Anthelmintic Resistance in Companion Animal Parasites

Diagnostic and Treatment Challenges

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KEYWORDS

• Anthelmintic resistance • Dogs • Ancylostoma caninum • Dirofilaria immitis
• Dipylidium caninum • Benzimidazoles • Macrocyclic lactones • Praziquantel

KEY POINTS

- Companion animal parasite control depends on only a few drug classes, with efficacy increasingly compromised by resistant populations emerging in *Ancylostoma caninum*, *Dirofilaria immitis* and *Dipylidium caninum*.
- Clinical diagnostic tests for resistance include fecal egg count reduction test and microfilarial suppression test; molecular tests only exist for a few parasite–drug combinations.
- Resistance-associated genes may accumulate in parasite populations long before clinical detection.
- Treatment options are limited, with current strategies relying on extralabel drug use and combination treatments.
- The spread of resistance is facilitated by limited diagnostic tools, absence of real time surveillance, pet movement and human behaviors.

INTRODUCTION

Helminth infections are a clinically significant problem in companion animals. Dogs and cats are definitive hosts for a variety of nematodes (roundworms), cestodes (tapeworms) and trematodes (flukes), with clinical consequences of infections ranging from asymptomatic or subclinical infections to severe disease.[1] Unlike food animal production where some parasite burden is tolerated, in companion animals, all parasite infections are treated. Anthelmintics are the major tool used to effectively manage helminths in dogs and cats for decades.[2]

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Abbreviations	
AAVP	American Association of Veterinary Parasitologists
AChR	acetylcholine receptor
FECRT	fecal egg count reduction test
LOE	loss of efficacy
MFST	microfilarial suppression test
ML	macrocyclic lactone
SNP	single nucleotide polymorphism

The major goals of anthelmintic usage in companion animals are as follows:

i. To eliminate adult stages of parasites, to reduce the morbidity caused by pathogenic helminths and prevent mortality,
ii. To achieve clinical cure in parasite-infected animals without damaging the host (principle of selective toxicity),
iii. In the case of *Dirofilaria immitis*, to prevent heartworm disease by targeting larval stages before they mature and reach their predilection sites in the pulmonary arteries,[3,4]
iv. To minimize environmental contamination by parasite eggs and larvae,
v. To prevent zoonotic infections and risk to humans, and
vi. To mitigate the psychological discomfort of human caregivers.

The spectrum of molecular targets for anthelmintic drugs is relatively narrow when compared to the number of targets exploited by antibiotics. Few new anthelmintics have been recently introduced to the market. Veterinary reliance on the repeated use of a small set of drug classes has made routine parasite control increasingly vulnerable to failure with the emergence of anthelmintic resistance. Drug resistant parasites have been reported in dogs but not yet in cats, which reflects the greater veterinary care that dogs receive. Resistance in companion animal parasites globally has been reviewed by other authors.[5]

In this review, we will discuss the anthelmintic drug classes used in dogs and cats in the United States and define anthelmintic resistance in this context. We will examine the manifestations of resistance in important canine helminths, focusing the discussion on history, epidemiology, diagnostic questions, and treatment challenges. Finally, we will explore the genetic and molecular basis of resistance and explore the drivers of anthelmintic resistance emergence and spread.

ANTHELMINTIC DRUGS CLASSES IN COMPANION ANIMALS

Anthelmintic drugs which are U.S. Food and Drug Administration (FDA)-approved for companion animal use belong to approximately 5 drug classes—the benzimidazoles, tetrahydropyrimidines, macrocyclic lactones (MLs), cyclodepsipeptides and isoquinolone-pyrazines, each with distinct spectra of activity (**Table 1**), mechanisms of action and resistance.

Benzimidazoles

Benzimidazoles were first introduced in the 1960s. Only fenbendazole and febantel are present in FDA-approved products for use in dogs,[6,7] and febantel is in products approved for cats.[8] Benzimidazoles bind to the parasite's β-tubulin isotype-1 protein, selectively inhibiting the polymerization of cytoplasmic microtubules and impairing cell division.[9–11] Benzimidazole resistance has been observed in nematode populations

Table 1
Anthelmintic drug classes and drugs used in companion animals, their labeled activity and parasites with emerging resistance

