

Faecal Diagnostics- When do they help and When do they stink?

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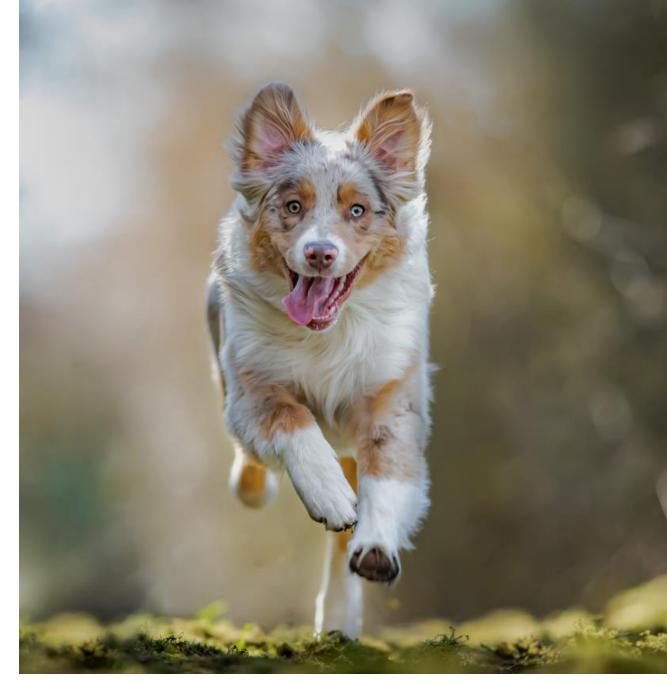
Disclosure

IDEXX Employee

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Agenda

- 1. Testing for enteric pathogens
- 2. Case Studies
- 3. Bacterial pathogens
- 4. Antimicrobial stewardship and faecal pathogens
- 5. Q&A





Enteric pathogens

- + Faecal analysis commonly performed in dogs and cats
- + Prevalence of Co, Gi and Tr was higher in pedigree cats compared to nonpedigree cats (DSH)
- + Prevalence decreased with increasing age for Co, Pa, Gi, Cr and Tr.
- + Co-infection was common
 - + ≥2 enteropathogens were detected in 62.5% of cats
 - + ≥4 enteropathogens were detected in 3.3% of cats





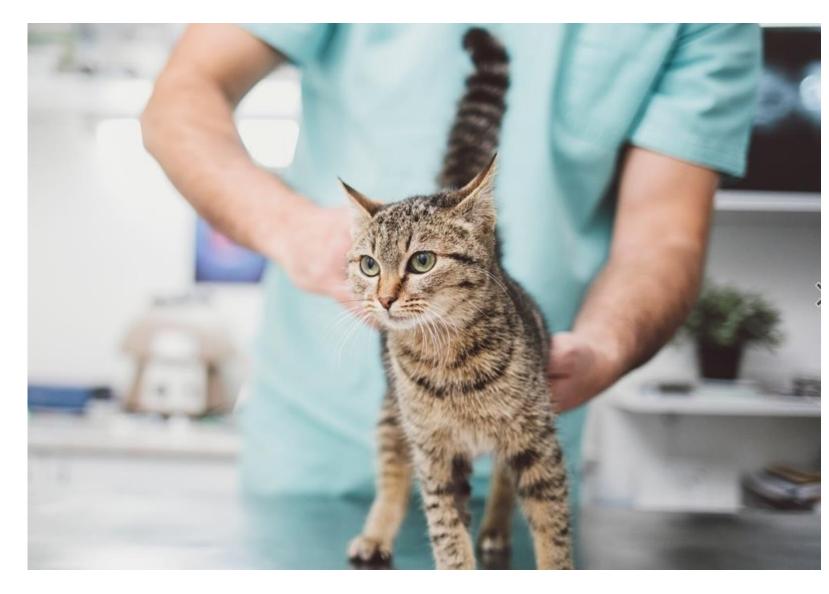
Enteric pathogens

 In previous study of dogs attending dog parks in USA one or more enteropathogens were detected in 114 of the 300 dogs (38%).



