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Identification and treatment of *Strongyloides stercoralis* infection in a Boston Terrier dog from south-eastern Australia

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Keywords

Canine; *Strongyloides stercoralis*; ivermectin; qPCR, Baermann

Abstract

Strongyloides stercoralis, the causative agent of strongyloidiasis is a potentially zoonotic intestinal nematode endemic to northern Australia. Strongyloidiasis is typically observed in immunocompromised hosts and is characterised by gastrointestinal signs, respiratory symptoms and a failure to thrive. In immunocompromised hosts, hyperinfection syndrome and disseminated infections can prove life-threatening. A 24-month-old Boston Terrier dog was referred for investigation of chronic small and large intestinal watery hematochezic diarrhoea, emaciation, and hematemesis. Small intestinal histology identified a nematode despite consecutive negative faecal flotations. A real-time polymerase chain reaction and Baermann test subsequently confirmed infection with *S. stercoralis*. The dog received an oral parasiticide comprising milbemycin oxime and afoxolaner every month for the 11 months prior to diagnosis. Despite fenbendazole being reported as successful in the treatment of canine strongyloidiasis, a course of fenbendazole failed to clear the infection. Eradication of *S. stercoralis* infection was confirmed after the administration of off-label ivermectin fortnightly for 12 doses. Attention should be paid to this nematode as the failure of routine copromicroscopic methods to diagnose *S. stercoralis* infections can result in misdiagnosis, mistreatment and progression of the disease. Off-label ivermectin may be an alternative to fenbendazole for the treatment of *Strongyloides* spp. infection in dogs.

Introduction

Strongyloides spp. are small (3 – 8 mm), slender nematodes that embed into the mucosa of the small intestine. *Strongyloides stercoralis* has a complex life cycle with both free living and parasitic cycles.¹ Transmission may occur via penetration of the skin or mucosae by third stage larvae present in the soil or via transfer of infective larval stages through the mammary

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glands if the bitch is infected in very late gestation or during lactation.¹ Once infected, the larvae undergo hepato-pulmonary followed by tracheal migration to develop into adults in the intestines. Parthenogenetic females produce eggs that hatch to rhabditiform, or first stage larvae, before passage in the faeces. Alternatively, first stage larvae can moult to filariform larvae and re-infect the host through the colonic mucosa or percutaneously through the perianal skin (autoinfection).¹ The prepatent period from infection to larval shedding for *S. stercoralis* is 6-10 days.

Strongyloidiasis may result in self-limiting, acute or chronic forms of the disease. In immunocompetent animals, the infection is mostly asymptomatic.¹ In immunocompromised hosts, an exacerbation of the auto-infective cycle leads to high adult worm burdens in the intestinal tract, resulting in hyperinfection syndrome. In hyperinfection syndrome, overwhelming numbers of migrating larvae can present as gastrointestinal and respiratory signs.² In severely immunocompromised patients, such as those treated with high doses of glucocorticoids, disseminated strongyloidiasis can ensue. In this case, larvae migrate beyond the gastrointestinal tract and lungs to disseminate through various organs leading to multi-systemic disease, septicaemia and death.³

The Baermann method is the test of choice for *S. stercoralis* larval isolation and identification.^{3,4} Other available clinical diagnostic techniques include agar plate culture, serological and molecular diagnostic assays.³ Faeces should be tested multiple times (three times over the course of five to seven days) as larval shedding is intermittent.⁴ In companion animal clinical practice, the failure of clinicians to request multiple Baermann's tests likely means the prevalence of *Strongyloides* spp. is underreported.⁵ *Strongyloides* spp. were identified in 21.9% of canine faecal samples collected from Indigenous communities across northern Australia.⁶ Additionally, the prevalence of *Strongyloides* spp. in canine faecal samples contaminating parks in urban centres of Australia was 7.0%, 0.1% and 0.3% for tropical, sub-tropical and temperate climates, respectively.⁵

Several case reports have shown variable success in treating *S. stercoralis* in dogs using ivermectin, fenbendazole and moxidectin, alone or in combination, and in all cases repeated treatments were necessary.^{3,7,8}

This report describes a case of *S. stercoralis* hyperinfection in a dog and highlights the failure of faecal flotation to diagnose strongyloidiasis given larval stages found in faeces do not readily suspend in the recommended solutions. This case report demonstrates the necessity to consider strongyloidiasis as a differential in refractory chronic enteropathy cases with

compliant de-worming histories. Multiple doses of ivermectin effectively treated infection in the presented case with eradication of the parasite confirmed by molecular diagnostic testing.

