Veterinary Parasitology 274 (2019) 108911

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Successful use of secnidazole to manage a giardiosis outbreak in a shelter

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ARTICLE INFO

Keywords:
Giardia duodenalis
Antigen
Secnidazole
Treatment
Canine
Clinical signs

ABSTRACT

Giardia duodenalis is a common parasite in dogs in shelters where new introductions, including numerous juvenile individuals, are ongoing. A safe and effective single dose parasiticide is highly desirable for shelters experiencing disease caused by *G. duodenalis* (giardiosis). Secnidazole is an efficacious, low-cost medication used for the treatment of giardiosis in humans and has the advantage of requiring only a single oral dose. The aim of this study was to determine retrospectively the effectiveness of secnidazole on dogs of all ages during an outbreak of giardiosis in a shelter. Patients recruited into this retrospective study were divided into two groups. Group A consisted of adult dogs and weaned dogs (> 10 weeks-of-age). Group B was comprised of puppies (< 10 weeks-of-age). Giardiosis resolved in all 14 patients in Group A within 13 days following a single oral dose of secnidazole (30 mg/kg). There were no individuals with both gastrointestinal signs and a positive *G. duodenalis* antigen test at the time of the first and second follow-up examination. For the young puppies in Group B, giardiosis was reduced by 90% (9/10) within 22 days following two consecutive doses of secnidazole (30 mg/kg; 2 weeks apart). No adverse reactions were observed in any patients treated with secnidazole. Secnidazole is an effective and easily administered drug for the treatment of clinical canine giardiosis.

1. Introduction

Giardia duodenalis (also known as *G. intestinalis* or *G. lamblia*) is one of the most common parasites in dogs and cats, especially in shelters, pounds and pet stores. In these often overcrowded environments, many young dogs are often stressed, particularly where new introductions are on-going (Bouzid et al., 2015; Palmer et al., 2008; Sommer et al., 2018). The parasite inhabits the upper small intestine where it can cause dysbiosis (Beatty et al., 2017; Šlapeta et al., 2015). Environmentally resilient cysts are shed in faeces, contaminating the environment (Einarsson et al., 2016). Clinical signs associated with giardiosis include small bowel diarrhoea, vomiting and weight loss. In some cases, however, dogs and cats infected with *G. duodenalis* can remain clinically unaffected (Ballweber et al., 2010; Tysnes et al., 2014). The disease is not usually life-threatening, but animals that are immature, immunocompromised or have co-morbidities are at risk of developing more severe signs, sometimes with a rapid onset. Under shelter conditions, a high prevalence of *G. duodenalis* is often recorded using a variety of screening methods (Šlapeta et al., 2015). A robust, point-of-care (PoC) test kit detecting *G. duodenalis* coproantigen was found to be suitable for both screening and rapid cage-side diagnostic testing (Uiterwijk et al., 2018). Microscopy and PCR, that rely on the demonstration of *G. duodenalis* cysts or DNA in faeces, are less suitable for shelter conditions due to low sensitivity and high cost, respectively (Ballweber et al., 2010).

When managing clinical giardiosis, the primary aim is to relieve the animal of debilitating clinical signs, such as diarrhea, vomiting and weight loss; although in the shelter situation, an additional challenge is to prevent ongoing environmental contamination with infectious *G. duodenalis* cysts (Ballweber et al., 2010; Da Silva et al., 2011; Tangtrongsup and Scorza, 2010). Once a diagnosis is made using a combination of clinical signs and PoC testing, the success of therapeutic intervention(s) is measured by the elimination of symptomatic disease as opposed to the elimination of detectable *G. duodenalis* cysts, antigen or DNA in faeces (Olson et al., 2010). Although the majority of infected dogs are outwardly normal, they act as asymptomatic carriers and contribute to *G. duodenalis* spread through faecal contamination of the environment, food bowls and water sources (Tysnes et al., 2014). In shelters, which often involve high population densities within a confined space and the movement of animals between enclosures, there is a high risk of developing overt disease, especially if environmental accumulation of *G. duodenalis* cysts goes unrecognised.