When is faecal testing helpful

- + Young animals
- + Multicat/dog households, cattery, kennel settings
- + Acute diarrhoea
- + Chronic diarrhoea
- + Wellness screens
- + Surveillance





What tests are available

+ Faecal culture

+ Microscopy

+ PCR

+ Antigen testing





Direct smear

- + Simple and quick, requires fresh sample (30 min)
- + Saline superior to water to preserve fragile trophozoites
- + Allows observation of motile organisms





Faecal flotation

+ Can be passive or combined with centrifugation

- + Increased sensitivity with centrifugation
- + Will be false negative if no eggs in sample
 - + Intermittent shedding, single-sex infection, prepatent period
- + Centrifugation flotation results in recovery of significantly more eggs than simple flotation



Faecal cultures in chronic diarrhoea

+ Previous study found that faecal cultures failed to distinguish between diseased and healthy dogs, and a high level of interlaboratory variation for culture was found.



Werner M, Suchodolski JS, Lidbury JA, Steiner JM, Hartmann K, Unterer S. Diagnostic value of fecal cultures in dogs with chronic diarrhea. J Vet Intern Med. 2021 Jan;35(1):199-208. doi: 10.1111/jvim.15982. Epub 2020 Dec 4. PMID: 33277779; PMCID: PMC7848338.



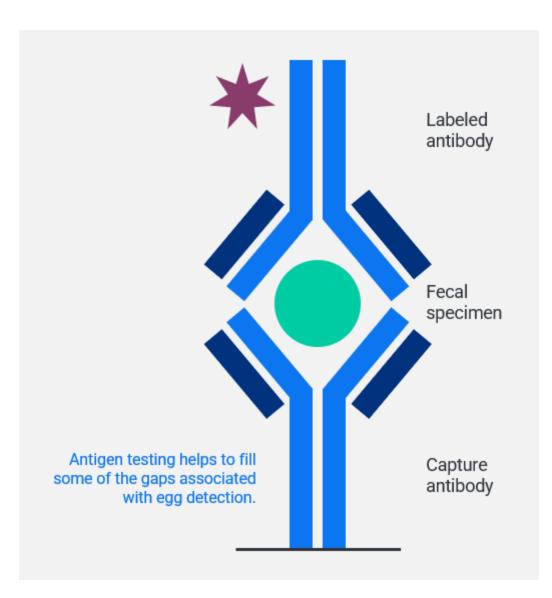
Faecal Dx antigen testing

- + Antigen testing for
 - + Hookworm
 - + Roundworm
 - + Whipworm
 - + Flea tapeworm
 - + Cystoisopora



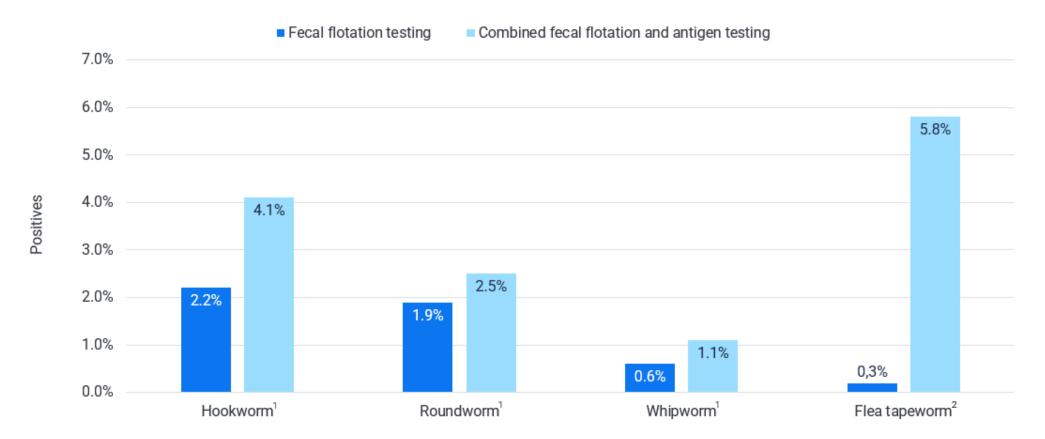
Parasite antigen testing

- Method for detecting proteins coproantigens—secreted or excreted by parasites in the intestinal lumen
- Uses unique markers for hookworm, whipworm, roundworm, flea tapeworm and cystoisopora—produced by the worms and not the eggs





