

"Quenching the Stench on Infectious Diarrhea in Dogs and Cats" – A Critical Appraisal of Diagnostic Testing

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Diarrhea is generally regarded as one of the most consistent clinical signs of intestinal disease in dogs and cats, and one of the most frustrating maladies for veterinarians to diagnose and manage. The clinical documentation of enteropathogenic bacteria causing diarrhea in dogs and cats is clouded by the presence of many of these organisms existing as normal constituents of the indigenous intestinal flora. The bacteria most commonly incriminated in canine and feline diarrhea include *Clostridium perfringens*, *Clostridium difficile*, *Campylobacter* spp., pathogenic *Escherichia coli*, and *Salmonella* spp. It is important to appreciate that these bacterial species are but a small representation of putative pathogenic enteric bacteria and additional studies are warranted to elucidate the role of less well defined species such as *Anaerobiospirillum* spp., *Yersinia* spp., and other yet uncharacterized organisms.

Veterinarians are faced with a quandary when attempting to diagnose dogs with suspected bacterial-associated diarrhea because **the isolation rates for putative bacterial enteropathogens are often similar in diarrheic and nondiarrheic dogs**, and because the incidence of bacterial-associated diarrhea in dogs is extremely variable. In addition, canine fecal enteric panels are expensive (\$80-100), time-consuming, and require technical expertise. Most small animal veterinary practitioners do not have the facilities or expertise to perform these tests, which necessitates mailing fecal specimens to commercial veterinary diagnostic laboratories for evaluation. **There is no universal consensus among veterinary diagnostic laboratories as to which bacterial species should be evaluated, or which diagnostic assays should be utilized.** In addition, **sensitivities and specificities of commercial toxin assays commonly utilized in dogs and cats have not been validated to date.** The indications for performing fecal enteric panels on diarrheic dogs are poorly defined, resulting in indiscriminate testing, and misinterpretation of results.

PEARLS AND PITFALLS IN DIAGNOSTIC TESTING

FECAL EXAMINATION

The diagnosis of gastrointestinal parasites in dogs and cats is an integral component of small animal practice and the guidelines below should be followed to maximize the diagnostic yield of fecal examinations.

1. Examine fresh fecal specimens:

Fecal flotations should be performed on **fresh** fecal specimens (< 1 hour old) whenever possible to ensure that eggs, oocysts, and larvae do not develop beyond their diagnostic stages. Fresh fecal specimens can be refrigerated for up to 96 hours to facilitate preservation of eggs, oocysts, and cysts if immediate examination cannot be performed. Fecal specimens can also be placed in 10% buffered formalin if more than one hour will elapse before analysis or

refrigeration. Specimens fixed in formalin are suitable for concentration techniques, acid-fast stains, and immunoassays.

2. Perform direct wet smears:

Direct wet smears are indicated for recovery of trophozoites of *Giardia* and other trichomonads such as *Tritrichomonas foetus* and *Pentatrichomonas hominis*. They must be done with saline and performed using fresh feces (body temperature, < 1 hour old). Trophozoites in older specimens will lose their motility and degenerate, becoming unrecognizable. The main limitation of direct wet smears is sample size, with the result that negative smears are not uncommon with low parasite burdens.

3. Perform centrifugation fecal flotations:

Fecal flotations are excellent for recovering common nematode ova, oocysts of coccidia (including *Cryptosporidium* spp.), and *Giardia* cysts. The most important considerations for fecal flotations include: 1) choice of flotation solution and its specific gravity, 2) selection of standing versus centrifugal flotation, and 3) transfer of the meniscus.

Flotation solutions: The flotation solution must have a specific gravity high enough to float most common parasite ova and low enough to avoid distortion of protozoal cysts. Three solutions in common use are zinc sulfate (s.g. 1.18 to 1.2); Sheather's sugar (s.g. 1.27); and sodium nitrate (s.g. 1.2). Sodium chloride is an unacceptable flotation medium, even when used with centrifugation, as it will not float *Trichuris* ova. Aqueous zinc sulfate ($ZnSO_4$) with a specific gravity of 1.18 to 1.2 has been widely recommended because it will float cysts, oocysts, and most helminth eggs with a minimum of distortion and fecal debris.