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https://doi.org/10.1016/j.vetpar.2019.08.005
Received 27 June 2019; Received in revised form 12 August 2019; Accepted 13 August 2019

Fenbendazole and metronidazole are the most commonly used drugs for treating symptomatic giardiosis in companion animal veterinary practice (Zajac et al., 1998). Drontal Allwormer (Bayer), which contains the prodrug febantel, which is metabolised to fenbendazole, is the only registered drug available in Australia that claims to control *G. duodenalis* for companion animals (Barr et al., 1998; Bowman et al., 2009). Both drugs need to be given over a period of three to five days to be effective. Under shelter conditions, such treatment regimens can lead to poor compliance and diminished therapeutic success (Tangtrongsup and Scorza, 2010). Therefore, a safe and effective single dose parasiticide is highly desirable for such shelter scenarios. The advantages of using secnidazole to treat giardiasis in humans are that it is effective, inexpensive and requires a single dose (Almirall et al., 2011). The use of secnidazole has been to date largely anecdotal in veterinary practice, with limited evidence for further adoption (Da Silva et al., 2011; Karahalli and Ural, 2017; Volpato et al., 2018).

The aim of this retrospective study was to determine the effect of secnidazole on symptomatic dogs during an outbreak of giardiosis, using veterinary records from an animal shelter. Due to the scale of the outbreak, secnidazole was selected as a drug of choice for its ease of administration. Information concerning dogs' age, history, treatment and post-treatment monitoring of clinical signs, as well as *G. duodenalis* antigen in faeces was retrieved for the eight-week period of the outbreak. Case notes were reviewed throughout this period and patient health records were searched for potential adverse effects following secnidazole administration.

2. Material and methods

2.1. Study site and patients' veterinary records

The retrospective study design encompassed a period from January to May 2019 and included a group of dogs housed in a shelter in suburban southwestern Sydney, New South Wales, Australia (Table 1). The shelter is a private facility that accepts surrendered, neglected and abandoned companion animals on an open admission basis, following a systematic intake protocol. There is an on-site veterinary clinic that serves both private clients and shelter residents, as well as an annex for isolation of animals with suspected or confirmed infectious diseases. The shelter has 72 canine enclosures divided into four limited-access dog wards. The veterinary clinic has one recovery ward, one general ward and two isolation wards. All adult dogs residing in the shelter are housed individually in a compartmentalised indoor/outdoor kennel with a polished concrete floor to facilitate cleaning. Puppy littermates are housed together. The shelter operates a large foster network with over 400 cats and dogs under its care. The handling and medicating of the patients that were involved in this study was performed by registered Australia veterinary practitioners under the New South Wales Veterinary Practice Act 2003 No 87 and associated legislation, prior to the retrospective evaluation of the veterinary records. All dogs are owned by the shelter until adopted. The shelter director has provided access to veterinary records for the purpose of this investigation.

Table 1
Characteristics of shelter dogs diagnosed with giardiosis in January-April 2019.

Characteristic	No. and (%) of study population
Age (at secnidazole administration)	
< 10 weeks	10 (42%)
3-11 months	7 (29%)
> 1 year	7 (29%)
Date of giardiosis diagnosis	
January	2 (8%)
February	19 (79%)
March	2 (8%)
April	1 (4%)

The study patients were canine shelter residents afflicted with giardiosis. There is no formal definition of 'giardiosis' in veterinary medicine. This shelter defines giardiosis as a condition afflicting an animal that presents with both characteristic clinical signs (small bowel diarrhoea weight loss with/without vomiting) and a positive *G. duodenalis* antigen test result using faeces as the diagnostic specimen. Thus, a dog with a positive *Giardia* antigen test result and no clinical signs (absent vomiting, normal stools) is not consider to have giardiosis. A rectal swab was used as the diagnostic specimen for puppies that were group housed. Veterinary records were collated for dogs for which both antigen test results and clinical observations (before and after secnidazole administration) were retrievable.

