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Multiple anthelmintic drug resistant *Ancylostoma caninum* in foxhounds

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ABSTRACT

Ancylostoma caninum is the most common and important gastrointestinal nematode of dogs in the United States. Despite recent reports of *A. caninum* isolates resistant to all classes of anthelmintics, little is known about the frequency and extent of this anthelmintic resistance. The study aim was to evaluate the efficacy of three commercial anthelmintic products in the treatment of foxhound dogs with a history of persistent *A. caninum* infections. In the first phase of this study, 35 foxhounds were randomly divided into three treatment groups: moxidectin/imidacloprid (MI), pyrantel pamoate/febantel/praziquantel (PFP), and emodepside/praziquantel (EP). Fecal samples were collected on day 0, 11, and 33 post-treatment (PT), and hookworm eggs were quantified using the mini-FLOTAC technique with a multiplication factor of 5 eggs per gram (EPG). The fecal egg count reduction (FECR) on day 11 PT was 65% (95% CI: 62%–68%) for MI, 69% (95% CI: 66%–72%) for PFP, and 96% (95% CI: 94%–97%) for EP. On day 33 PT, the FEC in the MI and PFP groups returned to almost the same values as on day 0, while in the EP group, the FEC remained low. Since MI and PFP proved ineffective, 32 animals were randomly divided into two groups in the second phase. They were treated either with a combination of MI/PFP or EP. The FECR at day 13 PT for the combination MI/PFP was 89% (95% CI: 87%–91%) and 99% (95% CI: 98%–99%) for EP. These results suggest that this *A. caninum* population is resistant to multiple anthelmintics. Although the combination of MI/PFP improved the anthelmintic efficacy, the FECR remained below 90%. Future studies are indicated to evaluate further the epidemiology of persistent hookworm infections in dogs in the US and to identify more effective treatment protocols as they pose a significant health risk to canine and human health.

1. Introduction

Ancylostoma caninum, the canine hookworm, is the most significant and widespread gastrointestinal nematode in dogs in the United States. This parasite continues to be one of the most identified endoparasites in dogs and its prevalence has remained consistent over the years (Drake and Carey, 2019; Sweet et al., 2021). In a retrospective study analyzing fecal flotation results of dogs from 10 veterinary diagnostic laboratories in the US, Ancylostomatidae eggs were found in 5.63% of cases (Sobotyk et al., 2021). Similarly, researchers demonstrated a 47% increase in the annual prevalence of *A. caninum* from 2012 to 2018 in an extensive study of over 39 million fecal samples (Drake and Carey, 2019). This rise in prevalence emphasizes the necessity of effective control and treatment of *A. caninum*.

Ancylostoma caninum is a hematophagous parasite of the small intestine of canids. The main pathologies induced in definitive hosts are iron-deficiency anemia and enteritis, but other clinical signs include hypoalbuminemia, hematochezia, and/or melena (Epe, 2009; Kalkofen, 1987). These parasites bite and attach to the intestinal mucosa and suck a plug of tissue into their buccal capsule to feed on both blood and tissue (Jimenez Castro et al., 2020; Kalkofen, 1987). This parasite also has zoonotic potential, as it is implicated in *cutaneous larva migrans* (Bowman et al., 2010). Transmission routes include oral, percutaneous, ingestion of paratenic hosts, and transmammary to neonatal puppies. *Ancylostoma caninum* larvae can encyst in somatic tissues in a hypobiotic state. After anthelmintic treatment, arrested larvae erupt to repopulate

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the small intestine and develop into adults (Epe, 2009; Kalkofen, 1987). This unique feature of *A. caninum's* pathogenicity is referred to as "larval leak" and is often associated with persistent hookworm infections. In recent years, however, there has been a significant rise in cases of hookworm infections that are refractory to treatment with U.S. Food and Drug Administration (FDA) approved anthelmintic drugs (Jimenez Castro et al., 2020). In the United States, the anthelmintics currently approved for hookworm treatment are febantel, fenbendazole, moxidectin, milbemycin oxime, and pyrantel. Moreover, it has been conclusively shown that some populations of *A. caninum* are multiple anthelmintic drug resistant (MADR) to benzimidazoles, macrocyclic lactones and pyrantel (Hess et al., 2019; Jimenez Castro et al., 2019; Kitchen et al., 2019). This trend coincides with data in other species, as anthelmintic resistance in several nematodes has been described in ruminants and horses (Coles et al., 2006; Kaplan, 2020; Nielsen, 2022).