Centrifugation vs Gravity (Standing) flotation: Gravitational (standing) flotation is the mainstay of many veterinary clinics. The use of this method has been perpetuated by the advent of commercial devices (e.g., Ovassay®, Fecalyzer®, Ovatector®) which allow the collection and processing of specimens with a minimum of handling by the client and technician. Flotation with centrifugation is considerably more efficient than standing flotations, which may not detect parasite stages shed in low numbers. Quantitative comparisons have shown that egg counts achieved using flotation with centrifugation was 2.4 to 6.0 times higher compared to standing flotation.

Meniscus transfer: Once the flotation procedure is complete, the meniscus containing the parasite stages should be transferred by coverglass to a clean glass slide after approximately **10 minutes**. The meniscus should be transferred by lifting the coverslip directly off the fluid surface and placing it on a slide. Meniscus transfer using a loop or glass rod is the poorest method of meniscus transfer and reduces the sensitivity of any flotation technique because only a small portion of the parasites recovered is actually transferred to the slide for examination.

4. Understand benefits and limitations of immunoassays:

Giardia infections in adult dogs and cats are often subclinical or associated with a transient softening of the stool early in the infection; however, acute diarrhea tends to occur in puppies and kittens shortly after infection. Feces are often malodorous and pale, and may contain mucus. *Cryptosporidium* spp. infection of puppies and kittens or immunosuppressed

animals can cause a spectrum of severity of disease, ranging from a nonclinical carrier state to mild, transient diarrhea, cholera-like illness, or prolonged severe life-threatening malabsorption syndrome.

The diagnosis of *Giardia* infection traditionally has depended on microscopic identification of trophozoites or cysts in feces from affected animals. However, microscopic diagnosis of *Giardia* infection can be difficult, because cysts may be shed intermittently and because cysts are so delicate. Many artifacts (grass pollen, yeast, etc.) mimic to varying degrees the morphology of *Giardia* cysts, and care must be exercised in differentiating these from *Giardia*. A recent survey evaluating the sensitivity of fecal flotation for detection of *Giardia* in dogs confirmed the poor performance of current in-house microscopy testing for *Giardia* compared to ELISA. Microscopy following fecal flotation only identified half of infected dogs, and falsely diagnosed up to 25% of uninfected patients. The need for accurate identification of these parasites in diarrheic animals is important when one considers that the organism is a zoonosis, and that failure to detect these parasites in diarrheic animals often leads to injudicious antibiotic therapy, which can exacerbate the diarrhea.

Direct immunofluorescence (DIF) is often the standard against which other tests for *Giardia* are measured. The Merifluor® *Cryptosporidium/Giardia* assay (Meridian Diagnostics, Inc., Cincinnati, Ohio) uses a fluorescein isothiocyanate (FITC)-labeled monoclonal antibody directed against cell wall antigen of *Giardia* cysts (not trophozoites) in the feces. A positive result is indicated by apple green fluorescence of the cyst. Morphologic identification is necessary for this technique. The test has been shown to have excellent specificity and sensitivity in humans, although it requires a fluorescent microscope which is typically available in large reference laboratories or Universities. Specimens sent to commercial laboratories for DIF should be fixed in 10% buffered formalin. Meridian's DIF combines the *Giardia*-specific and *Cryptosporidium-parvum*-specific antibodies in one reagent, so specimens can be examined for both parasites with a single test.