Drug Class	Drug	Spectrum of Activity	Parasites with Emerging Resistance
Benzimidazoles	Fenbendazole, Febantel	Ascarids (*Toxocara canis, T. cati, Toxascaris leonina*), hookworms (*A. caninum, Uncinaria stenocephala*), whipworm (*Trichuris vulpis*) and tapeworm (*Taenia pisiformis*)	*A. caninum*
Tetrahydropyrimidines	Pyrantel pamoate	Ascarids (*Toxocara canis, T. cati, T. leonina*) and hookworms (*A. caninum, U stenocephala, A. tubaeforme*).	*A. caninum*
MLs	Ivermectin, Eprinomectin, Selamectin, Moxidectin, Milbemycin oxime	*D. immitis* larvae Ascarids (*Toxocara canis, T. cati, T. leonina*), hookworms (*A. caninum, U. stenocephala*), whipworm (*T. vulpis*), fleas (*Ctenocephalides felis*), mites (*Otodectes cyanotis*) and several ticks	*A. caninum, D. immitis*
Cyclodepsipeptides	Emodepside	Ascarid (*T. cati*) and hookworms (*A. tubaeforme*)	None
Isoquinolone-pyrazines	Praziquantel, Epsiprantel	Tapeworms *(Dipylidium caninum, T. pisiformis, T. taeniformis, Echinococcus granulosus* and *E. multilocularis).*	*D. caninum*

associated with mutations in the β-tubulin isotype-1 gene,[12] specifically single nucleotide polymorphisms (SNPs) at codons 134,[13] 167,[14] 198[15] and 200[16] (**Box 1**).

Tetrahydropyrimidines

Pyrantel, a tetrahydropyrimidine, was introduced in the 1960s.[17] FDA-approved products for dogs and cats contain pyrantel pamoate (embonate). Pyrantel acts as a potent agonist at nematode nicotinic acetylcholine receptors (AChRs) and causes spastic paralysis, resulting in helminth expulsion from the body following normal host intestinal peristalsis.[18–21] Evidence suggests that pyrantel resistance may involve (i) the expression of multiple AChR subunits with differing susceptibilities,[22] (ii) reduced AChR

Box 1
Single nucleotide polymorphisms

An SNP is a change of a single nucleotide (A, C, G, or T) in the DNA sequence. Conventionally, single nucleotide changes at a specific location in a gene must be present in at least 1% of the population to be considered an SNP. In anthelmintic resistance literature, this term is applied more broadly. Point mutations at resistance loci are routinely referred to as SNPs, reflecting their established biological significance rather than a strict adherence to population frequency criteria.

How SNPs arise: SNPs in parasites are thought to arise due to DNA replication errors. During cell division, DNA polymerase may very rarely incorporate a mismatched nucleotide, and if the mismatch is not repaired by proof reading mechanisms, the nucleotide change is passed to daughter cells. Other mechanisms such as nucleotide changes due to deamination, CpG mutation hotspots and exogenous mutagens, which are well-studied causes of SNPs in vertebrates, are not thought to apply to helminth parasites. Anthelmintics do not induce SNPs, rather they kill susceptible parasites, leaving only those harboring resistance-associated SNPs to survive.

subunit transcription in resistant isolates,[23] or (iii) SNPs in both nicotinic AChR subunits and modulatory proteins.[21,24]

Macrocyclic Lactones

MLs, a class of potent endectocides, were introduced in the 1980s.[25] FDA-approved products containing ivermectin, selamectin, milbemycin oxime, and moxidectin are available for use in dogs, while products containing eprinomectin, milbemycin oxime selamectin and moxidectin are approved for used in cats. The activity of ML-containing products is pharmacokinetics-dependent and varies by host and parasite species. MLs act on nematode glutamate-gated chloride channels (GluCl) causing irreversible hyperpolarization and spastic paralysis which prevents locomotion.[26–28] They act at pharyngeal GluCls, resulting in worm starvation and death.[28,29] MLs also inhibit protein release by nematodes,[30] leading to increased susceptibility of parasites to the host immune system.[31] Resistance to the MLs is polygenic[32] and parasite species–dependent. In some nematodes, SNPs associated with resistant phenotypes have been conclusively demonstrated.[33,34] ML interaction with P-glycoproteins in several nematodes has been documented *in vitro*,[35–37] but its exact role in clinical resistance has not been fully elucidated.

Cyclodepsipeptide

Emodepside, the most recently marketed anthelmintic drug class, was introduced in 2005.[38] FDA-approved products containing emodepside are available only for cats in the the United States. Emodepside acts at nematode latrophilin receptors[39] and at calcium-activated, voltage-sensitive potassium channels (Slo-1K), resulting in hyperpolarization and paralysis.[40,41] There have been no reports of resistance to emodepside in nematodes.