Antigen testing



Sweet S, Hegarty E, McCrann DJ, Coyne M, Kincaid D, Szlosek D. A 3-year retrospective analysis of canine intestinal parasites: fecal testing positivity by age, U.S. geographical region and reason for veterinary visit. *Parasit Vectors*. 2021;14(1):173. doi:10.1186/s13071-021-04678-6
Elsemore D, Bezold T, Geng J, Hanna R, Tyrrell P, Beall M. Immunoassay for detection of Dipylidium caninum

coproantigen in dogs and cats; J Vet Diagn Invest. 2023 Jul 25:10406387231189193

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Utility of antigen testing

+ Positive results in healthy adult dog on preventative treatment

- + Consider efficacy of wormer used
- + Consider if treatment frequency is adequate
- + Consider possible resistance
- + Retreat and repeat test in 2 weeks
- + Positive result in dog with diarrhoea
 - + Treat with appropriate medication
 - + If no improvement, consider further investigations





Case-Tilly

- 7 month old FN BSH
- 3 week history of diarrhoea
- No vomiting, good appetite
- Physical examination unremarkable





Faecal Results

PARASITOLOGY

ALERT TEST

Giardia Antigen (#) * Faecal Microscopic Exam RESULT UNITS Giardia antigen detected. Giardia cysts present.

REF.INTERVAL

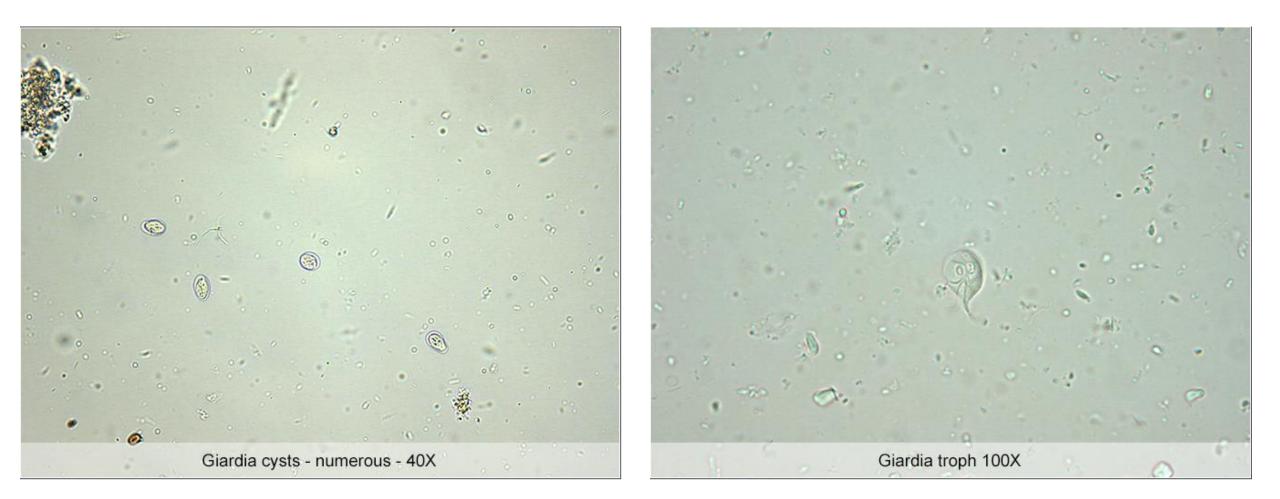


Life cycle of Giardia

- + Direct life cycle with repeated, asexual reproduction of trophozoites in the small intestine and intermittent production of resistant cysts that are passed in the faeces, initially often in large numbers.
- + Infection is by the oral uptake of cysts.
- + Trophozoites attach to the epithelial cells after infection and cause reduced absorptive capacity and altered intestinal permeability.
- + The prepatent period is 4–16 days. Patency usually persists for several weeks or months.



Giardia microscopy





Clinical signs

- + Asymptomatic
- + Diarrhoea +/- mucus
- + More severe signs in young or immunocompromised animals





Diagnosis

- + Due to intermittent shedding pooled faecal samples are recommended for analysis
- + Faecal microscopy
- + SNAP Giardia test
 - + Detects soluble Giardia antigen in canine and feline faeces
- + ELISA
- + Direct immunofluorescence microscopy
- + PCR



Treatment

+ Fenbendazole should be first line therapy

- + Metronidazole can cause neurological side effects
- + Can affect intestinal microbiome
- + Dose recommended is (50 mg/kg/day) for 3–10 days. A 3-day treatment duration in line with product licences is often not sufficient; if required, longer courses of treatment would be off licence.

ESCCAP . Control of Intestinal Protozoa in Dogs and Cats, Guideline 06, 2nd ed. Malvern: (2018).