These records were acquired from the following sources: the computerised hospital software (Idexx Cornerstone Veterinary Software, Idexx Australia), patient cage cards and a PoC antigen test result logbook. Dogs included in this study satisfied the following criteria: (i) tested positive using a *G. duodenalis* antigen test on one or more occasions; (ii) had diarrhoea and weight loss, with or without the presence of vomiting, initially coinciding with a positive faecal antigen test; (iii) were treated with secnidazole and followed for at least two weeks. Dogs that did not meet any one of the aforementioned criteria were excluded from the study.

2.2. Secnidazole therapy and follow-up

Secnidazole was formulated at Sydney South Compounding Chemist, 62/64 Broadarrow Road, Narwee NSW 2209 Australia (sydneysouthcompounding.com.au) as either a flavoured suspension (60 mg/mL; chicken flavour) or capsules (30 mg, 150 mg, 200 mg and 300 mg). Secnidazole was used at a dose of 30 mg/kg orally on an empty stomach. Patients remained in isolation after secnidazole administration to minimise environmental contamination of the shelter; moreover, so they were monitored daily. Five to 14 days post-treatment (median 12 days), a faecal sample was collected for *G. duodenalis* antigen testing. This process continued until two consecutive negative PoC test results were obtained, or until all clinical signs had resolved. At that point, patients were placed back into the shelter system. In addition, the shelter was closed to new admissions for a short period during the peak of the outbreak, from 23 February to 9 March 2019.

2.3. Husbandry protocols

The shelter feeds all clinically healthy dogs commercial kibble (Purina Supercoat or Proplan). Dogs developing diarrhoea or vomiting are fed commercial dry kibble and canned 'prescription diets' (dry and canned Hill's I/D) and a veterinarian is notified. When a giardiosis outbreak was first suspected, the shelter's inpatient flow and disinfection protocols were reviewed, and emphasis was placed on minimising hosing of enclosures and instead utilizing disposable bedding daily to avoid dissemination of cysts to adjacent runs. A quaternary ammonium disinfectant (Trigene Disinfectant, Medichem Australia) was used in place of bleach for routine cleaning of shelter surfaces, and an accelerated hydrogen peroxide product (Oxivir, Diversey, Australia) was used in the veterinary clinic. Symptomatic patients and puppies housed together were bathed individually 3 days following secnidazole administration with Natural Shampoo (Dermcare Australia). Remaining patients were to be bathed immediately before being placed back into the shelter system; this process, however, was not well-regulated.

2.4. Faecal examination for the presence of Giardia duodenalis

Dogs in the shelter displaying signs suggestive of giardiosis were subjected to faecal *G. duodenalis* antigen testing. Faecal samples collected from individual dogs by the shelter or veterinary staff were processed on-site using a *G. duodenalis* antigen test. The SNAP *Giardia* Test (Idexx Australia) and/or Anigen Rapid *Giardia* Ag Test

(RG1804DD, Bionote, South Korea) were used during the outbreak. Recent comparison of diagnostic techniques estimated median sensitivities and specificities for four tests with Bayesian latent class analysis as 71.9% (95% posterior probability interval (PPI), 60.9–81.9) sensitive and 99.6% (95%PPI, 98.5–99.9) specific for the SNAP *Giardia* Test (Uiterwijk et al., 2018). Publicly available comparison of the SNAP *Giardia* Test and Anigen Rapid *Giardia* Ag Test using 176 faecal samples demonstrated a perfect match between the two tests; moreover, the limit of detection for Anigen Rapid *Giardia* Ag Test was declared to be 125 cysts/100 µL (Evaluation of the Anigen Rapid *Giardia* Ag Test Kit, Jeju National University, South Korea; data on file with Bionote, South Korea). To compare *in-house* reliability of the two assays (SNAP *Giardia* Test *v* Anigen Rapid *Giardia* Ag Test) at detecting *G. duodenalis* antigen, we retrieved 10 frozen stored faecal samples from the outbreak and retested them using the two test kits.