Isoquinolone-pyrazine

Two isoquinolone-pyrazine drugs are effective against companion animal cestodes: praziquantel, introduced in 1975[42] and epsiprantel, introduced in 1989.[43] FDA-approved products containing praziquantel or epsiprantel are available for both dogs and cats. Praziquantel can cause rapid and sustained influx of calcium ions into parasite cells, resulting in spastic paralysis and tegumental damage. More recently, praziquantel has been shown to bind transient receptor potential channels.[44]

Resistance mechanisms in cestodes and trematodes are being actively studied, but no monogenic determinant has been identified.

Other Drugs

Other drugs that were available for companion animal use previously but are no longer available in the Canada and United States include nitroscanate and diethylcarbamazine. A narrow spectrum compound, piperazine, is also available.

DEFINITION OF ANTHELMINTIC RESISTANCE

Anthelmintic resistance is a genetically heritable trait that enables a population of a given parasitic species to survive exposure to a drug dosage that would previously have been able to eliminate a majority (>95%) of the individuals in the population. Major features of drug resistance in helminths are as follows:

i. Resistance-associated genetic variations are vertically transmitted to the offspring of surviving helminths. Unlike bacteria, which can horizontally transfer genes coding for novel resistance proteins (such as beta-lactamase genes on plasmids), anthelmintic resistance operates through modifications (such as SNPs and overexpression) to pre-existing genes and proteins.
ii. When a drug is used, a selective pressure is applied, and only resistant parasite individuals survive.[45]
iii. Resistance is difficult to reverse and develops only against the drug class to which the parasites are exposed.[46]
iv. Side-resistance is typical, that is, resistance to one compound confers resistance to other members of the same drug class. In parasitology, this is often referred to as *cross-resistance*, although this term technically refers to resistance that arises from the same genetic change but affecting multiple drug classes. True cross-resistance is not known to be common in parasitic helminths.
v. Resistance is observed at the population level, with resistant allele frequencies shifting from rare occurrences to widespread in a population following selection.
vi. Resistance-associated genetic variations may be present and accumulate in parasite populations long before detection using diagnostic tests. This results in resistance appearing to emerge abruptly at a high prevalence despite having been increasing over time.

CLINICAL ANTHELMINTIC RESISTANCE IN COMPANION ANIMAL HELMINTHS

Multiple Anthelmintic Drug Resistance in Ancylostoma caninum

Of the intestinal helminths, *A. caninum*, has attracted the greatest attention due to its high prevalence (2.79% in 2025[47]) in dogs and capacity to cause clinical signs in all age groups. Multiple anthelmintic drug resistance (MADR) in this species is now a well-documented veterinary emergency and public health concern in affected regions.

History and epidemiology of hookworm drug resistance

The earliest reports of pyrantel resistance in *A. caninum* emerged from Australia in 1987.[48] High-level resistance against pyrantel was confirmed in controlled trials in 2007.[49] High frequencies of resistance-associated β-tubulin SNPs were reported recently.[50] In the United States, MADR was reported in 2019, including 1 Florida isolate resistant to fenbendazole and ivermectin,[51] and 3 isolates from Florida/Georgia resistant to pyrantel, fenbendazole, and moxidectin.[52] Oral emodepside (European Medicines Agency [EMA] formulation) was effective in these isolates. *In vitro* assays

and post-treatment egg counts on 219 *A. caninum* samples from greyhounds exhibited MADR to benzimidazoles, pyrantel, and MLs.[53]

Next generation sequencing studies have revealed benzimidazole resistant *A. caninum* populations in the United States. Despite considerable genetic diversity in *A. caninum* populations when barcoding genes are analyzed,[54,55] benzimidazole selection has enriched β-tubulin SNPs in the population. Early MADR samples showed only SNPs at codon 167 (TTC > TAC, F167Y; phenylalanine to tyrosine change).[52] In hookworms from greyhounds, this SNP was detected in 99% of samples in some kennels.[53] A broader study of 685 samples identified SNPs at codon 167 (54% frequency) and a novel SNP at codon 134 (CAA > CAT, Q134H; glutamine to histidine change) (16.4%).[13] While these studies have revealed the widespread distribution of known β-tubulin SNPs, many lack the clinical or phenotypic data associated with each sample.