Enzyme immunoassays (EIA): Many veterinarians and reference laboratories have resorted to using ELISA tests that rely upon detection of *Giardia* cyst wall protein I (GCWP 1). The ELISA tests are advantageous because they are generally easy to perform and results are easy to interpret. In addition, the test does not rely upon morphological identification of cysts or oocysts via microscopy, thus saving technician time and potentially avoiding false negative interpretations. The EIA tests can also detect GCWP 1 in the absence of detectable cysts. However, virtually every commercially available EIA is marketed for human use, and there is a paucity of studies appraising their performance characteristics in diarrheic animals. Recently, a novel SNAP® *Giardia* Test Kit (IDEXX Laboratories, Inc., Westbrook, Maine) for detection of GCWP 1 in canine and feline feces was released. The SNAP® *Giardia* Test is a rapid in-house enzyme immunoassay that can be performed on fresh feces, previously frozen feces, or feces stored at 2-7 C for up to 7 days. This test represents the first commercially available EIA designed specifically for dogs and cats, and has the added advantages of simplicity, rapid availability of results (8 minutes following mixing of the conjugate solution with feces), and low cost. A recent study completed in the author's laboratory studied the performance characteristics of the SNAP test on 344 cats from four regional cat shelters in Northern California. The sensitivity of the SNAP test for detection of *Giardia* spp. was **85.3%**, and the specificity was

100%. Of importance, the sensitivity of the SNAP test for detection of *Giardia* increased to **97.8%** when the test was done in parallel with a centrifugation flotation. These results underscore the value of this simple, in-house assay, and also emphasize the importance of combining a centrifugation flotation with the SNAP test.

FECAL ENTERIC PANEL FOR ENTEROPATHOGENIC BACTERIA

Fecal culture is typically a low-yield diagnostic procedure in dogs with diarrhea because the clinical documentation of enteropathogenic bacteria causing diarrhea is clouded by the presence of these organisms in apparently healthy animals. If bacterial enteritis or enterocolitis is suspected, the feces should be cultured for specific pathogens such as *Salmonella*, *Campylobacter jejuni*, and *Clostridium difficile*. In addition, commercially available ELISAs for the detection of *C. perfringens* enterotoxin and *C. difficile* toxins (A and/or B) should be routinely performed in all fecal enteric panels. The author strongly discourages the implementation of partial enteric panels (e.g., culture only for *C. perfringens* and *C. difficile*), and veterinarians should routinely request a comprehensive enteric panel whenever the concern exists for enteropathogenic bacterial diarrhea in a dog or cat. Fecal enteric panels should be reserved for animals developing diarrhea after kenneling or show attendance, in animals with an acute onset of bloody diarrhea in association with evidence of sepsis, or in diarrhea outbreaks occurring in more than one pet in a household. Lastly, *Campylobacter* spp. *Salmonella* spp., and *C. difficile* are potentially zoonotic organisms, and can cause disease in immunocompromized people. Caution should be heeded in the implementation and interpretation of PCR-based enteric panels.

Recommendations for Diagnosing Specific Enteropathogens:

Clostridium perfringens:

1. Fecal isolation of *C. perfringens* is of no clinical utility, as the organism is a normal commensal and is typically cultured from over 80% of clinically healthy dogs.
2. *Clostridium perfringens* is associated with a wide array of clinical manifestations, including a life-threatening hemorrhagic gastroenteritis (HGE) to a milder, self-limiting enteritis.
3. Fecal endospore enumeration is not a reliable test for establishing *C. perfringens*-associated diarrhea in the dog as there is no association between endospore counts and detection of CPE (enterotoxin), or between endospore counts in diarrheic and nondiarrheic dogs.
4. Although an association has been documented between detection of CPE in feces and clinical signs of diarrhea, the involvement of other virulence factors acting alone or synergistically warrants further investigation.
5. *Clostridium perfringens* CPE is labile, and delayed submission of fecal specimens to a microbiology laboratory could result in false negative test results.
6. The commercial ELISA kit that is currently used for all veterinary patients is a human-based ELISA that has never been validated in any animals to date.
7. Based on the results of several recent studies, **the optimum diagnostic approach for canine *Clostridium perfringens*-associated diarrhea is the use of a CPE ELISA in conjunction with PCR for detection of enterotoxigenic strains (*cpe*).**

8. **Detection of *cpe* alone via PCR is insufficient for a diagnosis of *C. perfringens*, as 30% of PCR-positive fecal specimens are negative for the enterotoxin via ELISA.**
9. The antibiotics of choice for the management of *C. perfringens*-associated diarrhea include ampicillin, tylosin, and metronidazole.