Treatment

- + Anthelmintic treatment is only one part of management
- + Essential to reduce contamination with cysts and prevent reinfection
 - + environment should be cleaned, dried and disinfected with chlorine bleach, chloroxylenol or quaternary ammonium compounds. The areas should then be allowed to dry for 48 hours before reintroducing pets.
 - + Bedding should be washed at 60°C or above
 - + Patient should be cleaned, especially the perianal area to remove any cysts



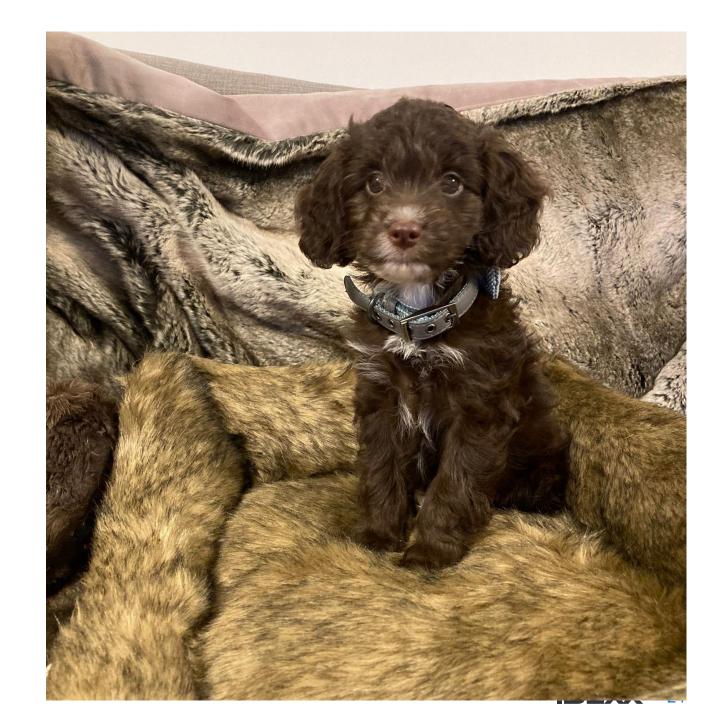
Retesting

- + Not indicated if clinical signs resolved
- + If clinical signs not resolved despite treatment, then repeat sampling recommended
- + Ideally test no more than 5 days after the completion of treatment to establish if infection is persisting. A later positive test, e.g. 2–4 weeks after completion of treatment, can also indicate reinfection has occurred.



Case-Tigger

- 3-month-old Cockerpoo
- BIOP for 2 weeks and had watery diarrhoea since
- Eating well and playful
- Had primary vaccinations and 3-day course of fenbendazole
- Physical examination unremarkable



Faecal analysis

PARASITOLOGY

ALERT TEST RE Giardia Antigen (#) Gi Cryptosporidium Antigen by ELISA Cr (#) Faecal Microscopic Exam Cy

RESULT UNITS REF.INTERVAL Giardia antigen not detected Cryptosporidium antigen not detected via EIA

Cystoisospora sp. oocysts present.



Faecal analysis

+ Cystoispora detected on microscopy



Cystoisospora canis oocysts, 35µm x 30µm, Canine, 40X



Cystoisopora

- + Previously known as Isospora
- + Infection caused by this parasite is called Coccidiosis
- + Host specific
- + Not zoonotic
- + Protozoa which infects dogs and cats

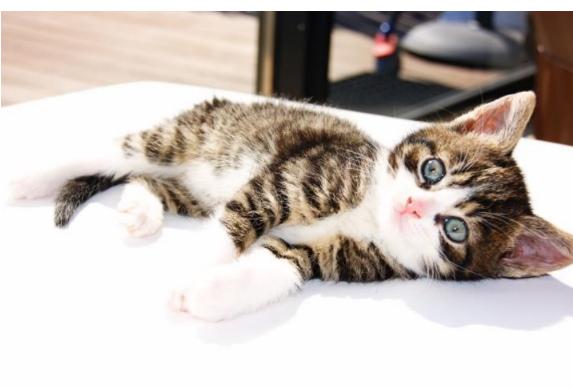


Cystoisospora canis oocysts, 35µm x 30µm, Canine, 40X



Clinical signs of coccidiosis

- + Healthy adult cats and dogs usually show no clinical signs even if shedding oocysts
- + Young, immunocompromised or stressed animals are most likely to develop clinical signs
- + Most infections occur in puppies and kittens under 4 months of age
- + Can result in vomiting, diarrhoea, depression, anorexia and dehydration.
- + Rarely can result in haemorrhage
- + Potentially fatal in very young, untreated animals