The epidemiologic context in which MADR *A. caninum* emerged in the United States is of explanatory importance. It is suspected that resistance arose in racing greyhound kennels.[56] Greyhounds were often housed together on sand or grass paddocks, enabling hookworm larval accumulation and continuous, high-intensity exposure and reinfection. They were frequently and routinely dewormed, often with the same drug classes. Over time, the treatments progressively lost efficacy, selecting for *A. caninum* populations which survived and reproduced, allowing a gradual shift toward resistance across multiple drug classes.[57]

Challenges for the diagnosis of drug resistant A. caninum

The current recommendation from the American Association of Veterinary Parasitologists (AAVP) for diagnosing drug resistant *A. caninum* against any drug class, is to perform a fecal egg count reduction test (FECRT).[58] If the treatment is effective, the expected reduction should be greater than 95%. If the reduction is less than 75%, resistance should be strongly suspected.

Considerations for fecal egg count reduction test interpretation

i. The timing of the FECRT is critical.[53] The first fecal sample must be collected immediately before treatment, and the second at 10 to 14 days after treatment. Retesting before 10 days post-treatment may provide inaccurate reduction percentages due to temporary inhibition of egg production, while testing after 21 days should be avoided because of the risk of internal autoinfection (known as larval leak) or external reinfection from contaminated environments.
ii. FECRT requires paired fecal samples and cannot be performed on a single sample. A single quantitative value has no diagnostic interpretation.
iii. The quantitative test chosen must be sensitive enough to detect subtle variations in egg counts. Mini-FLOTAC or the modified McMaster tests are recommended for accuracy and precision.[59]

Molecular tests. An allele-specific quantitative polymerase chain reaction (qPCR) targeting the SNP (F167Y) in the β-tubulin isotype-1 gene for detecting benzimidazole resistance is commercially available. Retrospective analyses using a commercial qPCR panel (KeyScreen, Antech), demonstrated the benzimidazole resistance-associated F167Y SNP in 10.6% of 511 samples from 2020 to 2021[60] and 14.44% of *A. caninum* infected dogs from 2022 to 2023.[61] However, this sensitive approach merely detects the presence of even low numbers of SNPs within a population of parasites; it does not necessarily mean that the entire population of parasites is phenotypically drug resistant. Currently, there are no molecular tests available for MLs and pyrantel resistance.

Status of the current treatment recommendations and challenges for multiple anthelmintic drug resistance A. caninum

The current recommendation from the AAVP for the treatment of drug resistant *A. caninum* is triple therapy.[58] Triple therapy refers to the use of the following FDA-approved drugs at labelled doses within a 24-hour period: (i) oral product containing febantel, praziquantel, and pyrantel and (ii) topical moxidectin. When administered repeatedly over 3 to 4 months, this combination stops *A. caninum* egg shedding.[62]

Some studies have used feline emodepside in an extralabel manner,[57,63] under the provisions of Animal Medicinal Drug Use Clarification Act of 1994 (AMDUCA). However, oral use of the topical feline emodepside product in dogs is not bioequivalent to approved oral formulations available in other jurisdictions and produces a 3x higher maximum concentration (Cmax) with prolonged retention in the body.[64] Therefore, caution is recommended in its use.

Additional precautions for extralabel use of emodepside are as follows:

i. Confirm that *A. caninum* is MADR using properly timed FECRT. If the infection is not MADR, the triple combination is recommended.
ii. Ensure that the dog is MDR1 (*ABCB1*) homozygous normal since emodepside can cause neurotoxicity in homozygous mutant (*ABCB1* nt230 (del4)) dogs.[65]
iii. Verify that the dog tests negative for both heartworm antigen and microfilaria since emodepside is an adulticide *in vitro*[66] and has microfilaricidal activity. Tests with the highest sensitivity for *D. immitis*—a heat-treatment antigen test and a modified Knott's test for microfilarial testing are recommend before emodepside treatment.
iv. Avoid administering emodepside with food, as this increases absorption.[67]
v. Monitor dogs for adverse effects for at least 12 hours after emodepside treatment and provide supportive care if needed.

