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Enteropathogenic Bacteria in Dogs and Cats: Diagnosis, Epidemiology, Treatment, and Control

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This report offers a consensus opinion on the diagnosis, epidemiology, treatment, and control of the primary enteropathogenic bacteria in dogs and cats, with an emphasis on *Clostridium difficile*, *Clostridium perfringens*, *Campylobacter* spp., *Salmonella* spp., and *Escherichia coli* associated with granulomatous colitis in Boxers. Veterinarians are challenged when attempting to diagnose animals with suspected bacterial-associated diarrhea because well-scrutinized practice guidelines that provide objective recommendations for implementing fecal testing are lacking. This problem is compounded by similar isolation rates for putative bacterial enteropathogens in animals with and without diarrhea, and by the lack of consensus among veterinary diagnostic laboratories as to which diagnostic assays should be utilized. Most bacterial enteropathogens are associated with self-limiting diarrhea, and injudicious administration of antimicrobials could be more harmful than beneficial. *Salmonella* and *Campylobacter* are well-documented zoonoses, but antimicrobial administration is not routinely advocated in uncomplicated cases and supportive therapy is recommended. Basic practices of isolation, use of appropriate protective equipment, and proper cleaning and disinfection are the mainstays of control. Handwashing with soap and water is preferred over use of alcohol-based hand sanitizers because spores of *C. difficile* and *C. perfringens* are alcohol-resistant, but susceptible to bleach (1:10 to 1:20 dilution of regular household bleach) and accelerated hydrogen peroxide. The implementation of practice guidelines in combination with the integration of validated molecular-based testing and conventional testing is pivotal if we are to optimize the identification and management of enteropathogenic bacteria in dogs and cats.

Key words: *Campylobacter* spp.; *Clostridium* spp.; Diarrhea; *Escherichia coli*; *Salmonella*; Zoonosis.

In contrast to veterinary medicine, specific practice guidelines have been published for the diagnosis and management of infectious diarrhea in people in an effort to improve the cost-effectiveness of diagnostic testing and maximize the diagnostic yield for detection of bacterial enteropathogens.¹ The wide array of

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Abbreviations:

AHDS	acute hemorrhagic diarrheal syndrome
AIEC	adherent-invasive <i>Escherichia coli</i>
CDAD	<i>Clostridium difficile</i> -associated diarrhea
CDI	<i>Clostridium difficile</i> infection
CDT	<i>Clostridium difficile</i> binary toxin
CLOs	Campylobacter-like organisms
CPE	<i>Clostridium perfringens</i> enterotoxin
EAEC	enteroaggregative <i>Escherichia coli</i>
EHEC	enterohemorrhagic <i>Escherichia coli</i>
EIEC	enteroinvasive <i>Escherichia coli</i>
EPEC	enteropathogenic <i>Escherichia coli</i>
ETEC	enterotoxigenic <i>Escherichia coli</i>
FISH	fluorescence in situ hybridization
GC	granulomatous colitis
GDH	glutamate dehydrogenase
NAP1	North American pulsotype 1
NTEC	necrotoxicogenic <i>Escherichia coli</i>
PCR	polymerase chain reaction
RPLA	reverse passive latex agglutination assay
SE	Salmonella Enteritidis
TcdA	<i>Clostridium difficile</i> toxin A
TcdB	<i>Clostridium difficile</i> toxin B

potential and recognized enteropathogens in dogs and cats coupled with the demand for cost containment and rapid turnaround of results increases the need for judicious implementation of fecal testing. Thorough clinical and epidemiological evaluations must define the severity and type of illness (eg, fever, hemorrhagic diarrhea, nosocomial infection, inflammatory leukogram). In addition, it should be determined whether there are risk factors for exposure (travel history, ingestion of raw or undercooked meat products, contact with ill animals, recent antibiotic use), and whether the animal or owner is immunocompromised. These details are necessary to facilitate fecal testing and optimization of antimicrobial therapy. Information pertaining to the diagnosis and management of bacterial-associated diarrhea in dogs and cats is scattered among disease-specific peer-reviewed articles, anecdotal reports, and review articles and textbooks, underscoring the importance of this Consensus Statement.

Although fecal cultures are commonly requested in people with diarrhea, their usefulness has been questioned,^{2,3} and the diagnostic yield of such cultures often is thought to be quite low. A fecal bacteriologic panel was evaluated in 260 dogs with diarrhea and yielded 28 (10.8%) diagnostic results (\$650/positive test result).⁴ In addition, among these 28 positives, many may have been false positives, because causality was not established. This impressive cost derives from the relative insensitivity of tests for the most likely pathogens and the poor selection of specimens being cultured. The advent of real-time PCR panels for dogs and cats with diarrhea has provided a new paradigm for the rapid and sensitive detection of toxin genes or organisms associated with disease. Interpretation of these panels can be problematic, however, because virtually all of these bacterial organisms have been frequently isolated from the feces of clinically healthy dogs and cats.

For the purpose of this review, bona fide bacterial enteropathogens of dogs and cats include *Clostridium difficile*, *Clostridium perfringens*, *Salmonella*, *Campylobacter jejuni*, and *Escherichia coli* associated with granulomatous colitis (GC). The currently available methods to detect these enteropathogens lack sensitivity, and in some cases specificity. In addition, the problem of determining etiologies in cases of bacterial-associated gastroenteritis is magnified by the challenges of defining what exactly constitutes a pathogen. Many strains of non-*jejuni* *Campylobacter* spp., *C. difficile*, *C. perfringens*, and *E. coli* can be excreted in the absence of diarrhea.²⁻⁴

Clostridium difficile

Introduction

Clostridium difficile is a fastidious Gram-positive, anaerobic, spore-forming bacillus that is an important enteropathogen in many species, particularly humans. It exists in 2 forms: vegetative cells and spores. The

vegetative cells are the actively growing form responsible for disease in the intestinal tract. They are poorly tolerant of oxygen and other stressors, and die or sporulate quickly once outside the body. Spores are highly resistant, can survive in the environment for years, and are responsible for most or all transmission of *C. difficile*.