Clinical features

History

A 24-month-old male neutered Boston Terrier dog weighing 7.0 kg with a Body Condition Score of 3/9 was referred to a veterinary clinic in Canberra, Australia for investigation of chronic mixed intestinal hematochezic diarrhoea.⁹ There was no importation or travel history to or from tropical regions. Over the preceding month the dog had lost 10% of its bodyweight and experienced one episode of hematemesis. One-week prior to presentation, the dog experienced an episode of dyspnoea seen by an emergency clinician which resolved with an anti-inflammatory dose of dexamethasone 0.05 mg/kg intravenously (Dexason®, Ilium, NSW, Australia). Since acquisition, the dog had a chronic intermittent history of mixed intestinal diarrhoea, predominantly small intestinal, and loss of coat lustre.

Faecal examinations by flotation, abdominal radiography including barium series, trypsin like immunoreactivity (TLI Canine, IDEXX, >50 ng/mL reference range (RR) 5.4-32.0) and canine pancreatic lipase (canine pancreatic-specific lipase quantitative, IDEXX, 30 µg/L RR 0-200) testing were performed by the referring veterinarian, and no abnormalities were recorded. Inflammatory bowel disease (IBD) was suspected, and a dietary trial was initiated with a hypoallergenic diet (Hills prescription diet Z/D®, Hills Pet Nutrition, USA). An antimicrobial trial was also undertaken with metronidazole (Flagyl®, Sanofi, UK) at 10 mg/kg orally every 12 hours for 10 days. No clinical improvement was reported.

The dog had been receiving monthly parasite treatment with a combination milbemycin oxime and afoxolaner (Nexgard Spectra®, Boehringer-Ingelheim, Australia) at labelled dose for 11 months prior to presentation.

Clinical findings

During the clinical examination, moderate muscle wastage and an unkempt, seborrheic hair coat were identified. The animal was managed for hypovolemic shock based on the combination of bradycardia (64 beats per minute), hypothermia (36.7°C) and weak but synchronous femoral pulses with an inability to obtain systolic blood pressure readings.

Haematology (IDEXX Procyte DM™, Australia) revealed a mild normocytic and normochromic non-regenerative anaemia (HCT, 36.1%, RR 37.3-61.7). The leukogram demonstrated a mild

neutrophilia ($4.58 \times 10^9/L$, RR 2.95-11.64) with left shift and lymphopenia ($0.83 \times 10^9/L$, RR 1.05-5.10). An eosinophilia was noted two days later ($1.26 \times 10^9/L$, RR 0.06-1.23).

Biochemistry (IDEXX Catalyst DX™, Australia) revealed a panhypoproteinaemia (total protein 37 g/L, RR 52-82) due to hypoalbuminaemia (17 g/L, RR 23-40) and hypoglobulinaemia (20 g/L, RR 25-45 g/L). A single fasting bile acid was within normal limits (2.6 $\mu\text{mol/L}$, RR 0-29.9 $\mu\text{mol/L}$). A basal cortisol level of 41 nmol/L (RR 30-100 nmol/L) was obtained with a follow-up adrenocortical stimulation test non-supportive for hypoadrenocorticism (0 hour 122 (RR 30-100), 1 hour 283).

A faecal swab submitted for faecal PCR to a commercial diagnostic laboratory was negative for *Clostridium perfringens* enterotoxin A, *Salmonella* spp., *Cryptosporidium* spp., *Giardia* spp., canine enteric coronavirus, canine parvovirus 2 and canine distemper virus. Faecal culture and sensitivity revealed a light growth of *Candida albicans* but was negative for *Salmonella* sp., *Yersinia* sp., and *Campylobacter* sp. A pooled faecal flotation from samples collected over multiple days was performed and returned negative.

Abdominal ultrasound was performed with a 14-5 MHz linear array probe (Siemens, ACUSON S3000). Focal mucosal layer thickening was present in both the stomach (up to 5.2 mm) and small intestine (up to 4.5 mm) with jejunal lymphadenopathy and small volume peritoneal effusion. Abdominocentesis retrieved a transudate of low-to-no cellularity for which microbiological culture was negative.

An exploratory celiotomy was pursued for investigation of the gastroenteropathy and jejunal lymphadenopathy, with full thickness biopsies obtained of the stomach, duodenum, jejunum, ileum and jejunal lymph node. Tissues were hyperaemic in appearance, but there was no foreign matter or parasites evident. Samples were fixed in 10% buffered formalin, routinely processed, embedded in paraffin, stained with hematoxylin and eosin and sectioned for evaluation by light microscopy.