Priority research needs include improved diagnostics, novel drug candidates, improving the understanding of MADR hookworm biology and non-chemotherapeutic options for hookworm control.[68]

Macrocyclic Lactone Resistance in D. immitis

To understand resistance in *D. immitis*, it is important to understand how ML-containing heartworm preventatives work. FDA-labeled products containing ivermectin, moxidectin, milbemycin oxime, selamectin, or eprinomectin at different doses and administration routes are designed to prevent the maturation of larval stages to adult worms, thereby preventing heartworm disease.[69] A single dose of ML will eliminate third (L3) and early fourth (L4) larval stages acquired over the previous ~30 days[70] and for the labeled duration after administration (30 days, 6 months, or 12 months depending on the product). However, late L4 larvae (60–120 days old) are tolerant of the MLs, a phenomenon known as the susceptibility gap.[69] Microfilariae are generally eliminated slowly after repeated ML use over a period of time, with the exception of high-dose topical moxidectin, which has a microfilaricidal label. Therefore, if larvae survive beyond 30 days of age either due to noncompliant product use, or resistance, they can mature to adult *D. immitis*, leading to heartworm disease.[71]

History and epidemiology of heartworm macrocyclic lactone resistance

Failure of the MLs to kill *D. immitis* larvae in dogs resulting in heartworm disease was first reported in pharmacovigilance submissions to the US FDA Center for Veterinary Medicine in 1998,[72] followed by a drastic rise in such reports between 2000 and 2002, from regions where infections are enzootic—the Mississippi and Ohio River valleys, and other southern states. Initially, these reports were attributed to owner

noncompliance. The term *loss of efficacy* (LOE) was coined to describe cases where dogs received preventatives in accordance with the FDA product label without lapses in treatment compliance, and yet tested positive later.[72] In a retrospective study of medical records (2004–2011), only 1.7% of LOE cases were attributable to true biological resistance,[73] with experimental confirmation provided from 2 cases in 2014.[74]

Over the past decade, data describing the genetic makeup of ML-resistant *D. immitis* have improved. Early work linked an SNP (GG-GG genotype) in a P-glycoprotein (*Dim-Pgp-11*) transporter to ivermectin resistance.[75–77] Later, by comparing the genomes of resistant and susceptible isolates, 2 SNP markers with the highest correlation with clinical resistance were selected[34,78] and adapted to a droplet digital PCR method.[79] This was used to analyze field isolates from Missouri,[80] where a 95% prevalence of resistant SNP markers was detected, and other states east of the Rockies, where 36% of samples had susceptible markers, 31% resistant, and 33% mixed.[81] Despite the high prevalence of SNPs, clinical ML failure in the dogs harboring SNP-containing *D. immitis* microfilariae remains unclear. One proposed contribution is the slow-kill method for heartworm, which is hypothesized to select for ML resistance.[82] This method uses MLs at preventative monthly low doses leading to adult worm death over a long period and is economically attractive due to the cost of melarsomine. However, definitively demonstrating this is experimentally challenging.

Challenges for the diagnosis of drug-resistant D. immitis

A major challenge in diagnosing drug-resistant *D. immitis* is that ML-resistant stages (L3 and early L4) are inaccessible while in the subcutaneous tissue of the dog. Therefore, diagnosis typically relies on microfilariae present in blood as the surrogate stage. *In vitro* tests based on microfilarial motility, third-stage larval motility, or biochemical features are not yet able to differentiate resistant and susceptible isolates.[83–85] Molecular tests are not currently available to veterinarians.

The only clinical test for ML resistance is the microfilariae suppression test (MFST).[34,86] Similar to the FECRT, it involves quantifying microfilariae per mL of blood before and 7 days after treatment, to calculate the percent reduction in microfilarial numbers. Susceptible isolates should have a rapid reduction in microfilarial counts, while a reduction of less than 75% indicates resistance.[86]

Considerations for the microfilariae suppression test interpretation

i. Timing the MFST is important. Collect 5 to 10 mL of blood and perform a modified Knott's test to quantify microfilariae per mL of blood. Then repeat the quantification 7 days after administering high-dose ML treatment.
ii. A microfilaricidal dose of MLs must be administered. Options include moxidectin at 2.5 mg/kg topically, ivermectin at 50 μg/kg or milbemycin oxime at 1 mg/kg.
iii. The patient must be monitored for adverse events such as anaphylaxis or shock from rapid microfilarial death.
iv. The modified Knott's test is a sensitive concentration technique and is ideal for quantification. Other methods such as antigen tests or PCR will not provide quantitative data.
v. Recent preventative use may mask or exaggerate MFST values. Therefore, drug history must be carefully reviewed.