The pathophysiology of *C. difficile* infection (CDI, previously referred to as *C. difficile*-associated diarrhea or CDAD) is poorly understood, but essentially involves growth of toxin-producing strains of *C. difficile* in the intestinal tract and production of adequate levels of toxins to cause disease. *Clostridium difficile* strains can produce at least 3 toxins, but some strains possess no known toxins and these nontoxigenic strains are considered clinically irrelevant. The 2 best-investigated toxins are toxin A (TcdA) and toxin B (TcdB). These typically are produced together, although TcdA negative but TcdB positive strains are clinically relevant⁵ and have been identified in dogs.⁶ Some strains also may produce a binary toxin (CDT), the clinical relevance of which currently is unclear.

Clostridium difficile is one of the most important causes of hospital-associated infection in humans, and community-associated disease appears to be on the rise.^{7,8} A remarkable change in the epidemiology of CDI has occurred in the past 10 years, with observed increases in the incidence of disease, mortality, relapse, and recognition of community-associated disease.⁹ Much of this change is related to the emergence and international dissemination of "hypervirulent" strains, particularly ribotype 027 (also referred to as North American pulsotype 1 [NAP1]).¹⁰ A similar change in CDI in animals has not been observed, but NAP1 has been identified in a dog.¹¹

Is Clostridium difficile Pathogenic in Dogs and Cats?

The role of *C. difficile* in canine and feline enteric disease currently is unclear. An association between the detection of *C. difficile* toxins in feces and disease has been reported in multiple studies,^{4,12,13} and *C. difficile* has been identified as a cause of 10–21% of cases of diarrhea in dogs in the general population.^{4,12} There also is evidence suggesting that *C. difficile* may be involved in some cases of acute hemorrhagic diarrheal syndrome in dogs,⁴ but questions remain because causation has not been proven. An attempt to reproduce CDI in healthy adult dogs by administering *C. difficile* with or without antimicrobials was unsuccessful.¹⁴ Thus, it is unclear whether *C. difficile* is a leading cause of community-associated disease, an opportunist that can cause disease concurrently with other enteropathogens, or an incidental finding in dogs and cats.

Although its role in disease still is under debate, *C. difficile* can be found in 0–58% of healthy, non-diarrheic dogs and cats, particularly young animals and dogs that visit human hospitals.^{4,12,13,15–18} Shedding of *C. difficile* appears to be variable and transient, perhaps indicative of frequent exposure from food or

the environment and either passive intestinal transit of spores or short-term colonization.¹⁹

There is little information regarding CDI in cats, with only a single report of disease in 2 cats in a household.²⁰ Whether this indicates a lower susceptibility to disease, less frequent exposure, or underreporting because of less frequent testing is unclear. Colonization rates of 0–21% of cats in the general population have been reported,^{15,21,22} although colonization rates can be higher (9–38%) in cats in veterinary hospitals.^{16,23,24}

There has been limited study of risk factors for *C. difficile* colonization. Living with an immunocompromised owner,¹⁹ antimicrobial administration to dogs,²² antimicrobial administration to the owner,²⁵ contact with children,²⁵ and visiting human hospitals²⁵ are recognized risk factors in dogs. Based on the prevalence of colonization and relatively low incidence of CDI, it is clear that colonization does not necessarily indicate that disease is present or will develop. Various pathogen (eg, strain) and host (eg, age, immune status, antimicrobial exposure, and comorbidities) factors probably play a key role in determining whether colonization progresses to disease.

Incidence and Prevalence of Clostridium difficile Infection in Dogs and Cats

Limited information is available. Diagnosis of CDI was made in 15/100 (15%) diarrheic dogs presented to a veterinary teaching hospital or primary care veterinary clinic,¹⁸ 18/87 (21%) diarrheic dogs presented to primary care veterinary clinics,¹² and 26/254 (10.2%) diarrheic dogs at a teaching hospital.⁴ A combined canine and feline incidence rate of 2.5 cases per 1,000 admissions was reported in a veterinary teaching hospital, with a higher rate (19/1,000 cases) during a suspected outbreak.²⁶

Diagnosis of Clostridium difficile Infection in Dogs and Cats

Clinical Signs. Clinical signs that have been associated with canine CDI range from subclinical carriage to a potentially fatal acute hemorrhagic diarrheal syndrome.^{4,13} There does not appear to be a specific anatomic localization of clinical signs, and dogs with CDI commonly have signs of small and large intestinal diarrhea as well as diffuse disease characterized by concurrent involvement of the small and large intestine.^{4,13} Objective information regarding practical diagnosis of CDI in dogs and cats is lacking, although 2 main approaches can be used in an attempt to diagnose CDI: detection of the organism or detection of its main toxins (TcdA and TcdB).

Detection of Fecal Toxins. The current gold standard assay is the cell culture cytotoxicity assay (CTA), which detects TcdB activity in feces. This assay, however, is not readily available because it is time consuming, technically demanding, and costly.²⁷ Various commercial ELISAs have been shown to have good

correlation with the cytotoxicity assay in people, and are widely used to detect clinical disease. Moderate-to-poor sensitivity and specificity of commercial ELISAs compared with the cytotoxicity assay have been reported for dogs,¹⁸ resulting in limitations in both positive and negative predictive values. Concerns about poor positive predictive value can be alleviated somewhat by parallel detection of the organism, as discussed in the following section.

Detection of the Organism. Detection of the organism can involve isolation on selective culture media, PCR from stool, or antigen ELISA. Although real-time PCR is gaining more attention for diagnosis of CDI in humans, it is probably not an optimal sole test in dogs and cats because of the potentially high baseline colonization rate. If the baseline prevalence of colonization is zero (or near 0), then a rapid and sensitive test such as real-time PCR can be very useful. However, the higher the colonization rate, the greater the likelihood of false positive results. In humans, it is assumed that the colonization rate is low, and there is willingness to accept false positives in hospitalized patients because of the importance of identifying as many cases as possible to implement early treatment and infection control practices. This may not be analogous to the situation in dogs and cats, with typically milder community-onset disease and a potentially higher baseline colonization rate as discussed above. If adequately sensitive, real-time PCR from stool is perhaps best to rule out the possibility of CDI, provided the assay is properly validated and performed, an issue that is currently problematic.

Similar limitations exist with the use of culture. Culture has the added disadvantages of being unable to differentiate toxigenic from nontoxigenic (and clinically irrelevant) strains, taking several days and requiring anaerobic culture capacity. A negative fecal culture strongly suggests that CDI is not present. "Toxigenic culture," which combines culture with detection of toxin genes, eliminates problems with detection of nontoxigenic strains, but suffers the same limitations as those of real-time PCR. Antigen ELISA can be useful because it is easy to perform, rapid, and may be highly sensitive. This type of test detects "common antigen" (glutamate dehydrogenase [GDH]) that is present in toxigenic and nontoxigenic *C. difficile* strains and a few uncommon *Clostridium* species. This test has the same limitations as culture has in terms of detection of nontoxigenic strains and colonization, although a negative result for GDH can rule out infection with *C. difficile* if the test is highly sensitive.