***Clostridium difficile*:**

1. Fecal isolation of *C. difficile* is of some clinical utility, as the isolation rates for this organism are far lower (0-40%) compared to *C. perfringens*, and a negative culture performed on selective media essentially rules *C. difficile* out.
2. *Clostridium difficile* is associated with a wide array of clinical manifestations, including life-threatening hemorrhagic gastroenteritis (HGE) to a milder, self-limiting enteritis.
3. The lack of a standardized diagnostic assay makes interpretation of positive fecal toxin assays difficult. Although toxin A negative, toxin B positive strains have only been rarely isolated from dogs to date, the potential role of these toxin variant strains cannot be dismissed.
4. The apparently high incidence of ELISA positive, culture negative canine specimens obtained with some assays is concerning in light of the fact that these commercial assays have never been validated in the dog and may represent false positive results.
5. A recent study in the author's laboratory documented sensitivities ranging from 7-60% for a variety of human immunoassays for toxin A and toxin B in the dog.
6. PCR detection of toxin A or B genes in diarrheic fecal specimens does not appear to improve the diagnosis of *C. difficile*-associated diarrhea in the dog.
7. The antibiotics of choice for the management of *C. difficile*-associated diarrhea include metronidazole and vancomycin. The latter drug is rarely used, and is only indicated upon documentation of resistant strains of *C. difficile*.

***Campylobacter* spp:**

1. There is considerable debate regarding the pathogenicity of *Campylobacter*, given that the prevalence of *Campylobacter* is often higher in non-diarrheic animals compared to diarrheic animals.
2. *Campylobacter* is more often a problem in young dogs and cats (< 1 yr), living in crowded environments.
3. Clinical signs most commonly associated with *Campylobacter* include hematochezia with mucous-laden feces (colitis signs).
4. Stained fecal smears must be interpreted cautiously, as spiral-shaped bacteria could represent non-pathogenic *Campylobacter* spp. or *Helicobacter* spp. Positive stained fecal smears for *Campylobacter*-like organisms (CLO's) should always be backed up with fecal cultures.
5. The isolation of *Campylobacter* on selective culture media must be interpreted cautiously in diarrheic dogs and cats, as many non-pathogenic species can be isolated.
6. PCR-based methodologies are being increasingly utilized to facilitate differentiation of *Campylobacter* species, and improve our understanding of the pathogenicity of the organism.
7. PCR-based detection of *Campylobacter* in a diarrheic dog or cat does NOT denote a cause and effect phenomenon.

8. The antibiotics of choice for the management of *Campylobacter* infections include erythromycin and azithromycin.

***Salmonella* spp.**

1. *Salmonella* is isolated infrequently from diarrheic dogs and cats (1-2%), although the incidence is much higher (approximately 60%) in animals consuming raw diets.
2. *Salmonella* was isolated from 71% (57/80) of racing Alaskan dogs in the recent Iditarod race, but there was no association with diarrhea in the dogs.
3. *Salmonella* is more commonly associated with systemic disease in immunocompromized animals.
4. Systemic infections with *Salmonella* can be associated with fever, hemorrhagic diarrhea, and a neutrophilic leukocytosis (with/without a left shift) or leukopenia.
5. The diagnosis of *Salmonella* in dogs and cats is based on clinical signs, supportive laboratory results (CBC), and isolation of the organism on specific culture media.
6. PCR-based testing is being increasingly utilized in reference laboratories, although multi-center validation studies for detection of *Salmonella* in dog and cat feces are currently lacking.
7. Antibiotics are NOT warranted for animals lacking systemic signs, as there is concern of prolonging the carrier state or inducing antibiotic resistance. Antibiotics are warranted in animals with systemic manifestations of infection (hemorrhagic diarrhea, fever, obtundation, etc). Antibiotics should be based on sensitivity testing, although fluoroquinolones can be used prior to sensitivity results.