Status of the current treatment recommendations and challenges for macrocyclic lactone–resistant D. immitis

Many FDA-approved products rely on low doses of MLs to eliminate larval stages of *D. immitis*.[87] These low doses were selected for safety in *ABCB1* homozygous mutant dogs, which are sensitive to the normal therapeutic dose of ivermectin. In the face of

resistance, high doses of MLs, especially moxidectin (>3 μg/kg), retain efficacy against ML-resistant *D. immitis* lab isolates,[88,89] and are now encouraged over other MLs.[82]

Parasite refugia, which are subpopulations of *D. immitis* not exposed to MLs can slow the spread of resistance, but withholding heartworm preventatives in dogs is unethical due to the serious nature of heartworm disease. Retrospective analyses show variable preventative use in US dogs (39.5%[90] to 79%[91]), and on average dogs received 7.3 monthly doses annually.[92] Parasites harbored by dogs not exposed to drugs form a large refugia population and include larval stages in mosquitoes, adult worms and susceptible larvae in untreated dogs.[93] The current recommendation is to ensure rigid adherence to ML prophylaxis. A decreased reliance on the slow kill method is hypothesized to decrease selection for resistance.

To limit local transmission in endemic areas, mosquito repellents and insecticides are recommended.[82] Dogs should be tested for heartworm infection before international and regional travel to reduce the spread of ML-resistant *D. immitis*.[94] Current research needs include the need for model hosts, phenotypic assays and mechanisms of resistance.[95]

Praziquantel Resistance in Dipylidium caninum

D. caninum is the most common cestode of companion animals in the United States.[96] Increasing evidence suggests that isolates from dogs and cats are biologically and genetically distinct, and likely represent two separate species.[97–99] Praziquantel and epsiprantel are the only FDA-approved drugs for companion animal use in the United States. However, praziquantel-resistant isolates of canine *D. caninum* have emerged over the past decade.

History and epidemiology of praziquantel resistance

Praziquantel resistance in *D. caninum* was first documented in 2016 in cases involving Pomeranians and a Jack Russell terrier.[100] Additional cases have been reported from a Terrier mix,[101] and from Europe in a Can de Chira dog.[102] In recent years, the problem has increased, particularly in the mid-western, southern, and eastern United States (unpublished data). Resistance appears mainly in dogs with significantly fewer reports in cats. Preliminary whole genome sequencing data suggest that resistance is polygenic (unpublished data).

Challenges for the diagnosis of drug-resistant D. caninum

A major diagnostic challenge with *D. caninum* is that routine fecal exams may miss infections if limited to only floatation-based tests because of the density of the egg packets and the lack of a uterine pore.[103] Copro-antigen tests offer improved sensitivity over floatation methods.[104] Since *D. caninum* is a flea-borne pathogen, reinfection is likely to occur unless fleas are controlled in the host's environment. Thus, it is challenging to differentiate reinfection from true resistance.

Considerations to establish a diagnosis of praziquantel-resistant *D. caninum*

i. Resistant *D. caninum* may be clinically suspected if proglottid shedding persists within 7 to 10 days after repeated praziquantel and/or epsiprantel treatment despite effective flea control.
ii. The animal must receive effective flea control, typically an isoxazoline product, and flea burdens in the environment must be eliminated.
iii. Persistently shed proglottids should be confirmed as *D. caninum* by egg and proglottid morphology, or PCR.

Even then, diagnosis of resistance remains presumptive as no validated genetic tests for *D. caninum* resistance are currently available.

Status of the current treatment recommendations and challenges for drug-resistant D. caninum

At registration, praziquantel and epsiprantel at labeled doses were 100% effective at eliminating *D. caninum*.[105–107] Since both belong to the same drug class, resistance to one is often associated with resistance to the other.[100,102] Extralabel options are available under AMDUCA. These include use of high and/or repeated doses of praziquantel (up to 25 mg/kg), compounded combination of oxantel–pyrantel-praziquantel, nitroscanate, nitazoxanide or mebendazole.[100–102]

Preventing reinfections requires effective flea control using adulticides and environmental measures. Isoxazolines (such as afoxalaner, fluralaner, lotilaner, and sarolaner) have quick initial and residual speeds of kill, rapidly killing fleas before egg-laying can commence.[108] Some neonicotinoid and insect growth regulator products are also effective. Elimination of both fleas on animals and in the environment are required.

While canine and public health priorities dictate relatively little change in the use of anthelmintics with *A. caninum* and *D. immitis*, cestodes may offer a test case in application of selective therapy. While the use of monthly praziquantel is becoming more common due to the availability of combination products, it is less clear if routine monthly cestode treatments are necessary. The argument for routine treatment is the impact of *Echinococcus* spp. However, it would be preferable to emphasize more diagnostic efforts to clients than to simply pursue treatment because praziquantel does not have preventative effects on cestode infections.

