile* toxin A, *C difficile* culture, and *Campylobacter* spp culture. The same test was used to compare these variables with patient diarrhea status (absent vs present). The Student *t* test was used to compare mean age and weight between each of the 2 test outcome groups (negative vs positive) for each of the 5 subpopulations. For all comparisons, a value of $P < 0.05$ was considered significant.

Results

Antimicrobial administration—Of the 260 dogs with diarrhea, 47 (18%) had received antimicrobials within 1 month prior to the onset of diarrhea, and 119 (46%) had received antimicrobials after the onset of diarrhea and within the 1-month period before fecal analysis. Of the group-2 and -3 dogs with normal feces, 12 of 74 (16%) had received antimicrobials within the 1-month period prior to fecal analysis. Of the group-4 and -5 dogs with normal feces, 33 of 102 (32%) had similarly received antimicrobials. The differences in numbers of dogs that had received antimicrobials between group-1 dogs and groups-2 and -3 and between group-1 dogs and groups-4 and -5 were significant ($P < 0.001$). The antimicrobials included a wide range of types (n = 23), numbers, and schedules.

Clostridium perfringens—Of the 260 fecal samples collected from dogs with diarrhea, 104 were evaluated by use of the RPLA assay, and the following 154 samples were evaluated by use of ELISA for CPE. Two samples were insufficient for CPE assay. No significant associations were identified between positive ELISA or RPLA assay results and signalment, month of diagnosis, staple diet, or intestinal parasitism in any of the groups.

Prevalence of CPE in dogs with diarrhea tested via ELISA was 22/154 (14.3%), whereas prevalence of CPE in the control populations with normal feces was 4/24 (16.7%) and 5/50 (10%) for the hospitalized dogs and out-patient dogs, respectively; there were no significant differences among these values. There was no significant association between signalment and positive or negative ELISA results in any of the populations.

A significant association was found between type of diarrhea and results of ELISA when the group of dogs with diarrhea that was not characterized regarding location was excluded from analysis ($P = 0.049$). Thus, the percentage of dogs with positive results of ELISA that had signs of diffuse intestinal disease was significantly greater than those of dogs with clinical evidence of enterocolitis than in either enteritis or colitis alone.

The association between antimicrobial administration after the onset of diarrhea and negative results of ELISA for CPE approached significance ($P = 0.054$). No significant association was found between administration of antimicrobials and result of ELISA in the control populations with normal feces. Endospores were seen in 14 of 22 (63%) dogs with diarrhea and positive results of ELISA, whereas endospores were detected in 30 of 132 (22%) dogs with diarrhea and negative results of ELISA ($P = 0.001$). Despite this finding, among dogs with diarrhea and large numbers of endospores, 5 dogs had positive results of ELISA, whereas 8 dogs has negative results of ELISA.

Prevalence of CPE in dogs with diarrhea that were tested by use of RPLA was 47/104 (45.2%). Prevalence of CPE in the control populations with normal feces was 14/53 (26.4%) and 12/50 (24%) for out-patient and hospitalized dogs, respectively. Significantly more dogs with diarrhea had positive results of RPLA than did outpatient control dogs ($P = 0.02$) or hospitalized control dogs ($P = 0.011$).

In contrast with dogs tested by use of ELISA, no significant association was identified between anatomic lesion of the intestinal disease and positive or negative RPLA results with or without inclusion of the group with diarrhea that was not characterized regarding location. No significant association was found between antimicrobial administrations or lack thereof and positive or negative RPLA results in any of the 3 populations.

Endospores were seen in 17 of 47 (36%) dogs with diarrhea and positive results of RPLA, whereas endospores were seen in only 7 of 57 (12%) dogs with diarrhea and negative results of RPLA ($P = 0.011$). Among dogs with diarrhea in which large numbers of endospores were seen, 4 dogs had positive results of RPLA, whereas only 1 dog had negative results of RPLA. There was a significant association between clinical large intestinal disease and large numbers of endospore counts, whereas small intestinal disease was associated with few endospores ($P = 0.013$; **Fig 1**).

