

Macrocytic Lactone Loss of Efficacy against *Dirofilaria immitis*

Subjects: **Veterinary Sciences**

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Macrocytic Lactone Loss of Efficacy against *Dirofilaria immitis* is a problem of resistance development by *D. immitis* (Filarioidea: Onchocercidae), a nematode parasite that inhabits the pulmonary arteries of dogs and other carnivores causing heartworm disease, versus Macrocytic Lactones, i.e. the drug category used for prevention of heartworm disease.

Dirofilaria immitis

macrocyclic lactones

resistance

1. *Dirofilaria immitis* and Heartworm Chemoprophylaxis

The nematode parasite *Dirofilaria immitis* ("heartworm") is the agent of canine heartworm disease, one of the most severe parasitic diseases of dogs and other carnivores. *Dirofilaria immitis* is transmitted by the bite of infected mosquitoes and may also infect humans, typically causing "pulmonary dirofilariasis". Because of the impact of heartworms on the health of animals, the complexity, risk and cost of the treatment and the zoonotic implications, heartworm prevention in dogs is imperative [1][2][3]. Prevention is achieved by the administration of drugs containing macrocyclic lactones (MLs), i.e. ivermectin (IVM), selamectin (SEL), eprinomectin (EPR), abamectin (ABA) (licensed in Australia for use in dogs), milbemycin oxime (MO) and moxidectin (MOX). These products are very safe and highly effective, targeting the third and fourth larval stages (L3, L4) of the parasite (Table 1).

Table 1. Veterinary products with macrocyclic lactones, registered in the USA or Europe for heartworm prevention in dogs and cats *.

Active Molecule **	Target Species	Application Route/Administration	Product/Company	Combination Molecule(s)
Eprinomectin	cat	topical/monthly	Centragard ² /Boehringer Ingelheim	Praziquantel
			NexGard Combo ³ /Boehringer Ingelheim	Esafoxolaner, Praziquantel
			Broadline ³ /Boehringer Ingelheim	Fipronil, Praziquantel, (S)-Methoprene

Active Molecule **	Target Species	Application Route/Administration	Product/Company	Combination Molecule(s)
Ivermectin	dog, cat	oral/monthly	Heartgard ² /Boehringer Ingelheim Iverhart ² /Virbac Ivermectin ² /Cronus Pharma	-
		topical/monthly	Advantage DUO ² /Elanco	Imidacloprid
			Heartgard Plus ² /Boehringer Ingelheim Iverhart Plus ² /Virbac Tri-Heart Plus ² /Heska	Pyrantel
	dog		Panacur Plus ² /Intervet	Praziquantel, Fenbendazole
		oral/monthly	Iverhart Max ² /Virbac	Praziquantel, Pyrantel
			Heartgard Plus ³ /Boehringer Ingelheim Cardotek Plus ³ /Boehringer Ingelheim	Pyrantel
			Cardotek ³ /Boehringer Ingelheim	-
Milbemycin oxime			Interceptor ¹ /Elanco MilbeGuard ² /Ceva Sante Animale	-
Milbemycin oxime	dog, cat	oral/monthly	Interceptor Plus ¹ /Elanco Milbemax ³ /Elanco Milbactor ³ /Ceva Sante Animal Milprazon ³ /Krka Milquantel ³ /Krka Milpro ³ /Virbac	Praziquantel
			Sentinel ² /Intervet Program plus ³ /Elanco	Lufenuron
			Sentinel Spectrum ² /Intervet	Lufenuron, Praziquantel
	dog		Interceptor Plus ² /Elanco	Praziquantel
			Trifexis ¹ /Elanco	Spinosad