GENETIC AND MOLECULAR BASIS OF ANTHELMINTIC RESISTANCE

We now turn to the underlying genetic and molecular mechanisms that enable parasites to survive drug exposure. Anthelmintic resistance mechanisms vary by drug class and can be broadly categorized as target site changes, altered gene expression, and altered drug transport.[24]

i. *Target site changes:* This is the best characterized mechanism, exemplified by benzimidazole resistance. SNPs in the β-tubulin isotype-1 gene at codons 134, 167, 198, or 200 cause amino acid substitutions in the encoded β-tubulin protein,[13,46] which results in reduced drug binding, allowing microtubule polymerization to proceed in the presence of drug concentrations that would be otherwise inhibitory. This mechanism is robustly validated in livestock nematodes and appears to underlie benzimidazole resistance in *A. caninum*.[13]
ii. *Altered target receptor transcription:* Many of the ion channels targeted by anthelmintics are multimeric proteins with subunits that can polymerize in different stoichiometries.
iii. *Changes in transcription of receptor subunits* can contribute to resistance by altering the composition of dominant homo or heteromeric receptors.[24] Two Australian pyrantel resistant *A. caninum* isolates had decreased transcription of AChR subunits compared to susceptible isolates.[23]
iv. *Non–drug-specific mechanisms:* Degradation of anthelmintics by enzymes such as cytochrome P450 or drug efflux by adenosine triphosphate (ATP)-binding cassette transporters (ABCB1/P-glycoproteins) are implicated in ML resistance.[24] For example, ABCB1 inhibitors are known to enhance the effects of some anthelmintics such as MLs.[109] Measuring the contribution of nonspecific resistance

mechanisms in a population is more challenging because it relies on measuring activity rather than simply detecting a gene polymorphism.

Despite progress in identifying genes and potential mechanisms associated with anthelmintic resistance, these mechanisms vary considerably among parasite species, drug classes and selection environments, making it difficult to generalize a single model of resistance across helminths. Moreover, although some SNPs are known, that does not preclude the development of new mutations which are not detected by current diagnostic tests.

DISCUSSION

Understanding the drivers of resistance is essential for veterinarians aiming to mitigate its effect and delay its spread. The complexities of the interactions between parasite genetics, drug use, host management and the environment make anthelmintic resistance a challenge to characterize. However, basic principles of biology dictate that drug resistant phenotypes arise from genetically heritable traits. The use of anthelmintic drugs is not known to directly induce resistant phenotypes, rather, anthelmintics select for parasites with certain DNA mutations that favor survival.

A useful framework for discussing selection is genetic sweep dynamics, which describes how resistance-encoding alleles change in frequency. Gene mutations are the result of recombination and random genetic changes. Mutations in helminths may randomly arise following the onset of drug selection and rapidly become dominant, which is known as a *hard sweep*.[110] Alternatively, a pool of pre-existing mutants could exist before drug selection and be gradually selected over time, which is known as a *soft sweep*. Thus far in helminths, evidence suggests soft sweeps appear to be the more common scenario, while hard sweeps arise under certain local conditions.[111] Regardless of the genetic sweep vigor, the use of anthelmintic drugs is not known to directly induce mutations in parasite DNA. Thus, curtailing resistance relies on an understanding of selection pressure.

Selection for Resistance

The principal drivers impacting selection can be grouped as follows:

i. *Frequent treatments, improper dosing:* The single most powerful resistance driver is unnecessary or more frequent treatments than warranted. Each treatment is a selection event in which susceptible individuals are eliminated and resistant survivors are enriched in the post-treatment populations. Underdosing encourages accumulation of mutant alleles that favor survival in the presence of anthelmintics. The cumulative effect of repeated selection over successive generations increases resistance allele frequency until clinical failure occurs.
ii. *Parasite population size/dynamics:* Compared to livestock, anthelmintic resistance in companion animals has been slower to develop and spread. In livestock, the sheer number of individual worms is expected to generate a greater number of mutant alleles. Furthermore, parasite egg outputs from pets are ~60,000 times lower,[111] resulting in smaller local population sizes. Nevertheless, population size constraints can be overcome by high environmental contamination, frequent reinfections, high housing density and frequent anthelmintic treatment as seen in *A. caninum.*
iii. *Role of refugia:* The population of parasites not exposed to drugs in untreated hosts or in the environment can slow resistance development by diluting resistant

alleles. Balancing the use of anthelmintics for canine health needs with a desire to delay resistance is not a straightforward matter.