Clostridium difficile—*Clostridium difficile* was isolated from 47 of 260 (18.8%) fecal samples from dogs with diarrhea. The prevalence in control dogs with normal feces was 2/24 (8.3%; $P = 0.28$) and 3/50 (6%; $P < 0.001$) in the hospitalized and out-patient dogs, respectively. Overall, isolation of *C difficile* from feces by use of bacteriologic culture was significantly more likely in dogs with diarrhea than in all patients with normal feces (18% vs 6.7%; $P = 0.018$). There was no significant difference in rates of isolation from in-patients and out-patients with normal feces.

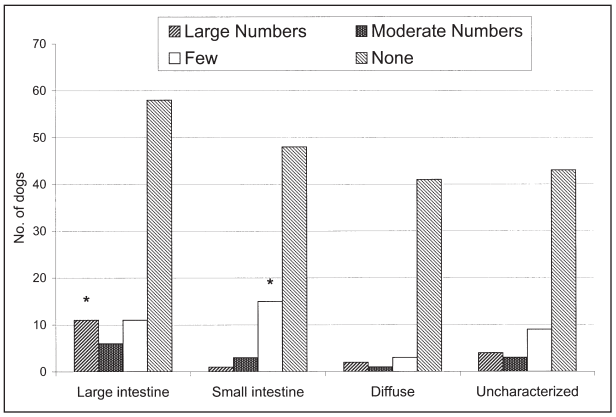

Figure 1—Number of clostridial endospores in the feces of 260 dogs with diarrhea attributable to involvement of various regions of the intestinal tract. *Significant (P = 0.013) difference, compared with other regions.

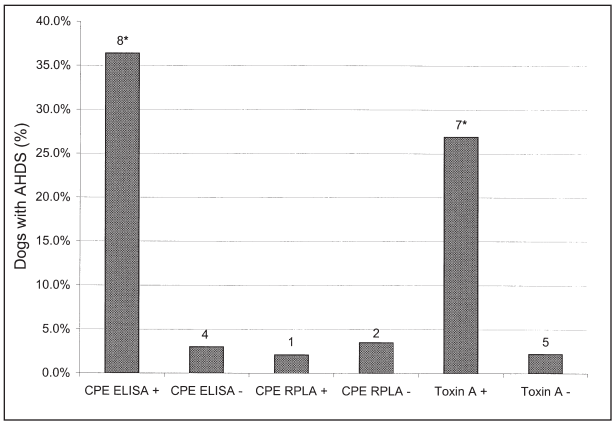

Figure 2—Prevalence of dogs with acute hemorrhagic diarrheal syndrome (AHDS) among groups of dogs that had positive (+) or negative (–) results of testing for *Clostridium perfringens* enterotoxin (CPE) via ELISA or reverse passive latex agglutination (RPLA) assay or had positive or negative results of testing for *C difficile* toxin A. *Significant ($P < 0.001$) difference from the group that had negative results for the same test. Numerals above the bars indicate the number of dogs with AHDS.

No significant association was identified between signalment, month of diagnosis, dietary staple, intestinal parasitism, anatomic location of the intestinal disease, and results of bacteriologic culture of feces for *C difficile* or evidence of toxin A in the feces in any group, whether diarrhea was present or not.

Among dogs that had received antimicrobials before the onset of diarrhea, 13 of 47 (27%) had positive results of culture for *C difficile*, whereas only 11 of 107 (10%) dogs that had not received any antimicrobials has a positive culture result (P = 0.0086). Similarly, among dogs that received antimicrobials after the onset of diarrhea, 26 of 119 (21%) had positive results of culture for *C difficile*, compared with the 11 of 107 (10%) dogs that had not received any antimicrobials (P = 0.02). No significant association was identified between exposure to antimicrobials and result of *C difficile* culture in the dogs with normal feces.

Prevalence of *C difficile* toxin A in dogs with diarrhea was 26/254 (10.2%; 6 samples were of insufficient quantity for assay), whereas prevalence in the combined control populations was 0/74 (P = 0.006), indicating a strong association between toxin A and diarrhea in dogs. No significant association was identified between the presence or absence of toxin A and a history of exposure to antimicrobials. *Clostridium difficile* toxin A was found in feces of dogs with CPE as detected by use of RPLA in 2 dogs and with CPE as detected by use of ELISA in 1 dog. In 2 of these dogs, no other explanation for the diarrhea was identified.