Combination Testing. Currently, the use of a combination of toxin testing by ELISA and organism detection (culture, antigen ELISA, or real-time PCR) is recommended for the diagnosis of CDI in dogs and cats. The chosen ELISA should detect both TcdA and TcdB because TcdA-negative, TcdB-positive strains have been reported to account for 0–41% of canine isolates.⁶ Positive toxin detection by ELISA and concurrent detection of organism are presumptive for a diagnosis of CDI. Detection of toxin, but failure to

identify *C. difficile*, should be interpreted with caution, particularly considering the relatively high sensitivity of organism and antigen detection methods compared with toxin ELISA. In such cases, it is plausible that the toxin ELISA result is false positive, rendering a diagnosis of *possible* CDI. Antigen or culture positive but toxin negative results are difficult to interpret because of the marginal sensitivity of available toxin tests. Such results should be considered to indicate a *possible* diagnosis of CDI in an animal with diarrhea and no other identifiable cause of diarrhea, but such testing cannot be considered definitive.

Management of *Clostridium difficile* Infection in Dogs and Cats

Current recommendations are based on a combination of extrapolation from other species, anecdotal data, and assumptions based on available microbiological and clinical data. In general, CDI is treated like any other diarrheal disease. Supportive therapy should be administered, based on clinical signs. If CDI is suspected to be antimicrobial-associated, antimicrobial therapy should be stopped if possible. Parenteral antimicrobial therapy rarely is indicated for CDI unless the animal has systemic illness.

Metronidazole (10–15 mg/kg PO q12h for 5 days) is commonly used, although it is unclear whether it is needed in all cases. Intravenous metronidazole (15 mg/kg q12h for 5 days) can be used if oral therapy is not an option. In humans, oral vancomycin often is used. However, because of the role of vancomycin for treatment of severe disease in humans and concerns about emergence of vancomycin-resistant organisms and lack of evidence of need in dogs and cats, we do not advocate the use of vancomycin for the treatment of CDI in dogs and cats. Other treatment options that have been used include intestinal adsorbents, probiotics, and dietary modification. Di-tri-octahedral smectite is a type of clay that adsorbs *C. difficile* toxins in vitro,²⁸ and is commonly used in the treatment of CDI in horses. Probiotic therapy has been evaluated in humans with CDI. Results have been somewhat conflicting and equivocal, however, and there currently is no clear answer regarding efficacy.^{29,30} Fecal transplantation is receiving attention and preliminary data from humans are very encouraging.³¹ However, this therapy is directed against recurrent CDI, something that does not appear to be a concern in dogs and cats. Increasing soluble fiber in the diet is commonly recommended for “clostridial” diarrhea, but evidence supporting this recommendation is lacking.

Zoonotic Implications of *Clostridium difficile* Infection

The risk of zoonotic transmission currently is unclear. Transmission of *C. difficile* from animals to humans has not been documented. However, because *C. difficile* is an important human pathogen and the strains of *C. difficile* that infect dogs often are indistin-

guishable from those found in people with CDI,^{19,32} it is prudent to consider *C. difficile* as potentially zoonotic.

Clostridium perfringens

Introduction

Clostridium perfringens is a Gram-positive anaerobic spore-forming bacillus. It is one of the most widespread pathogenic bacteria, and inhabits the gastrointestinal tract of humans and animals. The organism is divided into 5 biotypes, A to E, based on the possession of 1 or more of 4 major toxin genes: alpha (α), beta (β), iota (ι), and epsilon (ϵ). Each biotype also may express a subset of at least 10 other established toxins, including *C. perfringens* enterotoxin (CPE). Although all 5 biotypes can harbor the enterotoxin gene (*cpe*), the global distribution of enterotoxigenic strains is relatively low (~5%), and the majority of strains belong to type A,^{33,34} with only 1 published report documenting type C infection in 5 cases of peracute lethal hemorrhagic enteritis in dogs.³⁵ Enterotoxigenic *C. perfringens* type A has been associated with human food poisoning and sporadic diarrhea, canine acute and chronic large and small bowel diarrhea, and acute hemorrhagic diarrheal syndrome (AHDS).^{4,13,36,37} Although several studies have shown an association between the immunodetection of CPE in fecal specimens and canine diarrhea, the pathogenesis of *C. perfringens*-associated diarrhea in dogs and cats is not fully understood, because CPE also is detected in up to 14% of nondiarrheic dogs.^{12,13} A preliminary study showed a 2% prevalence of CPE in 54 nondiarrheic fecal specimens obtained from healthy cats (S.L.M., unpublished data).

A number of other virulence factors such as the beta2 (β 2) toxin also may play a role in diarrhea. These virulence factors may explain why the isolation of nonenterotoxigenic type A strains from animals with diarrhea does not preclude involvement in disease. *Clostridium perfringens* β 2 toxin has been associated with necrotic enteritis in piglets and typhlocolitis in horses.^{38,39} The role of β 2-toxigenic *C. perfringens* in dogs is less well understood. A single study evaluating 24 isolates from diarrheic dogs showed that 33% of isolates were positive for either the enterotoxin gene or the β 2-toxin gene or both (17%). Interpretation of these results is difficult due to the small number of dogs and lack of a control population.⁴⁰

Is *Clostridium perfringens* a Pathogen in Dogs and Cats?

This question is complicated by the fact that *C. perfringens* is a part of the normal canine intestinal microflora and is readily cultured from more than 80% of diarrheic and nondiarrheic dogs.^{12,13} The prevalence of *C. perfringens* in healthy cats appears to be lower than that in dogs, with isolation rates ranging between 43 and 63% (S.L.M., unpublished data). Canine *C. per-*

fringens-associated diarrhea has been attributed to *C. perfringens* enterotoxin (CPE), which has been shown to induce fluid accumulation and diarrhea when administered orally or directly into the intestinal lumen.⁴¹ The role of CPE in the development of diarrhea is unclear because CPE is detected in up to 34% of diarrheic dogs, and in 5–14% of nondiarrheic dogs.^{12,13} There appears to be an association between the detection of CPE in dogs with AHDS, because CPE was detected in 8/12 dogs (67%) with AHDS.⁴ In contrast to humans, in whom *C. perfringens*-associated diarrhea usually is a result of ingestion of enterotoxigenic isolates, *C. perfringens*-associated diarrhea in dogs appears more likely to be secondary to disruption of the intestinal microenvironment, enabling sporulation of commensal enterotoxigenic *C. perfringens*. The role of *C. perfringens* in cats is equally unclear because CPE was only detected in 9/219 cats with diarrhea (4.1%) and in 1 of 54 cats without diarrhea (2%) in a recent study (S.L.M., unpublished observation).