After selection has occurred and clinical resistance has emerged, there is little recourse to eliminate mutant alleles from a population. An important factor in the geographic spread of anthelmintic resistance is the transportation of infected animals by humans. The clinical impact of resistance depends on how efficiently it is diagnosed and controlled.

Barriers to Addressing Anthelmintic Resistance in Canine parasites

Despite our understanding of drug selection, there are a number of limitations that continue to drive the clinical impact of anthelmintic resistance including:

i. *Limited diagnostic tools for clinical use:* Few tests are available to confirm resistance. Those that exist are often labor-intensive, slow and require multiple sample collections. They do not require special equipment and could be done by practitioners. However, tests such as FECRT lack enough sensitivity to detect early-stage resistance when resistant parasites are few.[111] There is a significant lag between the initiation of selection and clinical detection. Molecular markers are still under development for several parasite-drug combinations and only a few are currently available. Even when they are available, PCR and sequencing approaches typically only identify the presence of resistant alleles, but not the proportion of individuals carrying those SNPs in the population
ii. *Limited real-time resistance surveillance:* There are no consolidated databases or testing performed for resistance surveillance in companion animal parasites.
iii. *Lack of universally agreed-on resistance thresholds:* Thresholds of resistance definitions are different for different parasites. For example, greater than 95% efficacy is expected for *A. caninum*, whereas heartworm preventatives are expected to have at least 99.5% efficiency.[112] Failure of either efficacy threshold is considered resistance.
iv. *Pet travel, population movement and the dissemination of resistance strains:* Global dog movement through rescue and rehoming networks and the international trade in dogs help disseminate resistant parasite populations into the broader pet dog population. Improved parasite testing and record keeping for animals being moved geographically would be helpful in monitoring resistance.
v. *Human knowledge, attitudes, and practices:* Anthelmintic use patterns are informed by veterinarians but largely determined by pet owners. Pet owners choose which products are administered, how often and if treatment guidelines are followed. Owners must report any suspected treatment failure and/or persistence of clinical signs to the veterinarian promptly for further diagnostic investigation. To prevent the spread of resistant parasite infections to other animals, owners must ensure that their pet is confirmed clear of infection before visiting shared spaces such as dog parks. For parasites transmitted by the fecal-oral route, proper fecal disposal immediately after defecation is recommended. For vector borne pathogens such as *D. immitis* and *D. caninum*, rigorous mosquito and flea control respectively are essential.

SUMMARY

Anthelmintic resistance in companion animal helminths represents a growing veterinary and public health challenge since the parasite control toolkit relies on a few drug classes. Resistance arises not from drug-induced mutations but from the

selection of pre-existing resistant alleles within parasite populations, a process which can be well advanced before detection by diagnostic tests. This review examines the drug classes available for companion animal use in the United States and resistance mechanisms documented against each. Parasites with emerging resistance of epidemiologic importance are examined including multiple anthelmintic drug resistant *A. caninum*, ML-resistant *D. immitis* and praziquantel-resistant *D. caninum*. Across these parasites, the ability to diagnose resistance in clinical practice remains constrained, due to reliance on functional tests as primary diagnostic tools and molecular testing existing for only a few parasite-drug combinations. When resistance is confirmed, therapeutic options remain limited, often reliant on combination or extralabel drug use. Conditions that allow resistance to spread include sparse availability of molecular diagnostics, absence of real-time surveillance, regional pet movement and treatment habits that favor routine over targeted drug use. Reducing further resistance emergence depends on using case-specific diagnostic tests, diagnostic-test guided treatments, and minimizing unnecessary treatments.

CLINICS CARE POINTS

- Anthelmintic resistance is emerging and driven by drug selection of resistant parasite populations.
- Suspected canine hookworm resistance can be confirmed with fecal egg count reduction test, and treated with triple combination therapy.
- Macrocyclic lactone resistance in canine heartworm can be confirmed with microfilarial suppression test. Moxidectin is expected to retain efficacy even against resistant isolates.
- Praziquantel-resistant flea tapeworm must be carefully confirmed to distinguish true resistance from reinfection. Extralabel drugs must be used with care.
- Reducing selection pressure is key to preserving efficacy. Resistance can be minimized by using diagnostics to guide treatment, avoiding repeated single class use and subtherapeutic dosing.
- Any suspected treatment failure and/or persistence of clinical sign must be reported by pet owners to veterinarians for additional diagnostic follow-up.

DISCLOSURE

The authors have nothing to disclose.

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