AHDS—There was a strong association between diagnosis of AHDS and detection of CPE via ELISA ($P < 0.001$) but not by use of RPLA assay **(Fig 2)**. In addition, there was a strong association between the diagnosis of AHDS and detection of *C difficile* toxin A ($P < 0.001$). Vomiting was reported in 5 of 9 dogs with CPE positive feces and 3 of 7 dogs with *C difficile* toxin A. Dogs were typically referred with a history of hemorrhagic diarrhea or hematemesis of < 24 hours' duration, developed acute hemorrhagic diarrhea suddenly in hospital, or died suddenly prior to the onset of diarrhea. Among the 8 dogs with AHDS that had positive results of ELISA for CPE, 4 were necropsied. Among the remaining 4 dogs, 3 recovered after parenteral administration of antimicrobials, plasma, and fluid therapy for 3 to 4 days, whereas 1 dog died but was not necropsied. Among the 7 dogs with positive results of ELISA for *C difficile* toxin A, 2 died while hospitalized and were necropsied. Both CPE and *C difficile* toxin A were identified by use of ELISA in the feces of 1 necropsied dog.

Common to all necropsied dogs were luminal hemorrhage throughout the small intestine with normal or slightly diarrheal colonic contents, thickened small intestine with mucosal or serosal hemorrhages, and superficial necrosis; all these lesions were most severe in the ileum. Mesenteric lymph nodes were enlarged in all dogs and grossly hemorrhagic on cross section in 2 dogs. The histologic diagnosis for all dogs was acute severe necrotizing hemorrhagic enteritis. Demarcation between normal and abnormal tissues was detected at the level of the ileocecal valve in 3 of the 4 dogs with CPE.

***Salmonella* spp**—*Salmonella* spp were isolated from the feces of 3 of 260 (1.2%) dogs with diarrhea. One of these dogs was referred with a 1-year history of intermittent diarrhea that had recently become hemorrhagic, but the dog was healthy otherwise. Results of CBC were within reference ranges. *Salmonella* ser Anatum was cultured, and the diarrhea resolved during a 3-week administration of trimethoprim-sulphadiazine and dietary management. The second dog was referred with a history of 3 episodes of vomiting and diarrhea during a 2-month period, the first 2 of which responded to conservative treatment. Bacteriologic culture of feces was performed at the time of the third episode and *S* Thompson was isolated. Results of CBC at that time were within reference ranges. No specific treatment was given, and the vomiting and diarrhea resolved with dietary management. The third dog developed diarrhea within 12 hours of being discharged from the hospital after having been sedated for fine-needle aspiration of an area of pulmonary consolidation. Profuse watery diarrhea developed in associa-

tion with fever (rectal temperature, 40 C [104 F]), signs of depression, and a moderate leukocytosis with left shift and toxic changes. *Salmonella* Enteritidis was recovered from the feces in association with positive results for CPE via ELISA. The diarrhea became hemorrhagic, which coincided with clinical deterioration and decreased Hct and serum albumin concentration. After 48 hours of treatment including administration of enrofloxacin, ampicillin, metronidazole, and fresh frozen plasma, the dog was euthanatized because of continued deterioration. No necropsy was performed. Among the 72 control dogs with normal feces, no *Salmonella* spp were isolated from any sample.