Incidence and Prevalence of Clostridium perfringens Infection in Dogs and Cats

Limited information is available about the incidence of *C. perfringens*-associated diarrhea in dogs and cats, given the challenges of proving cause and effect after detection of CPE in diarrheic animals, and the lack of tests to detect other potentially relevant toxins. The isolation rate of *C. perfringens* in healthy and diarrheic dogs is similar (>80%), although detection of CPE is more common in diarrheic dogs.^{12,13} The prevalence of *C. perfringens*-associated diarrhea in cats is much lower than in dogs, and CPE has been detected at a similar rate in cats with and without diarrhea (2.0–4.1%). *Clostridium perfringens* enterotoxin is commonly detected in dogs with AHDS (67%), but detection of CPE is lower in dogs with nonspecific enteropathies (30–34%).

Diagnosis of C. perfringens

No gold standard is currently available for the diagnosis of canine or feline *C. perfringens*-associated diarrhea. The optimal diagnostic approach for canine *C. perfringens*-associated diarrhea is the use of an ELISA to detect CPE in conjunction with PCR to detect enterotoxigenic strains.¹³

Clinical Signs. There are no pathognomonic signs indicative of *C. perfringens*-associated diarrhea in dogs and cats, and the spectrum of disease attributed to this organism varies greatly. Animals with *C. perfringens*-associated diarrhea can be presented with clinical signs of small intestinal or large intestinal disease or both.^{4,13} Severity of disease ranges from a mild, self-limiting diarrhea to a potentially fatal acute hemorrhagic diarrhea with severe inflammation of the intestinal mucosa in dogs.^{4,37}

Detection of the Organism. Culture of *C. perfringens* may be useful in procuring isolates for the application of molecular techniques to detect specific toxin genes

or for molecular typing to establish clonality in suspected outbreaks. Isolation of *C. perfringens* alone is not sufficient to diagnose *C. perfringens*-associated diarrhea due to the similar isolation rates in healthy and diarrheic animals.

Fecal Endospores. Because sporulation is coregulated with enterotoxin production, fecal endospore counting of Wright or Gram-stained fecal smears (≥ 3 spores per high power field) has been suggested as a tool to diagnose enterotoxigenic *C. perfringens*-associated disease,⁴² but several studies have reported no association between fecal endospore counts and the presence of diarrhea, or between spore counts and the detection of CPE in fecal specimens.^{12,13,43} Furthermore, it has been demonstrated that sporulation of enterotoxigenic strains continually occurs in both nondiarrheic and diarrheic dogs.¹³

Detection of Fecal Toxins

Fecal Enterotoxin Immunoassays. Fecal enterotoxin immunodetection is the most widely used diagnostic tool for *C. perfringens* in humans and animals. Two commercially available immunoassays currently are used in veterinary diagnostic laboratories: a reverse passive latex agglutination assay (RPLA)^a and an ELISA.^b Commercial veterinary diagnostic laboratories and veterinary institutions have used both assays, but performance characteristics have not been analyzed with canine feces, and there are concerns about sensitivity and specificity. Utilization of the RPLA has been associated with false positive results in people and dogs when compared with several different ELISA methods, thus adversely influencing the specificity of the RPLA.^{43,44} The sensitivity of immunodetection methods is extremely important because disease associated with CPE may be dependent on the concentration of CPE present in the intestinal lumen. This phenomenon is underscored by the finding that up to 14% of healthy dogs have detectable concentrations of CPE utilizing the ELISA.¹³

Molecular Techniques. The high prevalence of *C. perfringens* in healthy animals is a major limitation of fecal PCR assays, particularly those assays that target the α toxin gene that is of questionable virulence, and that is present in all *C. perfringens* strains. Assays targeting other genes of potentially greater virulence (eg *cpe*, *cbp2*) might be more useful, but there is inadequate evidence that PCR should be used as the sole diagnostic test. PCR is most useful as an adjunctive test in combination with ELISA detection of CPE. Analysis of fecal CPE and isolation and PCR detection of enterotoxigenic *C. perfringens* after a heat shock treatment were performed in 32 diarrheic and 100 nondiarrheic dogs.¹³ CPE was detected in fecal specimens by ELISA in 14% of nondiarrheic and in 34% of diarrheic dogs. Although this association was significant, the fact that more than half of the ELISA-positive specimens were from nondiarrheic dogs obscures the association. However, fecal specimens from nondiarrheic dogs were far less likely to be positive for both CPE and *cpe* (4%) compared with

specimens from diarrheic dogs (28%). Combining CPE detection by ELISA with PCR detection of enterotoxigenic strains currently is recommended to facilitate the diagnosis of *C. perfringens*-associated diarrhea. Real-time multiplex PCR assays are reliable for detection of *C. perfringens* toxin genes in animal isolates, and have the advantages of increased sensitivity and efficiency over conventional multiplex PCR assays.⁴⁵

Management of *Clostridium perfringens* Infection in Dogs and Cats

Animals that are systemically ill (eg, fever, hemorrhagic gastroenteritis, inflammatory or toxic leukogram) merit appropriate antimicrobial therapy. There is no documented evidence for the benefits of antimicrobial therapy in dogs with uncomplicated diarrhea associated with *C. perfringens*. Antibiotics that have been recommended for the treatment of canine *C. perfringens*-associated diarrhea include ampicillin, erythromycin, metronidazole, tylosin, and tetracycline,⁴⁶ but recent evidence has shown a high rate (21%) of in vitro resistance to tetracycline.⁴⁷ Most isolates were susceptible to ampicillin, metronidazole, and macrolide antibiotics, although resistant strains were identified. Preliminary studies have documented a high incidence (96%) of transferable tetracycline resistance in *C. perfringens* strains of porcine origin.⁴⁸