Active Molecule **	Target Species	Application Route/Administration	Product/Company	Combination Molecule(s)
Moxidectin	dog, cat	topical/monthly	NexGard Spectra ³ /Boehringer Ingelheim	Afoxolaner
			Credelio Plus ³ /Elanco	Lotilaner
			Prinovox ³ /Virbac	
			Advantage Multi ² /Elanco	Imidacloprid
			Imoxi ² /Vetoquinol	
	dog	oral/monthly	Advocate ³ /Elanco	
			Simparica Trio ¹ /Zoetis	Sarolaner, Pyrantel
			ProHeart ^{2,4} /Zoetis	
			Proheart 6 ² /Zoetis	
			Guardian ^{3***} /Elanco	-
Selamectin	dog, cat	inj./6 month	Afilaria ³ /Fatro, Support Pharma	
			Proheart 12 ² /Zoetis	
			Coraxis ² /Elanco	
			Bravecto Plus ¹ /Intervet	Fluralaner
			Revolution ² /Zoetis Revolt ² /Aurora Selarid ² /Norbrook Lab. Senergy ² /Chanelle Stronghold ³ /Zoetis Chanhold ³ /Chanelle Evicto ³ /Virbac Stronghold Plus ³ /Zoetis	-
Selamectin	cat	topical/monthly	Revolution Plus ² /Zoetis Stronghold Plus ³ /Zoetis Felisecto Plus ³ /Zoetis	Sarolaner

2. Bowman, D.D.; Atkins, C.E. Heartworm biology, treatment, and control. *Vet. Clin. N. Am. Small Anim. Pract.* 2009, 39, 1127–1158.

3. Theis, J.H. Public health aspects of dirofilariasis in the United States. *Vet. Parasitol.* 2005, 133,

* 157–180 Information retrieved from the European Medicines Agency (<https://www.ema.europa.eu/en>), accessed the 5th of August 2021), the U.S. Food and Drug Administration (<https://animaldrugsatfda.fda.gov/adafda/views/#/search>

1. Nowak, S. 5th August, 2020. Darithrin, DiS for Canines, and RhoSelzer, *P. For Heartworm disease. *** To be administered every 6 months. *Parasitol Drugs Drug Resist* 2021, 16, 16. Overview, intervention, and industry perspective according to the *Parasitol Drugs Drug Resist* 2021, registered in USA and Europe. ² Registered in USA only. ³ Registered in Europe only. ⁴ Registered in the USA, but no longer available.

5. Nolan, T.J.; Lok, J.B. Macrocyclic lactones in the treatment and control of parasitism in small companion animals. *Cur. Pharm. Biotech.* 2012, 13, 1078–1094.

Disclaimer: The authors have attempted to include all heartworm preventive products currently approved in the USA and Europe. ¹ Charles S. D. Arthur. Responsibility for laboratory evaluation of that efficacy of 10% but were not adopted the 25% mark of topical solutions (Advantage® Multi, Advocate®) for the treatment of *Dirofilaria immitis* circulating microfilariae in dogs. *Parasitol. Res.* 2015, 114 (Suppl. S1), S165–MLs are effective against L3 and L4 stages of *D. immitis* and kill them rapidly. MLs have no “forward” action (against future infections) but rather a “reach-back” efficacy (against past inoculations). Thus, the strategy of the 7. Hampshire, V.A. Evaluation of efficacy of heartworm preventive products at the FDA. *Vet. Parasitol.* 2005, 133, 191–195.

bites throughout the period of transmission and that monthly administration of MLs ensures that no worms will live

18. Richard, P.; Korchmar, A. ¹ ML resistance as a concern for heartworm control. What is the evidence? *Vet. Parasitol.* 2005, 133, 243–253. So an effect of MLs on microfilariae and this varies between the different molecules, dose rates and formulations ^[6].

9. Atkins, C.E.; Murray, M.J.; Olavessen, L.J.; Burton, K.W.; Marshall, J.W.; Brooks, C.C. Heartworm

‘lack of effectiveness’ claims in the Mississippi delta: Computerized analysis of owner

2. MLs Loss of Efficacy (LOE) Reports: initial scepticism, confirmation and tools developed for resistance detection

10. Evans, C.C.; Moorhead, A.R.; Storey, B.E.; Wolstenholme, A.J.; Kaplan, R.M. Development of an in vitro bioassay for measuring susceptibility to macrocyclic lactone anthelmintics in *Dirofilaria immitis*. *Int. J. Parasitol. Drugs Drug Resist.* 2013, 3, 102–108. Until 2011, claims of ineffectiveness of MLs, reported as ‘Lack of Efficacy’ (LOE), were generally attributed to owners’ non-compliance, or other reasons for inadequate preventative coverage. There was solid argumentation that a resistance problem is not likely to occur because of i) the great extent of infestation ii) the complexity of resistance development to MLs and iii) the possible big number of genes involved in resistance selection ^{[7][8][9][10]}.