***Campylobacter* spp**—*Campylobacter* spp were isolated from the feces of 13 of the 260 (5%) dogs with diarrhea; in 7 of these dogs characteristic organisms were seen in fecal smears, although large numbers of organisms were seen in only 1 of these dogs. Isolates from these 13 dogs included were *C jejuni* (n = 3), *C upsaliensis* (3), *C fecalis* (2), and *C coli* (2), and 3 were not speciated. When dogs with diarrhea were grouped according to age, prevalence of positive culture results in dogs ≤ 12 months old was 4/32 (12.5%) and in dogs > 12 months old was 9/228 (3.9%); this difference approached significance ($P = 0.062$). In 9 of these 13 dogs with positive culture results (3.5% of all dogs with diarrhea), no other cause of the diarrhea was identified, and their diarrhea was attributed to *Campylobacter* spp infection. With the exception of 1 dog that was euthanatized, all responded to antimicrobial administration within 4 days. There was a significant ($P = 0.027$) difference between the ages of these 9 dogs and dogs with negative results of culture. Mean ± SD age for these 9 dogs was 37.2 ± 44 months, compared with 77.5 ± 51 months for dogs with diarrhea and negative results of culture. Isolates from these 9 dogs included *C upsaliensis* (n = 2), *C coli* (2), *C jejuni* (2), and *C fecalis* (1); 2 isolates were not speciated. With 1 exception, duration of diarrhea was ≤ 7 days (median, 4 days). One dog was referred with a 4-year history of intermittent diarrhea. None of the dogs had received antimicrobials prior to the onset of diarrhea, whereas 4 dogs had received ampicillin between the onset of diarrhea and bacteriologic culture of feces, and 1 dog had received metronidazole. The anatomic location of the enteric lesion was described as large intestine in 5 dogs, with hematochezia reported in 4 of these dogs. Small intestinal or diffuse disease was reported in 1 dog each, and location was uncharacterized in 3 dogs. Vomiting was reported in 5 dogs, whereas only 1 of these had signs of large intestinal involvement. In contrast to the diarrheic population, *Campylobacter* spp were isolated from the feces of 3 of 24 (12.5%) hospitalized dogs with normal feces and 19 of 50 (38%) out-patients with normal feces (ie, 21 of all 74 [28.4%] dogs with normal feces). This difference between rates of isolation from dogs with normal and diarrheic feces was highly significant ($P < 0.001$). Of the 21 isolates from the dogs with normal feces, 2 (both from out-patients) were *C jejuni*; the remaining isolates were not speciated further. Differences in age or weight between dogs with positive or negative culture results were not seen in the 2 control populations, and no association was found between month of assay and positive or negative culture results in the dogs with diarrhea.

Discussion

A clear association was found between diarrhea and the prevalence of CPE as detected by use of RPLA but not by ELISA. This finding contrasts with a recent report by Weese et al,[16] who reported a significant association between CPE as detected via ELISA and diarrhea in dogs. Direct comparisons of sensitivity and specificity of the 2 assays cannot be made from results of our study, because they were performed on 2 separate populations over sequential but nonoverlapping time periods. This resulted from a change in diagnostic policy at the VMTH in October 1996. A temporal difference in disease prevalences between these 2 populations was unlikely because no association was detected between month of assay and diagnosis during the 3-year period during which the dogs with diarrhea were evaluated. However, it is plausible that this difference might be attributable to greater specificity of the ELISA assay, compared with the RPLA assay. In support of this are the findings of Berry et al,[12] who detected greater specificity but lower sensitivity for the ELISA, compared with the RPLA assay, on fecal specimens from humans. Specificity is preferable to sensitivity in identifying cases in which the enterotoxin is truly associated with disease. In this respect, retrospective studies of the type reported here have limitations. Given the absence of data relating to the presence or absence of the enterotoxin prior to the onset of diarrhea, no definite comment on causality can be made regarding the association between positive results of an assay and clinical disease. This is compounded by the high rate of positive results in dogs with normal feces.

Toxin A produced by *C difficile* was not identified in any of the 103 dogs with normal feces. This finding is in agreement with that of Weese et al,[16] who also determined that detection of toxin A in the feces was strongly associated with diarrhea in dogs. Prevalence of *C difficile* in the feces of clinically normal dogs was lower than that reported by Struble et al[17] from the same hospital. Additionally, the finding that isolation was more common from dogs with diarrhea was unexpected.

In many species, there is an association between antimicrobial administration and onset of *Clostridium*-induced enteritis.[18-20] The explanation for this is usually a disturbance of the normal flora that compete with and limit the growth of harmful bacteria.[21] In our study, we found an association approaching significance ($P = 0.054$) between antimicrobial administration and prevalence of CPE as detected by use of ELISA (but not RPLA) in dogs with diarrhea (but not dogs with normal feces): 20.8% (n = 72) of dogs that did not receive antibiotics had positive results for CPE via ELISA, compared with only 8.3% (60) of dogs that received antibiotics. No association was found between prevalence of *C difficile* toxin A and administration of antimicrobials in any of these populations. These findings contrast with those in other species and do not suggest that prior antimicrobial administration is asso-

ciated with increased risk of *Clostridium*-associated disease. However, in support of a potential association between antimicrobial administration and *C difficile*-associated disease, a positive association between prevalence of *C difficile* in bacteriologic cultures of feces and antimicrobial administration before and after the onset of diarrhea was found. Although this association was not apparent in dogs with normal feces, its importance is hard to determine. Although the number of dogs treated with antimicrobials was not small, the timing, dose regimes, types, and numbers of antimicrobials varied greatly. In equine and human patients, β-lactams and lincosamides have most frequently been associated with antimicrobial-induced clostridial enteritis.[19,22,23] A larger study in which these and other antimicrobials are given singly to a large number of dogs in consistent regimes might allow for separation of the different effects that various antimicrobials have on clostridial species.