The dog was hospitalised and received supportive care (Table 1) including antimicrobials to treat bacterial translocation and suspicions of sepsis. Dietary therapy included an ultra-low-fat diet of boiled chicken.

Diagnosis

Histopathology revealed helminthiasis with moderate, generalised eosinophilic enteritis of the duodenum, jejunum and ileum (Appendix 1). Intraglandular *Helicobacter* spp. were also present in the stomach. Lymph node biopsy showed sinus histiocytosis.

A repeat faecal flotation was submitted to a commercial laboratory, which returned a negative result. A course of fenbendazole was commenced (Panacur, Coopers, Australia 50 mg/kg per os daily for five days). Due to the presence of a nematode on histological examination, repeated negative faecal flotations and history of monthly anthelmintic prevention, a fresh faecal sample was then submitted to the Melbourne Veterinary School Parasitology Laboratory (Parkville, Australia) for a modified Baermann test and Taq-Man based multiplex qPCR for *Strongyloides* spp. and all canine hookworms (*Ancylostoma ceylanicum*, *Ancylostoma caninum*, *Ancylostoma braziliense* and *Uncinaria stenocephala*).

Parasitological techniques

A centrifugal faecal flotation was performed on 2 g of fresh faeces using sodium nitrate solution (specific gravity: 1.30) for the detection of helminth ova according to Zendejas-Heredia *et al* (2021).¹⁰ No parasitic ova were detected.

Five grams of faeces was subjected to a modified Baermann technique for isolation and identification of nematode larvae according to TroCCAP diagnostic guidelines.¹¹ Multiple larvae of *Strongyloides* spp. were identified.

Molecular diagnostic techniques

Genomic DNA was extracted from 200 mg of faeces in biological duplicates using a Maxwell RSC Pure Food GMO and Authentication Kit, Promega (Promega Corporation, US) as per manufacturer's instructions, with modifications as per Zendejas-Heredia *et al.* (2021).¹⁰ Following bead-beating and cell lysis, DNA purification proceeded in a Maxwell® RSC 48 Instrument (Promega) in a final elution volume of 100 µl. The DNA was then subjected to multiplex Taq-Man probe-based real time PCRs to detect *Strongyloides* spp. and canine hookworm according to Massetti *et al.* (2022).⁵ Assays were conducted in a Mic thermocycler (Bio Molecular Systems) in triplicates for each biological duplicate using a partial region of the canine 16S mitochondrial rRNA gene as an external DNA extraction control and equine herpes virus used as an internal assay control. Non-template controls were included with each run.^{10,12} PCR assays of the patient's sample were positive for *Strongyloides* spp. but negative for all canine hookworms.

Treatment

After persistent diarrhoea and no response to a course of fenbendazole (50 mg/kg/day orally for five days), permission was obtained from the owner to commence treatment with an off-label product. Ivermectin (Bomectin, Elanco, Australia) was administered at 200 µg/kg subcutaneously every two weeks for a total of 12 doses (24 weeks).

Outcome

The patient was hospitalised for seven days and was discharged home for outpatient care with continued medical therapy as outlined in Table 1.

The patient's diarrhoea improved after the seventh ivermectin treatment which was 14-weeks after the commencement of therapy. A naturally voided, undisturbed bowel movement at week seven was reported as grade 3 on the Waltham faecal scoring system.¹³

A faecal sample was collected three days after the eleventh ivermectin injection for repeat qPCR and Baermann testing (University of Melbourne Microbiology Laboratory, Australia) to determine the efficacy of ivermectin treatment. Both tests were negative for *Strongyloides* spp.

Finally, a repeat qPCR and Baermann two months later remained negative, indicating eradication of *S. stercoralis* infection.

The patient was weaned off the ultra-low-fat diet onto a formulated commercial hydrolysed protein diet (Hills prescription diet Z/D®, Hills Pet Nutrition, USA). The patient regained weight with the absence of diarrhoea at follow-up six months later. The treatment, clinical follow-up and results of faecal examinations are summarised in Table 1.

Discussion

Infections with *S. stercoralis* in domestic dogs in Australia are rarely reported and effective treatments have not previously been investigated. Despite its veterinary and public health significance, it was not until recently that a national study brought attention to the potential for *Strongyloides* spp. to infect pet dogs through exposure to contaminated faeces in urban parks.⁵ Although highly endemic in the tropics, this study detected *Strongyloides*-positive canine faecal samples as far south as Tasmania.⁵ In the presented autochthonous case, it is unknown how this dog, which was bred and resided in Canberra, Australia, with no travel history outside the state, contracted the infection. Exposure to free-living infective stages of the parasite in the soil is the most likely explanation. Continued active surveillance, reporting and mapping of cases by veterinarians across south-eastern Australia would provide more insight into the risk of strongyloidiasis to other pets and humans in this region.