***Salmonella* spp.**

Introduction

The salmonellae are Gram-negative, motile, non-spore-forming facultative anaerobic bacilli that belong to the family Enterobacteriaceae. The genus *Salmonella* consists of only 2 species, *Salmonella enterica* and *Salmonella bongori*. *S. enterica* is divided into 6 subspecies: *S. enterica* ssp. *enterica*, *S. enterica* ssp. *salmamae*, *S. enterica* ssp. *arizonae*, *S. enterica* ssp. *diarizonae*, *S. enterica* ssp. *houtenae*, and *S. enterica* ssp. *indica*.⁴⁹ Salmonellae are ubiquitous organisms that can infect or be isolated from a variety of mammals, birds, reptiles, and insects. The prevalence of *Salmonella* in dogs and cats has been studied over the years.^{50–53} Much of the available research highlights 1 particular confounding factor, namely that the prevalence in healthy dogs and cats can be similar to that observed in diarrheic animals.^{54,55} This observation dates back more than 50 years and, although still important, today it can be explained in part by geographic location and sample handling differences.⁵⁶

Is *Salmonella* Pathogenic in Dogs and Cats?

Salmonella is a pathogen for dogs and cats, but many cases are subclinical, and several questions remain unanswered.⁵⁷ For example, not all *Salmonella* strains are equally capable of causing disease. There is also the question of infectious dose, and a study of *S. Enteritidis* (SE) strains indicated that different

strains varied widely in their ability to cause infection in mice.⁵⁸ The LD50 after oral inoculation varied from 10² to 10⁸ organisms, and these observations indicated that clinical isolates of SE are highly heterogeneous in their ability to cause death in mice. Differences in virulence cannot be accounted for by the presence or absence of virulence genes because all SE strains examined in this study contained virulence genes. The logical conclusion is that *Salmonella* virulence involves multiple factors that are related to both the organism and the host. As mentioned previously, the prevalence of *Salmonella* among healthy dogs and cats has been shown to be similar, and shedding in healthy animals is thought to account in part for this phenomenon. This observation underscores the important point that mere isolation of *Salmonella* from a dog or cat does not denote cause or effect. Sled dogs are invariably fed raw meat diets, and *Salmonella* frequently is isolated from healthy and diarrheic dogs at the same prevalence (60–70%).⁵⁹ Little is known about the genetics of resistance of individual animals or species to infection by *Salmonella*. Similarly, the role of the host immune response remains unknown.

Incidence and Prevalence of *Salmonella* spp. Infection in Dogs and Cats

The prevalence of *Salmonella* in healthy dogs has been reported in most studies to range from 0 to 3.6%.^{4,60,61} The prevalence of *Salmonella* in diarrheic dogs and cats ranges from 0 to 3.5%^{4,62–64} and from 0 to 8.6%, respectively,^{62,65,66} whereas the prevalence range for *Salmonella* in stray or shelter dogs and cats is 0–51.4%.^{67–69} The prevalence of *Salmonella* also has been shown to be much higher in dogs that are fed raw food diets, and *Salmonella* was isolated from 80% of the diet samples and 30% of the stool samples in greyhounds fed raw chicken diets.⁷⁰ In addition, *Salmonella* was isolated from the feces of 18/26 (69%) healthy pre-race Alaskan sled dogs, and 19/30 (63%) diarrheic racing Alaskan sled dogs, underscoring the lack of an association between the isolation of *Salmonella* and clinical diarrhea.⁵⁹ This poses an important and perplexing problem and highlights the fact that the mere isolation of *Salmonella* from cats and dogs alone can be insufficient to make a diagnosis of *Salmonella*-induced enteritis.

Diagnosis of *Salmonella* spp. Infection

The traditional diagnosis of canine and feline salmonellosis is made based on isolation of the organism in conjunction with clinical signs and assessment of the potential risk factors such as hospitalization, age, environmental exposure, and antibiotic administration.

Clinical Signs. Salmonellosis is primarily an acute disease, although it should be suspected as a cause in any acute or chronic gastrointestinal illness in dogs and cats.⁷¹ The clinical signs are highly variable. Acute episodes of illness are thought to occur 3–5 days after exposure, but clinical signs also have been demon-

strated to occur after only 12 hours. Fever, malaise, anorexia followed by vomiting, abdominal pain, and diarrhea are common. The diarrhea frequently is watery or mucoid, and can be bloody in severe cases. The severity of infection in dogs and cats varies with the individual animal. Most dogs that shed *Salmonella* have no clinical abnormalities, although some dogs may manifest clinical signs of sepsis.⁵⁷

Culture. *Salmonella* are facultative anaerobes that grow readily at 37°C. There are a large variety of commercial media available, including MacConkey agar, XLD agar, and Brilliant Green agar. In addition, the unlimited combinations of pre-enrichment, selective enrichment (selenite F broth, tetrathionate broth, Gram-negative broth), and selective culture media make it practically impossible to determine which combination of methods is most likely to result in the successful isolation of *Salmonella* from feces. Most diagnostic laboratories use a combination of selective enrichment broth followed by subculture to 1 or more selective agar plates and will go on to identify presumptive *Salmonella* colonies using biochemical techniques. Isolates identified as *Salmonella* can be further discriminated by serotyping that is typically performed by specialized reference laboratories.

Molecular Techniques. PCR assays vary and can detect *Salmonella* in a variety of different matrices, from water to human stool samples.^{72–74} Despite the availability of rapid, cost-effective, real-time PCR methods that have been developed within the last few years, few clinical diagnostic laboratories have fully embraced this technology and there have been no multicenter validations for the use of PCR to detect *Salmonella* in dog or cat feces. It is recommended that PCR after overnight enrichment in a nonselective broth be adopted as the gold standard, and that all positive PCR samples be cultured using selective enrichment to isolate and identify the infecting organism.^{75,76} Assays that have been validated in the literature still must be verified as “fit for purpose” in the laboratory in which they will be used. However, this is not a laborious process and it is imperative that these steps be taken in an effort to better standardize the laboratory diagnosis of *Salmonella*. Given that the sensitivity of conventional culture is much lower than that of PCR,⁷⁷ multiple cultures should be performed if relying on this method alone. When culturing equine feces, it has been recommended that at least 5 serial samples be cultured to increase sensitivity (estimated at around 55%).⁷⁸ If the specificity of the test is estimated at 99% and the sensitivity of any culture method for dog and cat feces is greater than 45%, 3 consecutive negative cultures are needed to be 90% confident that the sample is truly negative and 6 cultures would be required to be 99% confident (H. Aceto, personal communication).