Current dogma states that both *C perfringens* and *C difficile* are causes of acute and chronic colitis.[6-9] However, results of our study did not reveal an association between either species and clinical large intestinal disease. Instead, a significant association was detected between clinical small intestinal or diffuse disease and CPE, as detected by use of ELISA. Despite the limitations that exist in the clinical characterization of diarrhea as being attributable to either the small or large intestine, it is the authors' opinion that this clinical categorization remains useful for initiating an initial diagnostic plan and empirical treatment and generating an initial differential diagnosis list.

A number of features suggest that both organisms might be associated with AHDS. Consistent reports in humans, horses, and experimental animals, and a report[24] that administration of CPE induces diarrhea when administered into the small intestine but not the large intestine support this notion. Detection of CPE via ELISA was strongly associated with AHDS, and identified 8 of 12 dogs with diagnostic criteria and 4 of 4 dogs that had peracute signs and died as a result of the disease. The low prevalence of AHDS in dogs tested by use of RPLA did not allow for meaningful assessment of that assay as a means to detect an association with CPE. All 4 dogs with AHDS that had positive results of ELISA and were necropsied had either more severe disease in the small intestine, especially the distal portions of the jejunum or ileum, or clear demarcation from healthy tissues at the level of the ileocecal valve. These findings are consistent with the findings that the rabbit ileum responds more strongly to a fixed dose of CPE than the jejunum, which responds more strongly than the duodenum.[25] Furthermore, McDonel and Demers[26] have demonstrated that although enterotoxin binding is seen on colonocytes from rabbits, there is little or no response in fluid and electrolyte transport from the colon when the toxin is applied directly. Katahira et al[27,28] reported cloning a cDNA for the so-called **CPE receptor (CPE-R)** and have documented that the expression of CPE-R mRNA in the murine intestine is restricted to crypt enterocytes of the small intestine.[27,28] Given that production of CPE is coordinated with sporulation, it is frustrating that fecal endospore enumeration has not been more useful. Indeed, the association between a positive ELISA or RPLA and endospore counts described here is in direct contrast to previous findings.[5,16] Nevertheless, the endospore counts reported in our study could not be described as being of great diagnostic value from a clinical perspective, and it is not the authors intent to promote the use of fecal endospore enumeration as a method of supporting a diagnosis of CPE-associated diarrhea in dogs. Possible reasons for this low value include the existence of *C perfringens* strains that do not contain the CPE gene, misidentification of *C perfringens* endospores, and the requirement for other host factors for induction of disease. When one combines the concepts that expression of the CPE-binding protein may not be present in the canine colon, that large endospore numbers are more likely to be present in cases of large intestine disease, and that the lesions of AHDS almost uniformly affected the small intestine only, this no longer appears so enigmatic. It may be that the location of enterotoxin production within the intestine is a critical factor and that intrinsic host factors that enable ileal sporulation are those that lead to disease. This would explain the finding of CPE in the feces of clinically normal dogs. Likewise, the increased numbers of endospores seen in dogs with large intestinal disease in this study may represent an effect of the diarrhea rather than a reason for it. Lastly, exclusion of assays for other toxins limits conclusions regarding the importance of these organisms in canine diarrhea.[29-31]

Prevalence of *Salmonella* spp in dogs with diarrhea and dogs with normal feces was lower than expected. Reported prevalence in randomly selected clinically normal dogs was 6.9% (n = 305),[32] 16.2% (98),[33] 27.6% (8,157),[34] and 18% (100).[35] More recently, prevalences of 2.3% (n = 212) and 1.6% (260) have been reported.[36,37] The most likely reason for the markedly lower rates of isolation in our study, relative to previous studies, is the change in feeding and housing arrangements that has occurred in domestic dogs in the last few decades. The low rate of isolation from dogs with diarrhea and the absence of isolation in dogs with normal feces suggest that these organisms are substantially less prevalent than previously, and indicates that they are rarely associated with diarrheal disease in dogs in northern California.