FECAL CYTOLOGY ON STAINED FECAL SMEARS

Stained fecal smears are commonly evaluated by veterinarians and veterinary technicians in an effort to identify the underlying cause of the diarrhea by looking for the presence of spiral-shaped bacteria, white blood cells, and fecal endospores associated with *Clostridium perfringens*. Unfortunately, the diagnostic yield of stained fecal smears is extremely low, and the author does not recommend their routine use in practice for several important reasons: 1) Fecal endospores are of no diagnostic value whatsoever, and 3 separate studies have documented the lack of association between the presence of the endospores and the presence or absence of diarrhea, and between the endospore count and enterotoxin results. Caution should be heeded in counting these endospores because healthy dogs with well formed stools can commonly have > 2-3 endospores / HPF; 2) Spiral-shaped bacteria are commonly found in fecal smears from healthy and diarrheic dogs, and the spiral-shaped bacteria are likely representative of a non-pathogenic *Campylobacter*-like organism or even *Helicobacter*. The problem is that there are over 18 different species of *Campylobacter*, many of which are non-pathogenic. The mere presence of spiral shaped organisms among other bacterial forms is of no clinical relevance whatsoever, and is not sufficient for a diagnosis of *Campylobacter*; 3) the author has recently completed a study evaluating the utility of stained fecal smears in diarrheic and non-diarrheic dogs at UC Davis, and was unable to find any correlation between the white blood cell count and fecal culture or toxin assay results.

CYTOLOGY OF RECTAL SCRAPINGS

Rectal scrapings followed by cytology can be helpful for diagnosing infiltrative disorders involving the rectum in dogs, and this procedure is far more informative than looking at fecal specimens for inflammatory cells. This procedure is relatively simple to perform, is cheap and non-invasive, and should be performed in dogs with suspected colonic or rectal lymphoma, rectal carcinoma, Pythiosis, Histoplasmosis, Protothecosis, and eosinophilic colitis or proctitis.

SIBO VERSUS ANTIBIOTIC-RESPONSIVE DIARRHEA (ARD)

There have been few topics in veterinary gastroenterology that have instigated as much controversy as the syndrome of small intestinal bacterial overgrowth (SIBO). In contrast to dogs, the syndrome of SIBO in humans is relatively well defined, and is characterized by a variety of clinical features, including macrocytic anemia, steatorrhea, and weight loss. The *sine qua non* for the diagnosis of bacterial overgrowth in humans is a properly collected and appropriately cultured aspirate from the proximal small intestine. Although quantitative culture of duodenal juice is the current gold standard for the diagnosis of SIBO in dogs, this technique has major limitations, which may partly explain the variability between different studies. The limitations in the diagnostic utility of measurement of serum folate and cobalamin concentrations for diagnosing SIBO in dogs was underscored in German and colleagues' study which failed to show a correlation between bacterial counts and serum vitamin concentrations. In addition, dietary levels of folate and cobalamin in commercially available pet foods can influence fasting serum levels in healthy dogs, leading to an increase in fasting serum levels. The diagnostic utility of measurement of serum folate and cobalamin concentrations is further compromised because serum folate concentrations can be normal or decreased in dogs with SIBO due to decreased absorption secondary to proximal small intestinal pathology, whereas serum cobalamin concentrations can be decreased by diseases other than SIBO, including exocrine pancreatic insufficiency and severe mucosal disease in the ileum. It appears that it is time to relinquish the current definition of SIBO based on quantitative culture of total bacteria, and instead focus on studying host-bacteria interactions and the immunopathologic effects of various enteric bacteria populations on the induction and perpetuation of disease. Gastroenterologists prefer to use the term, "antibiotic-responsive diarrhea" (ARD) in place of SIBO, and a recent study documented the benefits of the macrolide, tylosin, in dogs with apparent ARD.

Suggested Readings:

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