11. Bourguinat, C.; Keller, K.; Blagburn, B.; Schenker, R.; Geary, T.G.; Prichard, R.K. Correlation between loss of efficacy of macrocyclic lactone heartworm anthelmintics and T-glycoprotein genotype. *Vet. Parasitol.* 2011, 176, 374–381.

Soon after those reports, the first unequivocally resistant strains of *D. immitis*, originating from the Lower

12. Pulaski, C.N.; Malone, J.B.; Bourguinat, C.; Prichard, R.; Geary, T.; Ward, D.; Klei, T.R.; Guidry, T.; Smith, G.; Delcampagne, B.; et al. Establishment of macrocyclic lactone resistant *Dirofilaria immitis* isolates in experimentally infected laboratory dogs. *Parasites Vectors* 2014, 7, 494. Accordingly, tools have been developed to evaluate the susceptibility status of *D. immitis* strains. A simple, in-clinic, microfilariae suppression test (MFST), 14–28 days after ML administration ^[11], and a ‘decision tree’ (algorithm), including compliance and

13. Geary, T.G.; Bourguinat, C.; Prichard, R.K. Evidence for macrocyclic lactone anthelmintic resistance in *Dirofilaria immitis*. *Top. Companion Anim. Med.* 2011, 26, 180–192. preventives’ purchase history and testing group ^[14], may be applied for assessing any resistant nature of the parasite. On the molecular level, specific SNPs may be used as markers of ML resistance offering a basis for the

14. Moorhead, A.R.; Evans, C.C.; Kaplan, R.M. A diagnostic algorithm for evaluating cases of *D. immitis* resistance. It is suggested that ML resistance may be a polygenic trait and importantly, that there is probably a spectrum of resistant phenotypes. In this context, a specific 2 SNP model was found to be currently the best available diagnostic tool for the confirmation of clinically suspected cases ^[15].

15. Ballesteros, C.; Pulaski, C.N.; Bourguinat, C.; Keller, K.; Prichard, R.K.; Geary, T.G. Clinical

16. Bourguinat, C.; Keller, K.; Bhan, A.; Peregrine, A.; Geary, T.; Prichard, R. Macrocyclic lactone resistance in *Dirofilaria immitis*. *Vet. Parasitol.* 2011, 181, 388–392. validation of molecular markers of macrocyclic lactone resistance in *Dirofilaria immitis*. *Int. J. Parasitol. Drugs Drug Resist.* 2018, 8, 596–606.

According to the most recent information, resistant strains have been identified so far only in the area of the Lower Mississippi region in the USA ^{[11][12][16][15]}, while in Europe, no LOE/resistance claims have been reported. In

Angiostrongylus Days, Belgrade, Serbia, 5–7 July 2018. Abstract Number P14.

There are several factors rendering ML-resistance emergence a phenomenon that may be slow to occur in new areas or to expand from areas where it is already present. Nevertheless, we now know that this problem is already present, albeit apparently only in a part of the USA, and the expansion of resistance by the movement of infected *immitis* samples from heartworm positive dogs in Europe. Submitted 2021.

¹⁸See Anterior and Posterior Society of Cardiology Guidelines for the Prevention, Diagnosis, and

Management of Heartworm (Dirofilaria immitis) Infection in Dogs. Available online:

4. How to monitor and prevent Macrocytic Lactone Loss Effect on *Dirofilaria immitis*

4. How to monitor and prevent Macrocytic Lactone Loss of Efficacy on *Dirofilaria immitis*

20. European Society of Dirofilariosis and Angiostrongylosis. Guidelines for Clinical Management of Canine Heartworm Disease. Available online: <https://www.esda.vet/wp-content/uploads/2017/11/GUIDELINES-FOR-CLINICAL-MANAGEMENT-OF-CANINE-HEARTWORM-DISEASE.pdf> (accessed on 30 July 2021).

inquiring what the exact veterinary products used were, the intervals between administrations, possible missed or late dosages, prevention year-round or seasonal coverage, the exact doses and the chance that there was sharing of doses among pets of the same household and presuppose the presence of microfilariae in the circulation of the

microfilaria, and adult worms in microfilaremic dogs. *Vet Parasitol* 2014; 206: 5–13. dog. In case the prevention was applied correctly, the investigation of resistance should go further with the application of MFST with products registered as microfilaricidal. If MFST indicates any possibility of resistant parasites there is merit in further investigating the case in order to monitor the situation and track any expansion or emergence of a resistance problem. Until simple and inexpensive tests, that could be performed in the clinic, or

23. Bowman, D.D.; Drake, J. Examination of the "susceptibility gap" in the treatment of canine heartworm infection. Parasites Vectors 2017, 10 (Suppl. S2), 513. laboratories that are currently in a position of performing the required analyses (genotyping) and identifying ML-

laboratories that are currently in a position of performing the required analyses (genotyping) and identifying the *ME-24*-McTier-7th-in-Site-Parking PA-6 Glendale, Oak Hill, KY, Mohrshire, G.R.; Woods, D.J.; Meader, S.J.