2.5. Genotyping of Giardia duodenalis

Approximately 0.25 g of frozen stored faecal samples was used for DNA isolation at The Veterinary Pathology Diagnostics Services (VPDS), The University of Sydney, as described previously (Meggiolaro et al., 2019). Identification of *G. duodenalis* assemblage was determined using primers amplifying SSU rRNA genes (Feng and Xiao, 2011). To amplify SSU rDNA, we used RH11/RH4 followed by nested primers GiarF/GiarR. PCRs used MyTaq Red Mix (BioLine, Australia) in a final volume of 30 µL in a T100 PCR cycler (BioRad, Australia). All PCRs were run with a negative control of sterile PCR-grade water. PCR products were purified and bidirectionally sequenced using amplification primers at Macrogen Inc. (Seoul, Korea). Sequences were assembled and aligned with reference sequences representing *G. duodenalis* assemblage A–H using CLC Main Workbench 6.9.1 (CLC bio, a QIAGEN Company, Denmark).

3. Results

3.1. Giardia duodenalis causing clinical disease in a shelter

Clinical giardiosis in shelter dogs was diagnosed by the presence of soft faeces, diarrhoea, weight loss with/without vomiting, in addition to a positive *G. duodenalis* antigen test result. The initial diagnosis was therefore contingent on the simultaneous presence of both findings. Successful treatment was based on the antigen test becoming negative and/or clinical signs resolving. It was therefore possible for a patient to be considered cured, even if diarrhoea continued transiently after treatment, and only later resolving, while recurrence of a positive antigen test was not considered to represent recrudescent disease unless diarrhoea also recurred at the same time as the positive faecal antigen result.

A total of 24 dogs of varying ages and sizes that developed giardiosis from January to April 2019 met the inclusion criteria (Table 1; Supplementary Table S1). These dogs were treated using secnidazole and re-evaluated periodically for change in clinical status and sequential *G. duodenalis* antigen test results over the following 12 weeks (Table 1). Molecular genotyping based on amplification of SSU rDNA gene of *G. duodenalis* confirmed presence of dog adapted Assemblage C and D in all instances. The patients were further divided into two groups. Group A consisted of adult dogs and weaned puppies (> 10 weeks old, n = 14). Group B, consisted of younger puppies (< 10 weeks old, n = 10).

Group A was comprised of 14 dogs, ranging from 3 months to 10 years old (median age 9 months) (Table 2). All dogs had a positive *G. duodenalis* antigen test result and acutely developed clinical signs, including abnormal stools ranging from soft faeces to diarrhoea and weight loss. All dogs in this group were kennelled individually. Veterinary records demonstrated all dogs were fully vaccinated (C5; parvovirus, distemper, hepatitis, parainfluenza and *Bordetella bronchiseptica*) and had been given milbemycin oxime and praziquantel tablets every three months for intestinal anthelmintic prophylaxis and Advocate or Comfortis Plus every month for ectoparasite and heartworm prophylaxis. Two patients were positive for roundworm and one for hookworm eggs on faecal floatation. Both patients remained in isolation until clinical signs resolved. All dogs were clinically healthy prior to the diagnosis of giardiosis.

Table 2
Summary of giardiosis, diagnostics and clinical sign for dogs in Group A post secnidazole administration.

Group A*	T0 (n = 14)	T1 (n = 14)	T2 (n = 13)	T3 (n = 7)
Giardiosis	14 (100%)	0 (0%)	0 (0%)	0 (0%)
***Giardia duodenalis* antigen positive**	14 (100%)	4 (29%)	1 (7%)	0 (0%)
Diarrhea, vomiting, weight loss	14 (100%)	1 (7%)	2 (14%)	0 (0%)

* Secnidazole administered at T0.