Reported rates of isolation of *Campylobacter* spp from dogs with diarrhea were 18.7% (n = 197),[38] 10.5% (125),[39] and 16% (64),[40] whereas in these same studies the isolation rates from dogs with normal feces were 0% (61), 11.1% (54) and 9.5% (74), respectively. The disparity between these reports and our study may be partly explained by the age distribution of the study groups. Similar to our study, Flemming[38] and Hosie et al[39] reported a greater prevalence in dogs < 1 year old. However, the high prevalence of isolates from dogs with normal feces in our study is harder to explain. A temporal difference was unlikely to be the cause for the differences between the control dogs and dogs with diarrhea, because no association was found between month of assay and bacteriologic culture result. Previous isolation rates for *C jejuni* and *C coli* are comparable to those found in our study.[37,38,40] Isolation of *C*

upsaliensis from 2 of the dogs is important because these would not have been identified had initial colonies been discarded on the basis of a negative catalase reaction.[41] Lastly, although *C upsaliensis* and *C jejuni* have both been historically associated with diarrhea in dogs and humans, compelling evidence for their pathogenicity is still lacking.[42-47]

Given the high prevalence of positive results for *C perfringens*, *C difficile,* and *Campylobacter* spp in the dogs with normal feces, the specificity of bacteriologic culture of feces as a diagnostic test for establishing these species as a cause of diarrhea is low. The relatively high prevalence of CPE as detected by use of ELISA and the even higher prevalence as detected by use of RPLA suggest that sensitivity is not a limiting factor. It may be that a positive result for CPE via ELISA in conjunction with clinical signs of AHDS has high sensitivity and specificity, but this requires further analysis. In addition, given the severe, peracute nature of AHDS, there was a clear a priori indication from the clinical signs for parenteral administration of broad-spectrum antimicrobials in addition to fluid and blood products in some dogs prior to any diagnostic tests on feces. Because a positive or negative assay result is unlikely to alter management of the case, the value of the test, even for this putative disease, is questionable, regardless of the sensitivity and specificity.

At our VMTH, a fecal panel costs $70. Results from the toxin assays are generally available after 4 to 6 hours, whereas culture results are usually available after 24 to 48 hours. To estimate the diagnostic value, it is assumed that true positive cases represent those with no other diagnosis other than that obtained via positive results of the fecal panel, with resolution of diarrhea secondary to appropriate antimicrobial therapy. This gives a yield of 3 cases of salmonellosis, 9 cases of campylobacteriosis, 9 CPE-associated cases of AHDS, and 7 *C difficile* toxin A-associated cases of AHDS, or 28 of 260 (10.8%) fecal panels with a diagnostic result ($650/positive test result). In addition, it is likely that among these 28 true positives, any number may be false positives, since causality was not established. It has yet to be clearly established for all but *Salmonella* spp that these species are primary causes of disease in dogs, and we are yet to define the true nature of any such disease.

Results of this study reveal the convincing association between *C perfringens* enterotoxin and *C difficile* toxin A, as identified by ELISA, and clinical AHDS in dogs. No evidence was apparent to support the role of either organism in acute or chronic colitis in dogs. Furthermore, this study did not find any evidence to support the findings in other species of an association between prior antimicrobial exposure and development of *Clostridium*-associated disease in dogs. In contrast with previous reports published up to the mid-1980s, the prevalence of *Salmonella* spp was very low and this was not an important pathogen in this population. The finding of a higher prevalence of *Campylobacter* spp in dogs with normal feces further clouds understanding of the role these species play in canine diarrhea. On the basis of results of this study, the diagnostic value of indiscriminate bacteriologic culture of feces and toxin assessment in dogs with diarrhea appears low, and more data are sorely needed to improve the ability of clinicians to correctly identify disease caused by enteric pathogens.

[a]TechLab, Blacksburg, Va.
[b]Oxoid PET-RPLA, Unipath Co, Ogdensberg, NY.
[c]ImmunoCard Toxin A test, Meridian Diagnostic Inc, Cincinnati, Ohio.
[d]Triage Micro Biosite assay, Biosite Diagnostics, San Diego, Calif.
[e]CCFA, UCD Biological Media Service, Davis, Calif.
[f]Pro Disc, Remel, Lenexa, Kan.
[g]CVA, Hardy Diagnostics, Santa Maria, Calif.
[h]Anaeropack-Campylo, Remel, Lenexa, Kan.
[i]Hardy Diagnostics, Santa Maria, Calif.

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