The inability to reliably diagnose strongyloidiasis with routine faecal flotations means the prevalence is likely underreported in clinical practice. As a result, veterinarians are less likely to consider strongyloidiasis as a differential aetiology for gastrointestinal and respiratory disease in dogs which could lead to misdiagnosis and mistreatment. In this study, both the Baermann test and *Strongyloides*-spp. qPCR provided a definitive diagnosis using a single faecal sample. Although qPCR has been demonstrated more sensitive than the Baermann for the detection of *Strongyloides* spp. in faeces,¹⁴ its availability is restricted to research-based laboratories. The Baermann technique is widely accessible through commercial veterinary laboratories, and it is recommended that veterinarians specifically request this test for younger or immunocompromised animals with a suspected diagnosis of strongyloidiasis, until qPCR becomes more readily available.

Infection with *S. stercoralis* in dogs may be asymptomatic or could manifest with a spectrum of gastrointestinal and respiratory signs.¹ The dog in the present case demonstrated severe gastrointestinal signs and systemic shock but did not exhibit severe respiratory compromise, highlighting the variation in clinical presentation.¹⁵⁻¹⁷ Severe forms are typically encountered in immunosuppressed animals or in young dogs with no known factors of immunodeficiency.² While glucocorticoids are known to suppress the immune system and lead to disseminated strongyloidiasis in dogs,^{3,18} the presenting dog received only a single anti-inflammatory dose of glucocorticoid injection one week prior to diagnosis. The chronicity of this dog's gastrointestinal signs suggests that it harboured a chronic case of strongyloidiasis, though this is unconfirmed. The dog likely experienced acute clinical deterioration following iatrogenic corticosteroid administration. Co-infection with *Candida albicans* may have played a role in the course of the disease, although healthy dogs routinely culture *C. albicans* and their role in gastrointestinal health is currently poorly understood.¹⁹

In canines the optimal treatment for the eradication of *S. stercoralis* remains unknown. There are currently no veterinary products registered for the treatment of *S. stercoralis* and treatment recommendations are based on single cases, case series, or are extrapolated from human literature. Treatment of symptomatic dogs with imidacloprid/moxidectin spot-on or fenbendazole alone does not consistently eliminate infection.^{2,7} In this case, the dog did not respond clinically to treatment with fenbendazole, leading to the decision to use off-label ivermectin.²⁰ Ivermectin at 200 µg/kg has proven 100% effective in removing adult *S. stercoralis* from the intestinal tract of experimentally infected dogs, but not effective in removing third-stage larvae from parenteral sites.²¹ This is reflected in veterinary case reports which have shown that improvement of clinical signs and larval disappearance can vary

following subcutaneous ivermectin administration.^{3,8,15-17,22} Therefore, repeated fortnightly administration aimed to continually eliminate adults in the gastrointestinal tract as they developed from auto-infective and/or environmentally sourced larvae, given the prepatent period of 6-10 days. The successful eradication of parasitism provides evidence of ivermectin's efficacy, although further evidence from controlled clinical trials is necessary.

S. stercoralis-negative status was achieved after 12 doses of ivermectin in this case. It is unlikely that 12 doses were necessary for eradication given previous reports of single subcutaneous injections at the same dose being efficacious.^{15,17} Reports of ivermectin at an oral dosing regimen of 0.2 mg/kg for two consecutive days or 0.5 mg/kg for seven days was found to be similarly efficacious for clearance of larvae in faeces.^{8,16} In this case, clinical improvement did not occur immediately, likely owing to hyperinfection and the immunocompromised nature of the patient. Treatment was protracted and qPCR testing was only reperformed following clinical improvement due to logistical difficulties.

Veterinarians seeking to use ivermectin to treat strongyloidiasis should consider genotyping their dogs for the *ABCB-1* variant (previously MDR1) to avoid toxicosis. Ivermectin or multidrug toxicosis has been described in a wide variety of dog breeds.²³ This genetic variant has not been reported in Boston Terriers which is why testing was not pursued prior to administration of ivermectin in this case.²³

Dogs and humans share specific haplotypes of *S. stercoralis* which explains its zoonotic potential.^{1,3,24} Humans infected with *S. stercoralis* may experience pulmonary, cutaneous or gastrointestinal signs with a mortality rate up to 90% in immunocompromised individuals.¹ No humans in contact with the infected dog exhibited clinical signs of infection however, further testing was not performed. The risk of exposure of dogs and their owners to *S. stercoralis* can be mitigated through simple hygiene measures; including hand hygiene, immediate removal of dog faeces from the environment and ensuring owners do not expose their skin to areas in which the dog defaecates. In a One Health context, the potential for *S. stercoralis* to infect pet dogs should be emphasised to enable the management of its veterinary and public health impacts.