Group B consisted of ten puppies (9 and 10 weeks old at the time of diagnosis and secnidazole administration) (Table 3). Nine dogs in this group were littermates and were fostered in two homes. When foster carers first noticed diarrhoea, all puppies were transported to the clinic and housed together in three isolation enclosures. The siblings developed clinical signs acutely (diarrhoea and weight loss), all were positive for *G. duodenalis* antigen and 8/9 were positive for coccidia (*Cystoisospora* spp.). All nine siblings were treated with 5% toltrazuril solution (Bayer, Australia) at 15–20 mg/kg for three consecutive days. Three weeks later, the siblings remained both symptomatic and positive for *G. duodenalis* antigen, while coccidia were no longer evident on faecal flotation. The remaining unrelated puppy was never in contact with this litter and was housed individually. All ten puppies were vaccinated at 6, 9, 12 and 16 weeks of age, using a modified live virus vaccination containing canine parvovirus, canine adenovirus type 2 and canine distemper virus, and an intranasal modified live *Bordetella bronchiseptica*, canine adenovirus type 2 and parainfluenza (Broncho shield III) was given at 9 weeks of age.

3.2. Single dose of secnidazole for canine giardiosis dogs older than 10 weeks of age

Dogs (n = 14) were given a single dose of secnidazole (30 mg/kg orally, T0 in Table 2) and transferred to the shelter annex for isolation. All these dogs were kennelled individually. The first follow-up examination (T1) was conducted 5–13 days post-treatment (median 6 days). Clinical signs of giardiosis resolved in 13/14 dogs (93%) within this period (Table 2). The *G. duodenalis* antigen test was negative for 10/14 dogs (71%). A single dog (4 months old) continued to have soft faeces at the time of the first follow-up test, despite testing negative on *G. duodenalis* antigen test. Therefore, using the aforementioned criteria, no dogs were considered to have giardiosis at first follow up.

A second follow-up examination (T2) was conducted on day 10–22 post-treatment (median 12 days, Table 2). The number of dogs in Group A was reduced to 13, because one dog (normal faeces; negative antigen test) was euthanized for reasons unrelated to giardiosis. There was a persistent resolution of clinical signs in 11/13 (85%) of dogs and *G. duodenalis* antigen test was negative for 12/13 (93%). Two dogs (4 months and 1 year-of-age, respectively) developed diarrhoea during the period between the two follow-up examinations but tested negative on *G. duodenalis* antigen test. Therefore, using our shelter criteria, 13/13 dogs did not have giardiosis at the second follow-up examination.

A third and final follow-up examination (T3) was conducted on day 22–32 post-treatment (median 24 days). Following the previous follow-up examination, 6 dogs had been returned to general shelter population, reducing the number of dogs in Group A to 7. All 7 dogs were clinically well, with normal stools and testing negative on *G. duodenalis*

Table 3
Summary of giardiosis, diagnostics and clinical sign for dogs in Group B post secnidazole administration.

Group B*	T0 (n = 10)	T1 (n = 10)	T2 (n = 10)	T3 (n = 10)	T4 (n = 9)
Giardiosis	10 (100%)	3 (30%)	1 (10%)	1 (10%)	0 (0%)
***G. duodenalis* antigen positive**	10 (100%)	3 (30%)	1 (10%)	6 (60%)	4 (44%)
Diarrhea, vomiting, weight loss	10 (100%)	9 (90%)	10 (100%)	2 (20%)	0 (0%)

* Secnidazole administered twice: at T0 and approx. 14 days later (T1).

antigen testing (Table 2).