Although anthelmintic resistance is not considered to be as commonplace in small animals (von Samson-Himmelstjerna et al., 2021), few studies have been conducted to investigate this topic in most parasites. As for *A. caninum*, evidence suggests that greyhound racing farms in the Southern US are the origin of MADR hookworms. *Ancylostoma caninum* resistance is thought to have emerged in these populations due to the subtropical climate, the sandy soil on racetracks, the non-selective and routine use of anthelmintics, and the high density and constant movement of these dogs, all of which create ideal circumstances for hookworm transmission as well as an intense selection pressure for drug-resistant alleles (Jimenez Castro et al., 2021). The greyhound racing industry has been declining rapidly over the last decade, and since its demise, many greyhound rescue groups have worked diligently to adopt these dogs. There are growing concerns that this effort has contributed to the spread of MADR *A. caninum* to other canid populations, including wild canids, hunting dogs, and pets (Jimenez Castro et al., 2019). The emergence of MADR hookworms highlights the need to determine the extent of its prevalence and to develop new strategies for effectively treating this parasite, especially in dogs housed in groups like greyhounds and foxhounds.

This study aimed to evaluate the efficacy of three commercial anthelmintic products in treating foxhounds with a history of persistent infection with *A. caninum*.

2. Materials and methods

2.1. Animals/subjects

Thirty-five foxhounds from a kennel in New Jersey, aged between 7 months and 11 years old, with a 4-month history of persistent *A. caninum* infection despite anthelmintic treatment, were included in this study. Four of these animals were acquired from a kennel in Virginia 3 weeks before the beginning of this study. The history of those dogs was unknown. The rest of the animals had lived in the kennel for several years. All the animals were used recreationally to fox hunt, alternating hunting groups with each expedition. Dogs were on a flea/tick preventative with a topical solution of 6% fipronil, 44.8% permethrin, and 1.8% pyriproxyfen (Effitix® Plus, Virbac, France). They also received monthly heartworm prevention with a cattle formulation of ivermectin at a dose of 0.2 mg/kg orally (for the last 5 years) or topical moxidectin (for the 2 months prior to this study). During hunting activities, dogs had free access to wildlife carcasses and feces of several domestic animals (especially horses) and wildlife. All animals were confined to the same enclosure in a single big pen of concrete, with free access to a dirt outdoor area consisting mostly of mud. The concrete area was washed daily with water, and weekly with hot water plus bleach and dish soap. The mud area was not cleaned, but feces were picked up weekly. Ethics approval for this study was granted by the Virginia Tech Institutional Animal Care and Use Committee (Protocol 21–151). In addition, the owner of the hounds provided an informed consent document for the dogs' participation at the start of the study.

2.2. Molecular identification of parasite species

To confirm the molecular identification of *A. caninum*, eggs were purified from a subset of 4 dogs from each treatment group by centrifugal fecal flotation. Then, DNA was extracted using the DNeasy Blood and Tissue kit (Qiagen, Germany), according to the manufacturer's instructions. A region of the first and second internal transcribed spacers (ITS) plus the 5.8 gene was amplified using the primers NC5 5′-GTAGGTGAACCTGCGGAAGGATCATT-3′ and NC2 5′-TTAGTTTCTTTTCC-TCCGCT-3′, followed by enzyme digestion with *Hinf1* as previously described (Gasser et al., 1996).