Preventive efficacy of oral moxidectin at various doses and dosage regimens against macrocyclic lactone-resistant heartworm (*Dirofilaria immitis*) strains in dogs. Parasites Vectors 2019, 12, 444.

be implicated according to the American Heartworm Society and European Society of Dirofilariosis and 25. Kryda, K.; Six, R.H.; Walsh, K.F.; Holzmer, S.J.; Chapin, S.; Mahabir, S.P.; Myers, M.; Inskeep, T. Angiostrongylosis guidelines [\[19\]\[20\]](#), with special emphasis on fast interruption of parasite transmission with a) the Rugg, J.; Cundiff, B. Laboratory and field studies to investigate the efficacy of a novel, orally use of MLs licensed as microfilaricidal, b) the administration of antibiotics (doxycycline or minocycline) in order to administered combination product containing moxidectin, sarolaner and pyrantel for the remove the filarial endosymbiont *Wolbachia* *piplensis* which is critical for the survival, development and prevention of heartworm disease (*Dirofilaria immitis*) in dogs. Parasites Vectors 2019, 12, 445. reproduction of *D. immitis* [\[21\]\[22\]](#), c) the application of repellents and long-acting insecticides in order to avoid

260 **Spintharus, Dadd Heartwides, major boyelid jitters, and the spectre of resistance to prevention** worm
treat the United States the Parasites Vector of 2012 before 130 it [23].

27. Wolstenholme, A.J.; Evans, C.C.; Jimenez, P.D.; Moorhead, A.R. The emergence of macrocyclic lactone resistance in the canine heartworm, *Dirofilaria immitis*. *Parasitology* 2015, **142**, 1249–1259. currently available for this purpose. In areas where ML-resistance is established and breakthrough infections are Retrieved from <https://encyclopedia.pub/entry/history/show/37416>

confirmed, administration of high dose formulations of MOX may be of help, as it has been shown that MOX in all forms of products (per os, topical and injectable) has a better efficacy against resistant strains [24][25].

It is important to note that there are measures and strategies that can be implemented in an effort to prevent the development and spread of ML-resistance. In this context, it is important to adopt a tight testing schedule, i.e., at least once every year (preferably, every 6 months in areas where LOE cases are reported). The testing procedure is specific and includes both serology and the Knott's test, which is particularly critical in routine annual examinations of dogs under preventatives because even one couple of resistant adults will produce microfilariae while may give a negative antigen test.

The risk of promoting ML-resistance by the application of the so-called "slow kill protocols", i.e. therapeutic treatment by the use of continuous ML administration, has been suggested [26][13]. Nevertheless, in case a dog was not under prevention and is only infected with susceptible heartworms, the slow kill protocol would represent a promotion for resistance development only as an extreme and unlikely scenario [27]. In any case, it must be stressed that ML resistance in *D. immitis* can be selected on different stages of the parasites, i.e., the L3/L4 larvae (the target of ML administration as preventives), the microfilariae, and on adult parasites (because of the effects of MLs on their reproductive ability) when MLs are used in the presence of microfilariae and adult parasites.

Academics, clinical practitioners, and dog owners should be concerned and act together with the goal of monitoring and preventing the Macrocyclic Lactone Loss of Efficacy phenomenon. This battle starts with proper education and continues with best practices for infection prevention, adequate testing, accurate and prompt diagnosis, accurate investigation of the cases, and selection of best treatment protocols. The investigation of suspected resistance cases will allow distinction of infections that were established by susceptible parasites due to inadequate prophylaxis, from infections caused by truly resistant parasites. This would provide critical information about the actual spread of the phenomenon and its possible expansion or de novo emergence, while at the same time it would help increase practitioners' and owners' awareness and compliance [15].