Giardiosis resolved in all 14 patients (including the dog euthanased for unrelated reasons) within 13 days following a single dose of secnidazole, as there were no individuals with both gastrointestinal signs and a positive *G. duodenalis* antigen test in any of the follow-up examinations.

3.3. Reduced shedding of Giardia duodenalis after the administration of secnidazole in young puppies

The puppies (n = 10) were given one dose of secnidazole (30 mg/kg orally; T0 in Table 3) and housed in isolation. The first follow-up examination was conducted within 2 weeks post-treatment (T1; Table 3). Clinical signs of giardiosis, including abnormal faeces, ranging from soft and poorly formed to watery diarrhoea, persisted in 8/9 of the group-housed littermate puppies (n = 9) and the antigen test for *G. duodenalis* was positive for 3 (3/9; 33%) of the littermates. The individually housed puppy tested negative for *G. duodenalis* antigen but remained clinically affected, with signs persisting for 6 days post-treatment. By our criteria 30% of the puppies had persistent giardiosis, despite the first dose of secnidazole. A second dose of secnidazole 30 mg/kg was therefore administered to the littermates (n = 9) 14 days after the first dose.

A second follow-up examination was conducted on day 20–22 post initial treatment (median 22 days, T2; Table 3). All dogs had persisting clinical signs including soft faeces and diarrhoea, but only one littermate tested positive for *G. duodenalis* antigen; thus, by our criteria, only 1 puppy had persistent giardiosis. This indicates an 90% (9/10) eradication of giardiosis after the second dose of secnidazole.

A third follow-up examination was scheduled 27 to 34 days post initial secnidazole treatment (median 28 days, T3; Table 3). Clinical signs were recorded in 2/10 (20%) of puppies and antigen testing for *G. duodenalis* was positive for 6/10 (60%) of puppies (one puppy was both clinically affected and *G. duodenalis* antigen positive). The puppy that was housed separately from the litter had recovered clinically and tested negative for *G. duodenalis* antigen.

A fourth follow-up examination (T4) was scheduled for 43 days after the initiation of treatment (the first dose of secnidazole). By this stage, the individually isolated puppy, who had previously tested negative twice on *G. duodenalis* antigen tests, had been moved back into general shelter population. Of the remaining 9 puppies, 9/9 were considered to have normal stools. Despite a lack of clinical signs, 4/9 (44%) of patients tested positive for *G. duodenalis* antigen, but by our criteria these dogs did not have clinical giardiosis.

The prevalence of giardiosis was reduced by 90% (9/10) within 22 days following either a single dose (1 pup) or two consecutive doses (2 weeks apart; 9 puppies) of secnidazole. Although there was a recurrence in positive *G. duodenalis* antigen test results in the third and fourth follow-up examinations, the prevalence of symptomatic disease was reduced by 90% (9/10) within 22 days and clinical giardiosis was eradicated in 100% of cases by day 34 post-initial treatment, and eliminated within 43 days following initial treatment (Table 3).

No adverse reactions were observed in any patients treated with both single and sequential doses of secnidazole.

3.4. Pilot comparison of Giardia duodenalis antigen detection kits during the giardiosis outbreak

Two different *G. duodenalis* antigen tests were used during the giardiosis outbreak. The veterinary staff initially used the SNAP *Giardia* Test (Idexx Australia), while for follow-up testing, the Anigen Rapid *Giardia* Ag Test (RG1804DD, Bionote, Korea) was used. To evaluate the reliability of the Anigen Rapid *Giardia* Ag Test to confirm SNAP *Giardia* Test positive samples as antigen positive, we retrieved ten of the SNAP *Giardia* Test positive faecal samples and re-tested them with the Anigen Rapid *Giardia* Ag Test. A total of 10/10 (100%) samples that tested positive using the SNAP *Giardia* Test returned a positive antigen signal using the Anigen Rapid *Giardia* Ag Test.