2.3. Treatments

This study had two phases. In the first phase, using a random number generator, animals were divided into three groups (12, 12, and 11 animals, respectively) to evaluate the efficacy of three commercial anthelmintic products. In group 1 (MI), animals were treated topically with a combination of 2.5 mg/kg of moxidectin and 10 mg/kg of imidacloprid (Advantage® multi, Elanco, USA). Animals in group 2 (PFP) received an oral combination of 5 mg/kg of pyrantel pamoate, 25 mg/kg of febantel, and 5 mg/kg of praziquantel (Drontal® Plus, Elanco, USA). These two products are approved for treating *A. caninum* infections in dogs. Animals in group 3 (EP) were given orally a topical solution of 1 mg/kg of emodepside and 4 mg/kg of praziquantel (Profender®, Elanco, USA). This extra-label use of Profender was selected due to recent reports supporting its efficacy against persistent *A. caninum* infections in dogs (Jimenez Castro et al., 2020, 2022; Jimenez Castro and Kaplan, 2020). Dogs were confirmed heartworm negative before starting the treatment with emodepside. In phase 1, fecal samples were collected directly from the rectum on day 0 (treatment day) and days 11 and 33 PT. They were then shipped on ice to the lab and were processed within 48 h. Fecal egg counts (FEC) were subsequently performed using the mini-FLOTAC technique with a multiplication factor of 5 eggs per gram (EPG) of feces (Cringoli et al., 2017; Maurelli et al., 2014). The kennel and laboratory staff were blind to each dog's treatment. The fecal egg count reduction (FECR) was determined for each anthelmintic treatment (Wang et al., 2018). Anthelmintic treatment was effective when at least a 95% reduction in FEC was detected (Jimenez Castro and Kaplan, 2020).

Based on the results of the FECs on day 33 PT, a second phase of the study was planned to evaluate if combination therapy would increase the FECR. The second phase started one month after the end of the first phase. The same 35 animals from phase 1 were randomly divided into two treatment groups. However, due to the turnover of animals between kennels, we only were able to evaluate 32 dogs (15 and 17 animals, respectively). Dogs in group 1 (MI/PFP) were treated with a combination of MI (topically) and PFP (orally), and animals in group 2 were treated with EP (orally). All drugs were administered in the same doses described for phase 1. In phase 2, samples were collected on day 0 (treatment day) and day 13 post-treatment, and FECs were performed as described previously. Sample collection and laboratory methods were performed as indicated in phase 1.

2.4. Statistical analysis

To estimate the FECR, the online version of eggCounts (http://shiny.math.uzh.ch/user/furrer/shinyas/shiny-eggCounts/) was used. This online tool uses a Bayesian hierarchical model with paired pre- and post-treatment samples from the same animal to assess anthelmintic efficacy (Wang et al., 2018).

3. Results

For the molecular analysis, the restriction pattern in all the samples showed three different bands, similar to the results of Gasser et al.

(1996) (gel image not shown), confirming the identification of *A. caninum*.

In the first phase of the study, the FECR on day 11 post-treatment for MI and PFP were 65% (95% CI 62%–68%) and 69% (95% CI 66%–72%), respectively (Table 1). In contrast, EP resulted in a 96% reduction (95% CI 94%–97%) in the number of EPG (Table 1). On day 33 post-treatment, the values of EPG in MI and PFP groups returned to almost the same values as at the beginning of the study (613.8 vs 820.4 and 608.0 vs 708.3 for MI and PFP, respectively), while in EP the FEC was still low when compared with values at day 0 (240.0 vs 742.3).

In the second phase of the study, combination therapy (MI/PFP) reduced the FEC by 89% (95% CI 87%–91%), while EP alone caused a reduction of 99% (95% CI 98%–99%) (Table 2). No treatment-related adverse events were reported in this study.

4. Discussion

In this study, we used the fecal egg count reduction test (FECRT) to evaluate the efficacy of three anthelmintics products in a foxhound kennel with a history of persistent *A. caninum* infections. When products were used individually, MI and PFP did not show adequate efficacy, resulting in an FECR of <70%. These results suggest that this *A. caninum* population is resistant to benzimidazoles, macrocyclic lactones, and pyrantel pamoate. Although EP is not approved by the FDA for use in dogs, based on recent evidence of the high efficacy of this product in anthelmintic-resistant *A. caninum* isolates (Jimenez Castro et al., 2020, 2022; Jimenez Castro and Kaplan, 2020), the clinician (with owner's consent) decided to use Profender as an extralabel therapy in this dog population. This extralabel use followed the Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). Although the FECR in the group treated with EP (96%) indicates that emodepside was efficacious against *A. caninum* that are refractory to the treatment with benzimidazoles, macrocyclic lactones, and pyrantel pamoate, this reduction value is lower than 99.6%, previously reported for EP (Jimenez Castro et al., 2020, 2022). Four of the animals in this group had individual FECR values below 90% (see supplemental data) which explains why the average reduction in the group was not as high as expected. Although all drugs were administered by the clinician, the incomplete clearance of egg shedding in these animals could be caused by a suboptimal dose. It is also possible that dogs did not swallow the complete amount of the drug when administered. Another factor to consider is that we used the topical formulation for cats instead of the oral formulation for dogs, which is not commercially available in the US. There is limited data on the use of the cat topical formulation of emodepside by oral administration in dogs, therefore, the absorption and distribution of the drug might not have been optimal in this study.