Management of *Salmonella* spp. Infection in Dogs and Cats

It is widely accepted (although supportive scientific evidence is lacking) that the administration of antimi-

crobials is not warranted for uncomplicated episodes of salmonella infection, and only supportive therapy is recommended. In the event of systemic disease or an immunocompromised patient, antimicrobials may be necessary and a combination of ampicillin and enrofloxacin is advocated as empirical therapy. Treatment of an animal is not advocated if the owner is immunocompromised, although appropriate husbandry recommendations (see below) must be enforced. If culture results are available, antimicrobial susceptibility testing should be performed to optimize antimicrobial therapy if warranted.

Zoonotic Implications of *Salmonella* spp.

Salmonellosis is a disease of major zoonotic importance, and all *Salmonella* organisms, with the exception of those causing human typhoid fever, infect humans and animals. Foodborne outbreaks of nontyphoid salmonellosis can occur in people through contaminated products of animal origin (eg, meat, eggs, milk) that have been improperly prepared, stored, or handled before consumption. The practice of feeding raw meat to dogs increases the potential risk of transmission of *Salmonella* to people, underscoring the importance of excluding therapy dogs fed raw diets from animal-assisted intervention programs.⁷⁹ *Salmonella* infections in people also have been linked to contact with contaminated dry dog and cat food.⁸⁰

Campylobacter spp.

Introduction

Campylobacter spp. are Gram-negative, microaerophilic, curved, motile rods. There are 37 species and subspecies in the genus, although most are thought to be nonpathogenic. Many pathogenic campylobacter species such as *C. jejuni* ssp. *jejuni* and *Campylobacter coli* are thermophilic, and thus capable of growing at 42°C. Others such as *Campylobacter helveticus* and *Campylobacter upsaliensis* have no or variable thermotolerance. Quantitative PCR methods have demonstrated that domestic dogs can carry a wide range of *Campylobacter* species naturally.⁸¹

Table 1. Variations in *Campylobacter* prevalence as determined by culture and PCR in diarrheic and nondiarrheic dogs and cats.

<i>Campylobacter</i> spp.	Dogs		Cats	
	Culture	PCR	Culture	PCR
<i>Campylobacter jejuni</i>	0–45%	26%	0–16%	–
<i>Campylobacter upsaliensis/helveticus</i> *	0–53%	63/17%	4.5–35%	–
<i>Campylobacter coli</i>	0–5%	11%	0–1%	–
<i>Campylobacter lari</i>	0–1%	4%	0–1%	–
Any <i>Campylobacter</i>	0–87%	58–97%	0–75%	–

*Most early publications do not differentiate *C. helveticus* from *C. upsaliensis*.

Are Campylobacter spp. Pathogenic in Dogs and Cats?

Many studies have examined the association between diarrhea and the presence of *Campylobacter* in the feces. A majority of these studies have found similar isolation rates in healthy and diarrheic animals.^{82–84} Rare reports have described a positive association between diarrhea and isolation of campylobacter. In dogs <12 months of age, *C. jejuni* and *C. upsaliensis* had a prevalence rate in diarrheic animals over 2 times that of nondiarrheic animals, but this association was not observed in animals >1 year of age.⁸⁵ A recent study that used quantitative PCR to detect 14 *Campylobacter* species in DNA extracted from canine feces found that diarrheic animals had more detectable *Campylobacter* and increased diversity of *Campylobacter* species than did nondiarrheic animals.⁸¹

Experimental infection of puppies with *C. jejuni* has resulted in mild clinical disease, indicating that this organism has pathogenic potential, and naturally occurring campylobacteriosis has been documented.^{86–88} Inoculation of kittens with *C. jejuni* did not result in disease, although *C. jejuni* could be detected in feces for a few days postinfection.⁸⁹ Based on prevalence and experimental studies, it appears that clinical disease due to *C. jejuni* is more likely to occur in young animals rather than in adults. The evidence for disease causation by other species of *Campylobacter* such as *C. upsaliensis* and *C. helveticus* is less certain. Some species could represent commensal organisms, whereas others may be pathogenic. Additional factors such as stress, crowding, or other concurrent diseases may contribute to campylobacteriosis.

Incidence and Prevalence of Campylobacter spp. in Dogs and Cats

Isolation rates of *Campylobacter* from dog feces are highly variable (Table 1).^{4,68,81–85,90–100} The variability is likely due to differences in methodology, specimen collection and transport, the age of the animal, the geographic region, and season when testing is performed. Publications in the 1970s and 1980s centered on the prevalence of the thermophilic *C. jejuni* and *C. coli*. More recent publications indicate that *C. upsaliensis* is the *Campylobacter* most frequently isolated from dog feces.^{83,90–93} Despite the highly variable prevalence, dogs that are housed under crowded conditions such as kennels or shelters are more likely to be culture positive for *Campylobacter* than are household animals.^{94,95} Puppies <1 year of age also have a higher prevalence.^{82,91,95,96} Increased isolation rates also are observed in the spring and fall months depending on the study location.^{84,92,95} Additional factors associated with *Campylobacter* carriage include feeding of a home-cooked diet, feeding of table food scraps, and living with another dog that carried *C. upsaliensis*.^{92,93}

The prevalence of *Campylobacter* in cats is highly variable. When culture and identification conditions

are appropriate, *C. helveticus* and *C. upsaliensis* are the most common species identified in cats.⁹⁷ Similar to dogs, intensive housing has been identified as a risk factor for shedding in cats. Very few studies have compared shedding in cats with and without diarrhea, but there seems to be little difference in prevalence between these groups.^{83–85} Risk factors for healthy cats to shed *C. helveticus* and *C. upsaliensis* include age <36 months, sampling in winter months versus spring and summer, and cats with access to the outdoors that do not regularly use litter pans.⁹⁷

Diagnosis of Campylobacter spp.