4. Discussion

This study shows that a single dose of secnidazole (30 mg/kg orally) reduced both the prevalence of clinical signs associated with giardiosis and the shedding of *G. duodenalis* in adult shelter dogs. In puppies younger than 10 weeks old, two doses of secnidazole (30 mg/kg orally 14 days apart) were similarly effective. In both canine cohorts, clinical signs or coproantigen positivity could persist after treatment, although clinical disease referable to *Giardia* infection eventually resolved in every instance. Our retrospective study confirms the results of an experimental control study on 2 to 8-months old dogs afflicted with giardiosis (Karahalli and Ural, 2017), and confirms similar results can be obtained in real world situations. Secnidazole is considered a safe, efficacious and inexpensive single-dose medication for the treatment of giardiosis in humans, but is rarely used in veterinary medicine (Da Silva et al., 2011; Karahalli and Ural, 2017; Rossignol, 2010).

It is critical to emphasise that clinical signs do not typically resolve instantaneously after anti-*Giardia* therapy. Delayed resolution of clinical signs associated with giardiosis in weaned puppies is attributed to post-weaning changes in the physiology and microbiology of the gastrointestinal tract. A weaned puppy experiences a shift in gastrointestinal microbiota, with a 100-fold reduction in *Lactobacillus* spp. and an increase in bacterial diversity and richness, eventually developing an adult microbiome displaying increased resilience against environmental pathogens (Buddington, 2003; Guard et al., 2017). Studies in humans have found that the microbiome profile changes throughout neonatal development, only beginning to appear adult-like by 3 years old (Backhed et al., 2015; Yassour et al., 2016). Importantly, young puppies around the age of weaning (6–8 weeks) often experience sporadic episodes of diarrhoea unrelated to enteropathogens due to shifts in the microbiota and dietary changes (Guard et al., 2017). During weaning, many factors shape the development of the gut microbiome, including parental influences, environmental factors, diet and pathogen exposure (Guard et al., 2017). Any disruption to the colonisation phase, such as the use of anti-infectives or early weaning onto solid foods, has been shown to reduce the diversity of the microbiome and lead to increased risks of metabolic and immune diseases in humans (Mueller et al., 2015; Yassour et al., 2016). The persistence of gastrointestinal clinical signs observed in puppies in the current study could be attributed to any combination of the following: (i) administration of anti-infective secnidazole at a peri-weaning age, including the repeated dose, which may have disrupted the evolving microbiome and

led to a prolonged period of post-weaning diarrhoea, (ii) *G. duodenalis* infection disrupting the evolution of the gut microbial community, and (iii) concurrent infections (e.g. coccidiosis, ascariasis and ancylostomiasis in the present study). This investigation did not aim to determine the definitive causality of the diarrhoea. Nevertheless, comorbidities were ruled out based on clinical records, and all puppies were fully vaccinated. The interplay between parasites, including *G. duodenalis,* the microbiota and the immunological response of the gut in the pathogenesis of giardiosis, requires further experimental inquiry (Šlapeta et al., 2015; Tysnes et al., 2014).

During the described giardiosis outbreak, a second dose of secnidazole was administered to puppies. This decision was made due to persistent diarrhoea and weight loss, despite a 70% reduction in the prevalence of giardiosis within 2 weeks of the initial treatment. Although adverse effects of secnidazole have been documented in humans, the effects of a second dose of secnidazole has not been evaluated in small animals (Da Silva et al., 2011; Karahalli and Ural, 2017). Secnidazole and metronidazole belong to 5-nitroimidazole anti-infectives (Dingsdag and Hunter, 2018; Gillis and Wiseman, 1996; Tangtrongsup and Scorza, 2010). Metronidazole, however, is known to be associated with adverse effects in dogs and cats; moreover, and its margin of safety is considered narrow, with a therapeutic index of 1/100 in mice when administered at 30 mg/kg/day for two consecutive days (Caylor and Cassimatis, 2001; Dow et al., 1989). Adverse effects of metronidazole include neurological signs, such as tremors, rapid positional nystagmus, muscle spasm, ataxia, convulsions, as well as gastrointestinal signs (Olson et al., 2010; Scorza and Lappin, 2004). Metronidazole also interferes with olfactory acuity (Jenkins et al., 2016). While no adverse effects were recorded for secnidazole in dogs involved in this retrospective study, it is imperative to closely monitor for such effects when using secnidazole to treat giardiosis. This is especially true in a shelter setting, where dogs are less vigilantly observed compared to a private setting.