Table 1
Fecal egg count reduction for three treatment groups in phase 1.

Treatment group	Dogs (n)	Mean FEC[d] (Range)			FECRT[e] (95% CI)
		Day 0	Day 11	Day 33	Day 11
1 (MI[a])	12	820.4 (45–2045)	285.4 (0–2090)	613.8 (250–1585)	65% (62%–68%)
2 (PFP[b])	12	708.3 (80–2545)	221.3 (0–690)	608.8 (225–1065)	69% (66%–72%)
3 (EP[c])	11	742.3 (165–1735)	33.2 (0–140)	240.0 (0–945)	96% (94%–97%)

[a] Moxidectin + imidacloprid.
[b] Pyrantel pamoate + febantel + praziquantel.
[c] Emodepside + praziquantel.
[d] Fecal egg count.
[e] Fecal egg count reduction test.

Table 2
Fecal egg count reduction for two treatment groups in phase 2.

Treatment group	Dogs (n)	Mean FEC[d] (Range)		FECRT[e] (95% CI)
		Day 0	Day 13	Day 13
1 (MI[a]/PFP[b])	15	520.3 (55–2565)	61.0 (0–200)	89% (87%–91%)
2 (EP[c])	17	416.5 (80–1595)	4.7 (0–60)	99% (98%–99%)

[a] Moxidectin + imidacloprid.
[b] Pyrantel pamoate + febantel + praziquantel.
[c] Emodepside + praziquantel.
[d] Fecal egg count.
[e] Fecal egg count reduction test.

Due to the limited reduction in the hookworm FEC in the animals treated with either MI or PFP, the second phase of this study aimed to determine if combination MI/PFP therapy was effective in the same dog population. There have been conflicting reports on the efficacy of this anthelmintic combination in dogs with persistent *A. caninum* infections. Initial therapy with a combination of MI/PFP, followed by a monthly maintenance administration of MI, successfully eliminated persistent *A. caninum* eggs shedding in greyhounds (Hess et al., 2019). However, in another study, this combination treatment failed to eliminate persistent *A. caninum* infections in the same dog breed (Jimenez Castro et al., 2021). In the second phase of this study, the MI/PFP combination reduced FECs more than each product individually. This was expected since anthelmintic combinations with two or more drugs of different chemical groups have been shown to produce an additive effect, increasing the overall efficacy of the treatment (Kaplan, 2020). The average FECR was higher with the combination protocol than with each product individually, however, it was still insufficient (89%, 95% CI 87%–91%) to be considered effective (Jimenez Castro and Kaplan, 2020). Although the original protocol described for MI/PFP requires the administration of both products for several months (Hess et al., 2019), we only evaluated the efficacy of a single dose of this anthelmintic combination. It is also important to consider that of the 15 dogs treated with the drug combination in the second phase, five dogs showed a FECR of ≥ 93.8%. This suggests that a single dose of the triple anthelmintic combination may have adequate efficacy in some, but not all, persistent hookworm infections and this efficacy could increase if the combination is administered over time. Although we did not test this *A. caninum* population for molecular markers of benzimidazole resistance, the frequency of resistance genes in this population could also be low. On the other hand, EP was successful (99%, 95 CI 98%–99%) in clearing the *A. caninum* egg shedding in phase 2. Since we only evaluated the efficacy of emodepside at 13 days PT, it is possible that patent infections can appear after 2–3 weeks PT due to larval leak, even without reinfection.