Clinical Signs. In many cases, dogs are healthy carriers of *Campylobacter* species. Clinical signs in puppies <6 months or in those from stressful environments are variable and range from mild, loose feces to watery diarrhea or bloody mucoid diarrhea. Acute campylobacteriosis can be accompanied by anorexia, intermittent vomiting, and fever.^{82,88}

Culture. Many laboratories use a direct Gram-stained smear of feces to identify *Campylobacter*-like organisms (CLOs). Detection of small curved or “gull wing”-shaped bacteria only suggests the presence of CLOs, and should not be used as the sole method to diagnose campylobacteriosis because of the inability to differentiate between organisms of similar morphology such as *Arcobacter* or nonpathogenic campylobacters. Because *Campylobacter* isolation is performed from highly contaminated fecal or intestinal samples, it is necessary to use selective media. A variety of selective agars can be used; almost all contain antibiotics and antifungal agents. Incubation frequently is carried out at 42°C to select for thermophilic *Campylobacter*, but a temperature of 37°C should be used to ensure isolation of variable or nonthermophilic species. Biochemical and thermotolerance testing is used to differentiate *Campylobacter* species, but these results can be highly variable resulting in inaccurate identification.¹⁰¹

Molecular Techniques. Several molecular techniques have been described to identify and differentiate *Campylobacter* spp. These assays include direct sequencing of the 16S rDNA and comparison with databases such as GenBank, DNA hybridization with probes specific for different species, PCR amplification of specific regions of 16S rDNA or the *lpxA* gene, and amplified fragment length polymorphism.^{97,101–103} These tests have been examined for their ability to differentiate a variety of *Campylobacter* species such as *C. coli*, *C. jejuni*, *C. lari*, and *C. upsaliensis*.^{97,101–103} Recently, a cpn60-based direct fecal real-time PCR assay has been described that can detect 14 different *Campylobacter* species.⁸¹

Management of Campylobacter-Associated Diarrhea

The majority of cases are uncomplicated, self-limiting, and will resolve with supportive therapy alone. Because isolation of *Campylobacter* does not necessarily imply causation of clinical signs, treatment may not

be warranted and may further disrupt the intestinal microflora. However, in immunocompromised or febrile animals, or in animals with evidence of hemorrhagic diarrhea, antimicrobial treatment may be indicated.

Macrolides or fluoroquinolones are most commonly used to treat *Campylobacter* infections, although fluoroquinolones should be avoided in young animals due to their possible adverse effects on cartilage. Antimicrobial drug resistance has been reported to both of these drug classes, but routine antimicrobial sensitivity testing is rarely performed in veterinary diagnostic laboratories because it is difficult and time consuming. In 1 study that examined the MIC values of campylobacter isolates from dogs and cats, some resistance to enrofloxacin and ciprofloxacin was observed.⁸³ Extensive resistance to the fluoroquinolones has been reported in *Campylobacter* isolates from humans.¹⁰⁴ Consequently, macrolides are the preferred drug for treatment in humans.¹⁰⁵ Erythromycin, 10–15 mg/kg PO q8h, or azithromycin, 5–10 mg/kg PO q24h, can be given for 5–21 days as treatment. Azithromycin is better tolerated, but to the authors' knowledge, no published studies regarding efficacy of azithromycin for treatment of campylobacteriosis in dogs or its comparison with other macrolides or fluoroquinolones are available. In general, treated dogs will have a 50–73% response to treatment; 50% of cats will respond.^{54,106} It is not known whether treatment failures reflect resistance to the antimicrobial drug used or an incorrect diagnosis of *Campylobacter*-associated diarrhea.

Zoonotic Implications of *Campylobacter* spp.

Campylobacter spp. are well-recognized human pathogens, and the species most commonly causing diarrheal disease in humans include *C. jejuni*, *C. coli* and *C. upsaliensis*.^{105,107} The disease is more severe in immunocompromised individuals. The most common sequela of *Campylobacter* infection in humans are immune-mediated diseases such as reactive arthritis and Guillain-Barre syndrome, an acute progressive neuropathy characterized by myelin loss.¹⁰⁷

Campylobacter spp. are potentially zoonotic from dogs to humans, and epidemiologic analyses have established a relationship between *C. jejuni* enteric disease in humans with the presence of a dog, particularly puppies <6 months of age, in the same household.¹⁰⁸ A direct link has been established between pets and *C. jejuni*-associated diarrhea in people.^{99,109–111} Children and immunocompromised individuals exposed to young dogs or cats are most likely to become infected from contact with dogs or cats shedding *Campylobacter*. However, other sources of *Campylobacter*, such as food products, are the most common means for acquisition of this pathogen.

Enteric *Escherichia coli* Infections

Escherichia coli are pleomorphic Gram-negative, nonspore-forming rods belonging to the family Entero-

bacteriaceae. *Escherichia coli* are part of the normal intestinal microflora, but can be associated with gastroenteritis in the presence of bacterial virulence factors and impaired local or systemic immunity. Several distinct pathotypes of diarrheogenic *E. coli* are now recognized, and each pathotype is defined by a characteristic set of virulence factors acquired by horizontal gene transfer that act in concert to determine the clinical, pathologic, and epidemiologic features of the disease they cause. The 7 pathotypes include enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enterohemorrhagic *E. coli* (EHEC), necrotoxicogenic *E. coli* (NTEC), enteroinvasive *E. coli* (EIEC), enteroaggregative *E. coli* (EAEC), and adherent-invasive *E. coli* (AIEC) strains.^{112–114} Many strains have been isolated from dogs with and without diarrhea, and the role of many of these strains in disease causation in dogs and cats is poorly defined. In contrast, there is evidence for the role of AIEC strains in susceptible dog breeds such as the Boxer, French Bulldog, and the Border Collie.

***Escherichia coli* Associated with Granulomatous Colitis**

Granulomatous colitis (GC) or histiocytic ulcerative colitis of Boxer dogs was first described by Van Kruiningen in a kennel of Boxer dogs in 1965.¹¹⁵ The colonic lesion also has been described infrequently in the French Bulldog and the Border Collie. Historically, GC of Boxer dogs was considered an idiopathic immune-mediated disease with a poor prognosis. Poor response to immunosuppression, however, led to reassessment of antibiotic therapy, and there is now convincing evidence documenting dramatic improvement in clinical signs and histologic lesions of affected Boxer dogs treated with enrofloxacin.^{116–118} Documentation of periodic acid-Schiff (PAS)-staining positive macrophages in people with Whipple's disease together with the dramatic response to antibiotics in Boxer dogs with a similar enteropathy precipitated the search for an infectious agent.

Diagnosis of Granulomatous Colitis in Boxers

Clinical Signs. Affected Boxer dogs typically have a history of severe large bowel diarrhea that is often accompanied by marked weight loss, inappetence, and loss of body condition.