Conclusion

Strongyloidiasis is an infrequently reported disease of canines in Australia and can be challenging to treat. As none of the products registered to treat helminths in Australia have a label claim against *Strongyloides* spp., it is important that it is retained as a differential diagnosis in dogs with histories of comprehensive anthelmintic treatment. Canine chronic

enteropathy cases may benefit from subjecting faecal samples to Baermann and, where possible, qPCR screening for *Strongyloides* spp. as routine faecal flotation is unlikely to identify this parasite. Successful management of severe strongyloidiasis in dogs may require repeated adulticidal doses of off-label ivermectin to eliminate re-infection with auto-infective and environmental larvae. As *S. stercoralis* presents a zoonotic risk to pet owners and the general public, heightened awareness should be paid to the effective diagnosis and appropriate treatment for this parasite.

Conflict of interest statement

The authors declare no conflicts of interest or specific sources of funding for the work presented.

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Table 1. Clinical signs, treatment given and faecal microbiology/parasitology results during the course of fortnightly ivermectin treatments demonstrating clinical follow up.

Fortnight of treatment	Clinical signs	Comments	Treatment	Dosage	Microbiology/parasitology result
1	Profuse watery diarrhoea, mild abdominal pain, hyporexia, ptyalism, intermittent vomiting, lethargy	Dry coat	Fenbendazole PO Buprenorphine IV Gabapentin PO Enoxaparin sodium SC Omeprazole IV ^a Maropitant IV ^a Metronidazole IV ^a Enrofloxacin IV ^a Ampicillin IV <i>Enterococcus faecum</i> probiotic PO Ivermectin SC	50 mg/kg Q24h 5d 0.02 mg/kg Q6h 3d 10 mg/kg Q8h 5d 1 mg/kg Q8h 3d 0.8 mg/kg Q24h 14d 2 mg/kg Q24h 5d 10 mg/kg Q12h 14d 7.5 mg/kg Q24h 14d 40 mg/kg Q12h 7d 1x10 ⁸ CFU Q24h 200 µg/kg Q2w	Faecal <i>Candida albicans</i> First detection of <i>Strongyloides stercoralis</i>
2	Diarrhoea, intermittent hyporexia, mild lethargy	No change	Ivermectin SC <i>Enterococcus faecum</i> probiotic PO	200 µg/kg Q2w 1x10 ⁸ CFU Q24h	N/P
3	Diarrhoea, intermittent hyporexia, mild lethargy	No change	Ivermectin SC <i>Enterococcus faecum</i> probiotic PO	200 µg/kg Q2w 1x10 ⁸ CFU Q24h	N/P
4	Diarrhoea	Normal appetite and	Ivermectin SC <i>Enterococcus faecum</i> probiotic PO	200 µg/kg Q2w 1x10 ⁸ CFU Q24h	N/P

		near normal energy levels			
5	Diarrhoea	No change	Ivermectin SC	200 µg/kg Q2w	N/P
			<i>Enterococcus faecum</i> probiotic PO	1x10 ⁸ CFU Q24h	
6	Diarrhoea	No change	Ivermectin SC	200 µg/kg Q2w	N/P
			<i>Enterococcus faecum</i> probiotic PO	1x10 ⁸ CFU Q24h	
7	None	Formed stools, ravenous appetite, near normal energy levels	Ivermectin SC	200 µg/kg Q2w	N/P
8	None	No owner concerns	Ivermectin SC	200 µg/kg Q2w	N/P
9	None	No owner concerns	Ivermectin SC	200 µg/kg Q2w	N/P
10	None	No owner concerns	Ivermectin SC	200 µg/kg Q2w	N/P
11	None	No owner concerns	Ivermectin discontinued		First negative <i>Strongyloides stercoralis</i>
12	None	No owner concerns	None		N/P

20	None	No owner concerns	None	Second negative <i>Strongyloides stercoralis</i>
44	None	No owner concerns, optimal weight and normal energy levels	None	N/P

^aTransitioned from intravenous to oral formulation for outpatient care

PO: per os; IV: intravenous; SC: subcutaneous; Q: every so many hours; h: hour; d: day; w: week; N/P: not performed

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