Length of stay is the most significant risk factor for acquiring viral infectious disease in shelter animals in the United States (Dinnage et al., 2009; Edinboro et al., 2004; Holt et al., 2010). The waxing and waning periods of both diarrhoea and the shedding of cysts associated with undiagnosed *G. duodenalis* has been shown to increase environmental contamination (Tysnes et al., 2014). Asymptomatic animals continue to be exposed as they progress through the shelter environment, as demonstrated by a study involving 878 shelter dogs in London, 21% of which became infected with *G. duodenalis* (Upjohn et al., 2010). Due to the difficulty in identifying asymptomatic animals, limited budget and constant pressure to rehome animals expeditiously, routine *G. duodenalis* testing is challenging to implement.

If symptomatic patients are identified and test positive using a *G. duodenalis* PoC antigen test, they should be separated from healthy individuals prior to secnidazole treatment. Complete resolution of cyst shedding by cats and dogs was achieved at 6 to 8 days and 3 to 7 days after secnidazole treatment, respectively (Da Silva et al., 2011; Karahalli and Ural, 2017). As secnidazole is a medication used off-label for this purpose, patients displaying clinical signs suggestive of adverse reactions to the drug must be carefully monitored, documented and supported symptomatically. Sufficient staff resources and education are essential for appropriate care. There is little direct evidence of zoonotic transmission from companion animals to humans, with dogs shedding canine-adapted *G. duodenalis* Assemblage C and D (Ballweber et al., 2010; Feng and Xiao, 2011; Gruffydd-Jones et al., 2013). Numerous past studies failed to identify contact with pets as a risk factor for human giardiosis, while human-to-human transmission and contamination of water sources are considered to be far more likely (Efstratiou et al., 2017; Gray et al., 1994; Hoque et al., 2002, 2003; Hunter and Thompson, 2005; Warburton et al., 1994).

Total eradication of *G. duodenalis* from shelters is an unrealistic expectation (Tangtrongsup and Scorza, 2010; Upjohn et al., 2010). Under shelter conditions, undiagnosed giardiosis may lead to an increase in the prevalence of disease in puppies and immunocompromised animals, potentially leading to disease outbreaks (Raza et al., 2018). Secnidazole is an efficacious and easily administered drug for the treatment of clinical canine giardiosis and may be cheaper, safer and more practical when used in a shelter scenario, as opposed to other available options (Karahalli and Ural, 2017; Tangtrongsup and Scorza, 2010). If sufficient shelter resources exist, testing of shelter residents and judicious implementation of quarantine protocol on intake may prevent future giardiosis outbreaks.

Declaration of Competing Interest

JS, RM declare no conflict of interest. CR, SM were employed as veterinarians at the shelter. WC served as volunteer and casual veterinarian at the shelter during the outbreak. The shelter had no role in the decision to publish.

Acknowledgements

We would like to thank veterinary staff and nurses and shelter staff for cooperation with data retrieval. We thank Anne Fawcett (Sydney School of Veterinary Science, The University of Sydney) for advice and discussions, Maira Meggiolaro (Veterinary Pathology Diagnostics Services, The University of Sydney) for expert assistance with samples and Galib Braschler (Atkinson Center for a Sustainable Future, Cornell University) for language editing and proofreading. We thank the reviewers for their comments that improved the manuscript.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.vetpar.2019.08.005.

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