Laboratory Findings. Complete blood count changes often are mild and nonspecific, but dogs with severe GC can develop microcytic anemia caused by chronic blood loss.

Boxer dogs with GC commonly are hypoalbuminemic on serum chemistry panels.

Colonic Histopathology and Molecular Testing. Histopathologic lesions in Boxers with GC are pathognomonic and include mucosal infiltration with large numbers of PAS-positive macrophages, and evidence of mucosal ulceration and loss of goblet cells.^{115,116} Colonic biopsies are warranted in Boxer dogs with signs of

colitis to eliminate other causes of colitis and to optimize therapy when culture and sensitivity testing of mucosal biopsies is feasible. The identification of Gram-negative coccobacilli within macrophages can be confirmed using fluorescence in situ hybridization (FISH) probes.¹¹⁶ Colonic tissue culture can be used to isolate *E. coli* and optimize antibiotic selection given the increasing incidence of antibiotic resistance in Boxers.¹¹⁶

Management of *Escherichia coli* Associated with Granulomatous Colitis

Enrofloxacin (or other fluoroquinolones) is the drug of choice and should be administered at 10 mg/kg PO q24h for 8 weeks. A recent study documented that over 50% of dogs with GC harbored mucosal *E. coli* that were resistant to 1 or more antimicrobials, and resistance to fluoroquinolones was observed in 43%.¹¹⁸ Injudicious prior administration of fluoroquinolones may account for the relatively high incidence of antimicrobial resistance. This finding underscores the importance of continuing therapy for a full 8 weeks even if clinical signs resolve within 2 weeks, because cessation of therapy before complete eradication of *E. coli* might precipitate emergence of resistant strains. In addition, the lack of clinical response of dogs that were unresponsive to antimicrobials with efficacy against the *E. coli* strains in vitro (eg, amikacin, neomycin, amoxicillin-clavulanic acid) suggests that other factors such as drug distribution might impact eradication of AIEC. Administration of fluoroquinolones is associated with rapid resolution of clinical signs and also has been associated with resolution of the cellular infiltration characteristic of this disorder on colonic biopsy.¹¹⁷

Infection Control of Enteropathogenic Bacteria

The risk of transmission of canine and feline bacterial enteropathogens among animals in a veterinary hospital and between animals and humans is poorly understood and likely quite variable. Nosocomial transmission of *C. difficile* and *Salmonella* has been identified in small animal clinics,^{26,119} and outbreaks of human salmonellosis in clinic personnel have been documented.^{119,120} Contact with diarrheic animals has been identified as a risk factor for diarrhea (particularly campylobacteriosis) in humans in household studies.^{121–124} The risk of nosocomial and zoonotic transmission of *C. perfringens* probably is minimal, but cannot be dismissed.

All dogs and cats with idiopathic diarrhea or a diagnosis of infection with any of the bacteria described in this consensus statement should be considered potentially contagious. Basic practices such as isolation, use of appropriate personal protective equipment, and proper cleaning and disinfection practices are the main control measures. Handwashing is preferred over alcohol-based hand sanitizers because spores of *C. difficile* and *C. perfringens* are alcohol-resistant. If affected animals need to be walked, they

should be walked in an area where other patients are not walked and where feces can be promptly removed. Litterboxes should be cleaned and disinfected regularly. Gloves should be worn when handling litterboxes and hands washed after glove removal. *Clostridium difficile* and *C. perfringens* spores are highly resistant to most disinfectants, but susceptible to bleach (1:10 to 1:20 dilution of regular household bleach) and some oxidizing agents such as accelerated hydrogen peroxide.^{125,126}

Optimizing the Collection, Handling, and Shipping of Fecal Samples

Proper collection and preservation of feces frequently are neglected but are important requirements for the isolation of putative enteropathogens. Approximately 2–3 g of fresh feces should be collected into a clean, sealed, and leak-proof cup or sterile container and transported to the laboratory as soon as possible to maximize survival of *Salmonella* and *Campylobacter* spp. Specimens should be processed within 2 hours after collection. If the laboratory is on-site, no transport medium is required. Transport medium such as Cary-Blair or Amies gel should be used for specimens that cannot be cultured within 2 hours of collection. Rectal swabs are suboptimal for bacterial isolation given the limited volume of feces obtained. If rectal swabs are used, the specimen should be collected with a sterile swab, placed in Amies transport medium, and transported to the laboratory as soon as possible. Specimens should be kept cool at 4–10°C, but not frozen. Fecal specimens submitted for ELISA testing should not be placed in transport media.

Should Nondiarrheic Animals Be Screened for Bacterial Enteropathogens?

There is no indication for the testing of healthy nondiarrheic animals because all of the enteropathogens discussed previously can be found in healthy animals, and treatment of healthy animals is not indicated. Screening of healthy animals to eliminate the presence of an organism, sometimes requested for zoonotic pathogens, is not recommended because of a lack of clear measures in response to positive results and the concerns with sensitivity of the tests. Likewise, there is no indication for testing of healthy pets for *C. difficile* or *Campylobacter* spp. colonization in response to an owner being diagnosed with disease.

Conclusion

The lack of well-scrutinized practice guidelines for veterinarians that provide objective recommendations for implementing fecal bacterial testing, combined with the clinical documentation of enteropathogenic bacteria in diarrheic and healthy animals, has resulted in indiscriminate testing and misinterpretation of results. This problem has been compounded by the lack of validated immunoassays for dogs and cats, and the

acceptance of molecular-based testing as the “holy grail” in our diagnostic armamentarium. Molecular-based testing should be carefully integrated with conventional testing while recognizing the benefits and limitations of each modality.

Veterinarians should be cognizant of the fact that most bacterial enteropathogens are associated with self-limiting diarrhea, and the injudicious administration of antimicrobials could be more harmful than beneficial. Supportive therapy and appropriate hygiene control should be considered in all animals with suspected or confirmed bacterial-associated diarrhea (with the exception of *E. coli* associated with granulomatous colitis in which antimicrobial therapy is warranted), and antimicrobials should only be administered to animals manifesting systemic signs of illness.

Footnotes

^a *Clostridium perfringens* enterotoxin reverse passive latex agglutination assay (PET-RPLA), Oxoid Limited, Hampshire, UK

^b *Clostridium perfringens* enterotoxin test (ELISA), TECHLAB Inc, Blacksburg, VA

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