Macrocyclic Lactone Loss of Efficacy against Dirofilaria immitis

Subjects: Veterinary Sciences

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Macrocyclic 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 Macrocyclic Lactones, i.e. the drug category used for prevention of heartworm disease.

Keywords: 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 dirofilariosis”. 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
	dog	oral/monthly	Heartgard Plus[2]/Boehringer Ingelheim Iverhart Plus[2]/Virbac Tri-Heart Plus[2]/Heska	Pyrantel
			Panacur Plus[2]/Intervet	Praziquantel, Fenbendazole
			Iverhart Max[2]/Virbac	Praziquantel, Pyrantel
Milbemycin oxime	dog, cat	oral/monthly	Heartgard Plus[3]/Boehringer Ingelheim Cardotek Plus[3]/Boehringer Ingelheim	Pyrantel
			Cardotek[3]/Boehringer Ingelheim	-
			Interceptor[1]/Elanco MilbeGuard[2]/Ceva Sante Animale	-
			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	oral/monthly	Interceptor Plus[2]/Elanco	Praziquantel
			Trifexis[1]/Elanco	Spinosad
			NexGard Spectra[3]/Boehringer Ingelheim	Afoxolaner
			Credelio Plus[3]/Elanco	Lotilaner
Moxidectin	dog, cat	topical/monthly	Prinovox[3]/Virbac Advantage Multi[2]/Elanco Imoxi[2]/Vetoquinol Advocate[3]/Elanco	Imidacloprid
	dog	oral/monthly	Simparica Trio[1]/Zoetis	Sarolaner, Pyrantel
			ProHeart[2,4]/Zoetis Proheart 6[2]/Zoetis	-
		inj./6 month	Guardian[3]***/Elanco Afilaria[3]/Fatro, Support Pharma	
		inj./12 month	Proheart 12[2]/Zoetis	
		topical/monthly	Coraxis[2]/Elanco	
	cat	topical/monthly	Bravecto Plus[1]/Intervet	Fluralaner

Active Molecule **	Target Species	Application Route/Administration	Product/Company	Combination Molecule(s)
Selamectin	dog, cat	topical/monthly	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	-
	cat	topical/monthly	Revolution Plus[2]/Zoetis Stronghold Plus[3]/Zoetis Felisecto Plus[3]/Zoetis	Sarolaner

* 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 accessed the 5th of August 2021), and from [4] for Europe and the USA. ** For heartworm prevention. *** To be administered yearly, the first month of mosquito activity, according to the drug instructions in Europe. [1] Registered in USA and Europe. [2] Registered in USA only. [3] Registered in Europe only. [4] Registered in the USA, but no longer available.

Disclaimer: The authors have attempted to include all heartworm preventive products currently approved in the USA and Europe. However, they do not accept responsibility for not listing any products that may be available but were not found in their exploration of the market products.

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 periodic administration is based on the scenario that dogs are under continuous exposure to infective mosquito bites throughout the period of transmission and that monthly administration of MLs ensures that no worms will live to reach the pulmonary arteries [5]. MLs have also an effect on young adults, and adult worms, but this action is apparent after several, continuous, periodic administrations of the drugs. Finally, there is also an effect of MLs on microfilariae and this varies between the different molecules, dose rates and formulations [6].

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

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 refugia, ii) the complexity of resistance development to MLs, and iii) the possible big number of genes involved in resistance selection [7][8][9][10].

Soon after those reports, the first unequivocally resistant strains of *D. immitis*, originating from the Lower Mississippi area, have been genetically, in vitro and clinically confirmed [11][12]. 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 [13], and a “decision tree” (algorithm), including compliance and preventatives’ purchase history, and testing gaps [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 validation of clinically suspected resistant strains. 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].

3. Current situation in the USA and Europe and scenarios for the future

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 Europe, a small number of cases generated strong suspicion of resistance presence in the recent past [17], however, resistant *D. immitis* isolates were not genetically confirmed. Furthermore, a recent investigation of *D. immitis* clinical isolates, from Italy, Spain, and Hungary showed genotypes consistent with susceptibility [18].

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 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 dogs (or mosquitoes), or the de novo emergence cannot be ruled out. For this reason, vigilance and monitoring are essential and towards this direction academics, veterinarians and owners should work together.

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

The first indication to consider that a case is worth investigating for resistance is when a dog under consistent preventives becomes heartworm positive. In that case, a specific sequel of actions would help to get a clearer picture of the susceptibility nature of the parasites involved in a LOE suspected case [14]. These actions include 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 dog. In case the prevention was applied correctly, the investigation of resistance should go further with the application of MFST with a product 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 in routine diagnostic laboratories are available, samples could be obtained and sent to the few institutions and laboratories that are currently in a position of performing the required analyses (genotyping) and identifying ML-resistance, such as the Institute of Parasitology at McGill University in Canada.

Irrespective of whether there is confirmation of infection by a resistant strain or not, the treatment protocol should be implicated according to the American Heartworm Society and European Society of Dirofilariosis and Angiostrongylosis guidelines [19][20], with special emphasis on fast interruption of parasite transmission with a) the use of MLs licensed as microfilaricidal, b) the administration of antibiotics (doxycycline or minocycline) in order to remove the filarial endosymbiont Wolbachia pipientis which is critical for the survival, development and reproduction of *D. immitis* [21][22], c) the application of repellents and long-acting insecticides, in order to avoid mosquito bites, and d) consideration of shortening the pre-adulticide period described in the proposed heartworm treatment protocol, if the general condition of the dog permits it [23].

For the foreseeable future, chemoprophylaxis of dogs and cats with MLs against heartworm disease is not negotiable because of its detrimental nature, its zoonotic potential, and because MLs are the only drug class that is currently available for this purpose. In areas where ML-resistance is established and breakthrough infections are 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].

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