Cystoisospora Testing

- + Faecal microscopy
- + Faecal antigen test
 - + Detected from sporozoites, merozoites and occysts
 - + More sensitive and specific
- + Detects parasite specific antigen rather than detection of eggs
- + Antigen specific to cystoisopora and does not cross react with other coccidia that are not pathogenic e.g Eimeria
- + Part of faecal Dx antigen test
 - + Also includes hookworm, roundworm, whipworm and flea tapeworm



Treatment

- + Trimethoprim-sulphonamide at 15-30mg/kg BID PO for 5 days
- + Ponazuril
 - + 15mg/kg SID PO for 7 days in cats
 - + 30-50mg/kg SID PO for 1-7days in dogs
- + Toltrazuril
 - + 15-10mg/kg SID PO for 1-6days in dogs

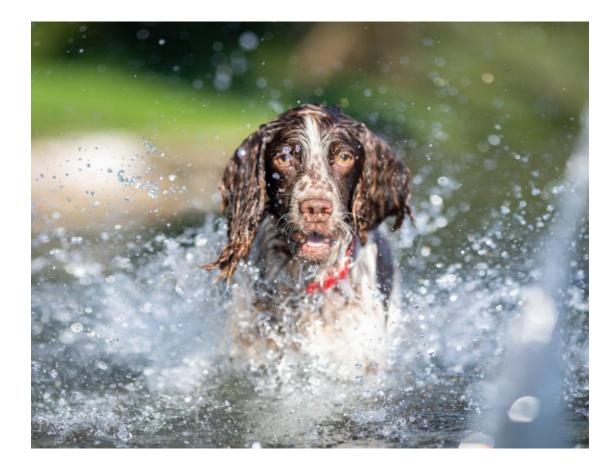
(Ref Infectious Diseases of the Dog and the Cat. 4th Edition. Craig E. Greene)





Prevention

- + Good hygiene is essential
- + Regular removal of faeces before sporulation
- + Occysts are highly resistant in the environment
- + Steam and pressure washing kennels/cages
- + Prevent predation





BSAVA Protect me poster

+ New addition which specifically states that antibiotics are not required for

- + Acute vomiting
- + Acute diarrhoea unless septic
- + Chronic diarrhoea
- + Gastric Helicobacter infections
- + Campylobacter, Salmonella, Clostridium perfringens or Clostridium difficile infections



Salmonella

- + Gram negative motile facultative anaerobic bacilli
- + Prevalence of Salmonella in cats and dogs is similar in both healthy and diarrhoeic animals.





Multi-drug resistant strains

+ In one study of 25 isolates of Salmonella there were high resistance rate noted

- + Tetracycline (92%)
- + Azithromycin (88%)
- + Cefazolin (84%)
- + Nalidixic acid (80%)
- + Ampicillin (80%)
- + Ceftriaxone (80%)
- + Streptomycin (76%).
- + Resistance to three or more antimicrobial agents was detected in 24 (96%) isolates.

Wei L, Yang C, Shao W, Sun T, Wang J, Zhou Z, Chen C, Zhu A, Pan Z. Prevalence and Drug Resistance of *Salmonella* in Dogs and Cats in Xuzhou, China. J Vet Res. 2020 May 12;64(2):263-268. doi: 10.2478/jvetres-2020-0032. PMID: 32587913; PMCID: PMC7305642.



Raw feeding

- + Salmonella has been shown to be more prevalent in dogs fed raw food diets.
- + In one study Salmonella was isolated from 80% of the BARF diet samples and from 30% of the stool samples from dogs fed the diet





Joffe DJ, Schlesinger DP. Preliminary assessment of the risk of Salmonella infection in dogs fed raw chicken diets. Can Vet J. 2002 Jun;43(6):441-2. PMID: 12058569; PMCID: PMC339295

Clinical signs

- + Acute disease
- + Usually within 3-5days but can be as soon as 12 hours after exposure
- + Pyrexia
- + Lethargy
- + Anorexia
- + Vomiting
- + Abdominal pain
- + Diarrhoea
 - + Watery, mucoid +/- haemorrhagic





Diagnosis

+ Culture

- + Grow readily at 37C
- + Can get sensitivity and specificity of isolate
- + Isolates further discriminated by serotyping

+ PCR

- + Need take into consideration clinical signs and potential risk factors
- + Possible risk factors
 - + Hospitalisation
 - + Age
 - + Antibiotic administration