Oral administration of moxidectin produces higher plasma concentrations, lower clearance, and higher volume of distribution than ivermectin (Al-Azzam et al., 2007). After four treatments at intervals of 28 days, moxidectin reaches a steady-state concentration with residual effects for up to one month (Bowman et al., 2016). Therefore, it has been hypothesized that after treatment with a combination of anthelmintics and clearing hookworm egg shedding, monthly administration of moxidectin could eliminate any dormant larvae that reactivate development to repopulate the small intestine (Hess et al., 2019).

A large animal ivermectin formulation has been extensively used in this kennel for approximately the last 5 years at a dose of 0.2 mg/kg. This extra-label use of ivermectin has been commonly used in the foxhound industry for economic reasons (Masters of Foxhounds Association, 2015). In greyhounds, there is evidence that ivermectin-resistant hookworms can also develop resistance to moxidectin quickly when this drug is used in already ivermectin-resistant populations (Jimenez Castro et al., 2021). Thus, in this particular

foxhound kennel, potential resistance to ivermectin might have developed first, and the recent use of moxidectin may have triggered the development of moxidectin resistance observed here.

Many factors can also affect the FECR in dogs after treatment of *A. caninum* infections (Morgan et al., 2022). There is limited research about the pharmacogenetic particularities in foxhounds; however, since differences in the specific responses to drug absorption and metabolism have been reported in different dog breeds (Fleischer et al., 2008), we cannot rule out a deficiency in the drug absorption or distribution in this study. Although there are significant and apparent differences between large and companion animals, epidemiological similarities between greyhound and foxhound husbandry are, to some extent, related to large animal husbandry practices (von Samson-Himmelstjerna et al., 2021). These practices, such as large numbers of animals housed and bred in confinement and undergoing intense and frequent deworming, decrease refugia and therefore increase the rate of anthelmintic resistance development.

Although less likely, other potential causes of the persistent *A. caninum* egg shedding in this kennel could be the contaminated environment in which the dogs are continually kept. Specifically, the hounds have constant access to dirt runs, which are likely the source of ongoing hookworm reinfection. Failures in parasite control programs are frequently associated with the constant reinfection of dogs from contaminated environments (Jimenez Castro et al., 2021; Ridley et al., 1994) and the reactivation of some dormant somatic larvae resulting in new patent gastrointestinal infections.

The density-dependent egg production phenomenon could also cause the inadequate FECR in dogs treated with either MI or PFP. There is evidence that female *A. caninum* adults that survive anthelmintic therapy produce relatively more eggs, as a result of the reduction of density-dependent constraints on their egg production (Kopp et al., 2008; Kotze and Kopp, 2008; Morgan et al., 2022). In these cases, the anthelmintic efficacy, measured by the FECRT, could be underestimated.

The high turnover rate of dogs between foxhound kennels is a risk for the spread of parasites, and this is especially important for an anthelmintic-resistant parasite population. Kennel management personnel should be aware of this risk and minimize it by testing animals before purchasing or selling them. New animals should also be treated, and a FECRT should be performed to measure the anthelmintic efficacy. Furthermore, in the presence of intensive deworming, hygienic measures such as the removal and proper disposal of feces in all areas of the kennel, remain critical to minimize environmental contamination and reinfection of dogs.

Although the presence of persistent infections by *A. caninum* is a major concern in the foxhound industry, there has been little to no research about this topic in these dog populations in the US. Therefore, active surveillance programs are needed to detect and prevent the spread of anthelmintic resistant parasites in foxhounds in the US.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijpddr.2023.07.001.

Declarations of interest: none.

References

Al-Azzam, S.I., Fleckenstein, L., Cheng, K.J., Dzimianski, M.T., McCall, J.W., 2007. Comparison of the pharmacokinetics of moxidectin and ivermectin after oral administration to beagle dogs. Biopharm. Drug Dispos. 28, 431–438. https://doi.org/10.1002/BDD.572.

Bowman, D.D., Grazette, A.R., Basel, C., Wang, Y., Hostetler, J.A., 2016. Protection of dogs against canine heartworm infection 28 days after four monthly treatments with Advantage Multi® for Dogs. Parasites Vectors 9. https://doi.org/10.1186/S13071-016-1293-Z.

Bowman, D.D., Montgomery, S.P., Zajac, A.M., Eberhard, M.L., Kazacos, K.R., 2010. Hookworms of dogs and cats as agents of cutaneous larva migrans. Trends Parasitol. 26, 162–167. https://doi.org/10.1016/J.PT.2010.01.005.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., Von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruysse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 136, 167–185. https://doi.org/10.1016/J.VETPAR.2005.11.019.

Cringoli, G., Maurelli, M.P., Levecke, B., Bosco, A., Vercruysse, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. Nat. Protoc. 12, 1723–1732. https://doi.org/10.1038/NPROT.2017.067.

Drake, J., Carey, T., 2019. Seasonality and changing prevalence of common canine gastrointestinal nematodes in the USA. Parasites Vectors 12, 1–7. https://doi.org/10.1186/S13071-019-3701-7/FIGURES/6.

Epe, C., 2009. Intestinal nematodes: biology and control. Vet Clin North Am Small Anim Pract 39, 1091–1107. https://doi.org/10.1016/J.CVSM.2009.07.002.

Fleischer, S., Sharkey, M., Mealey, K., Ostrander, E.A., Martinez, M., 2008. Pharmacogenetic and metabolic differences between dog breeds: their impact on canine medicine and the use of the dog as a preclinical animal model. AAPS J. 10, 110. https://doi.org/10.1208/S12248-008-9011-1.

Gasser, R.B., Stewart, L.E., Speare, R., 1996. Genetic markers in ribosomal DNA for hookworm identification. Acta Trop. 62, 15–21. https://doi.org/10.1016/S0001–706X(96)00015-0.

Hess, L.B., Millward, L.M., Rudinsky, A., Vincent, E., Marsh, A., 2019. Combination anthelmintic treatment for persistent *Ancylostoma caninum* ova shedding in greyhounds. J. Am. Anim. Hosp. Assoc. 55, 160–166. https://doi.org/10.5326/JAAHA-MS-6904.

Jimenez Castro, P.D., Durrence, K., Durrence, S., Sicalo Giannechini, L., Collins, J., Dunn, K., Kaplan, R.M., 2022. Multiple anthelmintic drug resistance in hookworms (*Ancylostoma caninum*) in a Labrador breeding and training kennel in Georgia, USA. J. Am. Vet. Med. Assoc. 261, 342–347. https://doi.org/10.2460/JAVMA.22.08.0377.

Jimenez Castro, P.D., Howell, S.B., Schaefer, J.J., Avramenko, R.W., Gilleard, J.S., Kaplan, R.M., 2019. Multiple drug resistance in the canine hookworm *Ancylostoma caninum*: an emerging threat? Parasites Vectors 12. https://doi.org/10.1186/S13071-019-3828-6.

Jimenez Castro, P.D., Kaplan, R.M., 2020. Persistent or suspected-resistant hookworm infections. Clinician's Brief 61–68.

Jimenez Castro, P.D., Mansour, A., Charles, S., Hostetler, J., Settje, T., Kulke, D., Kaplan, R.M., 2020. Efficacy evaluation of anthelmintic products against an infection with the canine hookworm (*Ancylostoma caninum*) isolate Worthy 4.1F3P in dogs. Int. J. Parasitol. Drugs Drug. Resist. 13, 22–27. https://doi.org/10.1016/J.IJPDDR.2020.04.003.

Jimenez Castro, P.D., Venkatesan, A., Redman, E., Chen, R., Malatesta, A., Huff, H., Zuluaga Salazar, D.A., Avramenko, R., Gilleard, J.S., Kaplan, R.M., 2021. Multiple drug resistance in hookworms infecting greyhound dogs in the USA. Int. J. Parasitol. Drugs Drug. Resist. 17, 107–117. https://doi.org/10.1016/j.ijpddr.2021.08.005.

Kalkofen, U.P., 1987. Hookworms of dogs and cats. Vet. Clin. North Am. Small Anim. Pract. 17, 1341–1354. https://doi.org/10.1016/S0195-5616(87)50005-5.