Treatment

- + Asymptomatic or uncomplicated cases do not warrant antimicrobial therapy
- + If evidence of sepsis then consider antimicrobial therapy, ideally based on culture and sensitivity.
- + Empirical therapy is generally initiated with combination of ampicillin and enrofloxacin in absence of sensitivity results.
- + Barrier nurse due to zoonotic potential



Dysbiosis and antibiotic use

- + Several studies have shown that antibiotics can lead to dysbiosis
- + Documented in dogs treated with tylosin, metronidazole and amoxicillinclavulanic acid





Risk of early antibiotic use

- + Study was designed to investigate whether antibiotic use during the first year of life is related to the development of chronic diarrhoea (later in life (during adulthood) in cats
- + A total of 11% (11/95) cats had chronic diarrhoea of which 91% (10/11) had received antibiotics.
- + Cats with chronic diarrhoea during adulthood were 19.9 times more likely to have received antibiotics before the first year of age

Early-life antibiotic exposure and susceptibility to chronic diarrheaduring adulthood in cats. EM Stavroulaki, GT Fosgate, KT Moraiti, PG Xenoulis. ECVIM abstract 2023



Effect of amoxicillin-clavulanic acid in dogs with uncomplicated acute diarrhoea

+ Prospective, placebo-controlled, double-blinded study

+ dogs randomly assigned to an antibiotic (AG) or a placebo (PG) group

+ There was no significant difference in the faecal dysbiosis index

+ The proportion of resistant faecal *E. coli* increased during treatment with amoxicillin-clavulanic acid and was still increased 3 weeks after treatment

Werner M, Suchodolski JS, Straubinger RK, Wolf G, Steiner JM, Lidbury JA, Neuerer F, Hartmann K, Unterer S. Effect of amoxicillin-clavulanic acid on clinical scores, intestinal microbiome, and amoxicillin-resistant Escherichia coli in dogs with uncomplicated acute diarrhea. J Vet Intern Med. 2020 May;34(3):1166-1176. doi: 10.1111/jvim.15775. Epub 2020 Apr 23. PMID: 32324947; PMCID: PMC7255678.

Acute diarrhoea and metronidazole

- + In a prospective, randomized, blinded clinical trial study in 2024, twenty-seven dogs with acute diarrhoea were treated with either metronidazole or a synbiotic (E. *faecium* DSM 10663; NCIMB 10415/4b170).
- + This study also found that metronidazole led to dysbiosis and resulted in a significantly increased concentration of *E. coli*, and a reduction in *Clostridium hiranonis* concentration (*Clostridium hiranonis* is a beneficial bacteria in the gut flora). No significant difference was noted regarding the clinical improvement. The authors concluded that metronidazole had a negative effect on the core microbiome without affecting clinical outcomes (Stübing et al 2024).

Stübing H, Suchodolski JS, Reisinger A, Werner M, Hartmann K, Unterer S, Busch K. The Effect of Metronidazole versus a Synbiotic on Clinical Course and Core Intestinal Microbiota in Dogs with Acute Diarrhea. Vet Sci. 2024 Apr 29;11(5):197. doi: 10.3390/vetsci11050197. PMID: 38787169; PMCID: PMC11125899.

Acute diarrhoea and metronidazole

- + In a 2022 study of dogs with acute non-infectious colitis, dogs were enrolled in a 30-day diet trial
- + Dogs were randomized into 3 placebo-controlled groups: group 1, easily digestible diet + placebo tablet; group 2, easily digestible diet + metronidazole tablet; and group 3, psyllium-enhanced easily digestible diet + placebo tablet.
- + This study found that the group given metronidazole were slower to respond to treatment and had increased dysbiosis (Rudinsky et al 2022).

Rudinsky AJ, Parker VJ, Winston J, Cooper E, Mathie T, Howard JP, Bremer CA, Yaxley P, Marsh A, Laxalde J, Suchodolski J, Perea S. Randomized controlled trial demonstrates nutritional management is superior to metronidazole for treatment of acute colitis in dogs. J Am Vet Med Assoc. 2022 Oct 6;260(S3):S23-S32. doi: 10.2460/javma.22.08.0349. PMID: 36191142.



Summary

- + Enteropathogens are common in cats and dogs
- + Healthy cats and dogs can have positive results on faecal testing
- + A positive test does not mean that this is the cause of the clinical signs
- + Imperative not to over treat, particularly for bacterial infections which can lead to inappropriate antibiotic use and antimicrobial resistance