Kaplan, R.M., 2020. Biology, epidemiology, diagnosis, and management of anthelmintic resistance in gastrointestinal nematodes of livestock. Vet. Clin. North Am. Food Anim. Pract. 36, 17–30. https://doi.org/10.1016/J.CVFA.2019.12.001.

Kitchen, S., Ratnappan, R., Han, S., Leasure, C., Grill, E., Iqbal, Z., Granger, O., O'Halloran, D.M., Hawdon, J.M., 2019. Isolation and characterization of a naturally occurring multidrug-resistant strain of the canine hookworm, *Ancylostoma caninum*. Int. J. Parasitol. 49, 397–406. https://doi.org/10.1016/J.IJPARA.2018.12.004.

Kopp, S.R., Coleman, G.T., McCarthy, J.S., Kotze, A.C., 2008. Application of in vitro anthelmintic sensitivity assays to canine parasitology: detecting resistance to pyrantel in *Ancylostoma caninum*. Vet. Parasitol. 152, 284–293. https://doi.org/10.1016/J.VETPAR.2007.12.020.

Kotze, A.C., Kopp, S.R., 2008. The potential impact of density dependent fecundity on the use of the faecal egg count reduction test for detecting drug resistance in human hookworms. PLoS Neglected Trop. Dis. 2, e297. https://doi.org/10.1371/JOURNAL.PNTD.0000297.

Masters of Foxhounds Association, 2015. Foxhound Kennel Notebook. Milwood, VA.

Maurelli, M.P., Rinaldi, L., Alfano, S., Pepe, P., Coles, G.C., Cringoli, G., 2014. Mini-FLOTAC, a new tool for copromicroscopic diagnosis of common intestinal nematodes in dogs. Parasites Vectors 7, 1–5. https://doi.org/10.1186/1756-3305-7-356/TABLES/4.

Morgan, E.R., Lanusse, C., Rinaldi, L., Charlier, J., Vercruysse, J., 2022. Confounding factors affecting faecal egg count reduction as a measure of anthelmintic efficacy. Parasite 29, 20. https://doi.org/10.1051/PARASITE/2022017.

Nielsen, M.K., 2022. Anthelmintic resistance in equine nematodes: current status and emerging trends. Int. J. Parasitol. Drugs Drug. Resist. 20, 76–88. https://doi.org/10.1016/J.IJPDDR.2022.10.005.

Ridley, R., Dryden, M., Gabbert, N.H., Schoning, P., 1994. Epidemiology and control of helminth parasites in Greyhound breeding farms. Compend. Continuing Educ. Pract. Vet. 16, 585–596.

Sobotyk, C., Upton, K.E., Lejeune, M., Nolan, T.J., Marsh, A.E., Herrin, B.H., Borst, M.M., Piccione, J., Zajac, A.M., Camp, L.E., Pulaski, C.N., Starkey, L.A., von Simson, C., Verocai, G.G., 2021. Retrospective study of canine endoparasites diagnosed by fecal flotation methods analyzed across veterinary parasitology diagnostic laboratories,

United States, 2018. Parasites Vectors 14, 1–10. https://doi.org/10.1186/S13071-021-04960-7/TABLES/6.

Sweet, S., Hegarty, E., McCrann, D.J., Coyne, M., Kincaid, D., Szlosek, D., 2021. A 3-year retrospective analysis of canine intestinal parasites: fecal testing positivity by age, U. S. geographical region and reason for veterinary visit. Parasites Vectors 14. https://doi.org/10.1186/S13071-021-04678-6.

von Samson-Himmelstjerna, G., Thompson, R.A., Krücken, J., Grant, W., Bowman, D.D., Schnyder, M., Deplazes, P., 2021. Spread of anthelmintic resistance in intestinal helminths of dogs and cats is currently less pronounced than in ruminants and horses – yet it is of major concern. Int. J. Parasitol. Drugs Drug. Resist. 17, 36–45. https://doi.org/10.1016/J.IJPDDR.2021.07.003.

Wang, C., Torgerson, P.R., Kaplan, R.M., George, M.M., Furrer, R., 2018. Modelling anthelmintic resistance by extending eggCounts package to allow individual efficacy. Int. J. Parasitol. Drugs Drug. Resist. 8, 386–393. https://doi.org/10.1016/J.IJPDDR.2018.07.003.