

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 **macrocytic 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 macrocytic 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 macrocytic 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	-
			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
			Heartgard Plus ³ /Boehringer Ingelheim	Pyrantel
			Cardotek Plus ³ /Boehringer Ingelheim	
			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
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
			Interceptor Plus ¹ /Elanco Milbemax ³ /Elanco Milbactor ³ /Ceva Sante Animal Milprazon ³ /Krka Milquantel ³ /Krka Milpro ³ /Virbac	Praziquantel
	dog	oral/monthly	Sentinel ² /Intervet Program plus ³ /Elanco	Lufenuron
			Sentinel Spectrum ² /Intervet	Lufenuron, Praziquantel
			Interceptor Plus ² /Elanco	Praziquantel
			Trifexis ¹ /Elanco	Spinosad

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

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* 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>

4. Blackthorn, S. 5. Harrington, J. 2020. Parfitt, D. S. For Kaminsky, R. the Selzer, *P.M. For Heartworm disease.*** To be administered by injection, and industry perspective according Parasitol. Drugs Drug Resist. 2021; 16: 154–160. 6. 7. 8. 9. 10. 11. 12. 13. 14. 15. 16. 17. 18. 19. 20. 21. 22. 23. 24. 25. 26. 27. 28. 29. 30. 31. 32. 33. 34. 35. 36. 37. 38. 39. 40. 41. 42. 43. 44. 45. 46. 47. 48. 49. 50. 51. 52. 53. 54. 55. 56. 57. 58. 59. 60. 61. 62. 63. 64. 65. 66. 67. 68. 69. 70. 71. 72. 73. 74. 75. 76. 77. 78. 79. 80. 81. 82. 83. 84. 85. 86. 87. 88. 89. 90. 91. 92. 93. 94. 95. 96. 97. 98. 99. 100. 101. 102. 103. 104. 105. 106. 107. 108. 109. 110. 111. 112. 113. 114. 115. 116. 117. 118. 119. 120. 121. 122. 123. 124. 125. 126. 127. 128. 129. 130. 131. 132. 133. 134. 135. 136. 137. 138. 139. 140. 141. 142. 143. 144. 145. 146. 147. 148. 149. 150. 151. 152. 153. 154. 155. 156. 157. 158. 159. 160. 161. 162. 163. 164. 165. 166. 167. 168. 169. 170. 171. 172. 173. 174. 175. 176. 177. 178. 179. 180. 181. 182. 183. 184. 185. 186. 187. 188. 189. 190. 191. 192. 193. 194. 195. 196. 197. 198. 199. 200. 201. 202. 203. 204. 205. 206. 207. 208. 209. 210. 211. 212. 213. 214. 215. 216. 217. 218. 219. 220. 221. 222. 223. 224. 225. 226. 227. 228. 229. 230. 231. 232. 233. 234. 235. 236. 237. 238. 239. 240. 241. 242. 243. 244. 245. 246. 247. 248. 249. 250. 251. 252. 253. 254. 255. 256. 257. 258. 259. 260. 261. 262. 263. 264. 265. 266. 267. 268. 269. 270. 271. 272. 273. 274. 275. 276. 277. 278. 279. 280. 281. 282. 283. 284. 285. 286. 287. 288. 289. 290. 291. 292. 293. 294. 295. 296. 297. 298. 299. 300. 301. 302. 303. 304. 305. 306. 307. 308. 309. 310. 311. 312. 313. 314. 315. 316. 317. 318. 319. 320. 321. 322. 323. 324. 325. 326. 327. 328. 329. 330. 331. 332. 333. 334. 335. 336. 337. 338. 339. 340. 341. 342. 343. 344. 345. 346. 347. 348. 349. 350. 351. 352. 353. 354. 355. 356. 357. 358. 359. 360. 361. 362. 363. 364. 365. 366. 367. 368. 369. 370. 371. 372. 373. 374. 375. 376. 377. 378. 379. 380. 381. 382. 383. 384. 385. 386. 387. 388. 389. 390. 391. 392. 393. 394. 395. 396. 397. 398. 399. 400. 401. 402. 403. 404. 405. 406. 407. 408. 409. 410. 411. 412. 413. 414. 415. 416. 417. 418. 419. 420. 421. 422. 423. 424. 425. 426. 427. 428. 429. 430. 431. 432. 433. 434. 435. 436. 437. 438. 439. 440. 441. 442. 443. 444. 445. 446. 447. 448. 449. 450. 451. 452. 453. 454. 455. 456. 457. 458. 459. 460. 461. 462. 463. 464. 465. 466. 467. 468. 469. 470. 471. 472. 473. 474. 475. 476. 477. 478. 479. 480. 481. 482. 483. 484. 485. 486. 487. 488. 489. 490. 491. 492. 493. 494. 495. 496. 497. 498. 499. 500. 501. 502. 503. 504. 505. 506. 507. 508. 509. 510. 511. 512. 513. 514. 515. 516. 517. 518. 519. 520. 521. 522. 523. 524. 525. 526. 527. 528. 529. 530. 531. 532. 533. 534. 535. 536. 537. 538. 539. 540. 541. 542. 543. 544. 545. 546. 547. 548. 549. 550. 551. 552. 553. 554. 555. 556. 557. 558. 559. 560. 561. 562. 563. 564. 565. 566. 567. 568. 569. 570. 571. 572. 573. 574. 575. 576. 577. 578. 579. 580. 581. 582. 583. 584. 585. 586. 587. 588. 589. 590. 591. 592. 593. 594. 595. 596. 597. 598. 599. 600. 601. 602. 603. 604. 605. 606. 607. 608. 609. 610. 611. 612. 613. 614. 615. 616. 617. 618. 619. 620. 621. 622. 623. 624. 625. 626. 627. 628. 629. 630. 631. 632. 633. 634. 635. 636. 637. 638. 639. 640. 641. 642. 643. 644. 645. 646. 647. 648. 649. 650. 651. 652. 653. 654. 655. 656. 657. 658. 659. 660. 661. 662. 663. 664. 665. 666. 667. 668. 669. 670. 671. 672. 673. 674. 675. 676. 677. 678. 679. 680. 681. 682. 683. 684. 685. 686. 687. 688. 689. 690. 691. 692. 693. 694. 695. 696. 697. 698. 699. 700. 701. 702. 703. 704. 705. 706. 707. 708. 709. 710. 711. 712. 713. 714. 715. 716. 717. 718. 719. 720. 721. 722. 723. 724. 725. 726. 727. 728. 729. 730. 731. 732. 733. 734. 735. 736. 737. 738. 739. 740. 741. 742. 743. 744. 745. 746. 747. 748. 749. 750. 751. 752. 753. 754. 755. 756. 757. 758. 759. 760. 761. 762. 763. 764. 765. 766. 767. 768. 769. 770. 771. 772. 773. 774. 775. 776. 777. 778. 779. 780. 781. 782. 783. 784. 785. 786. 787. 788. 789. 790. 791. 792. 793. 794. 795. 796. 797. 798. 799. 800. 801. 802. 803. 804. 805. 806. 807. 808. 809. 810. 811. 812. 813. 814. 815. 816. 817. 818. 819. 820. 821. 822. 823. 824. 825. 826. 827. 828. 829. 830. 831. 832. 833. 834. 835. 836. 837. 838. 839. 840. 841. 842. 843. 844. 845. 846. 847. 848. 849. 850. 851. 852. 853. 854. 855. 856. 857. 858. 859. 860. 861. 862. 863. 864. 865. 866. 867. 868. 869. 870. 871. 872. 873. 874. 875. 876. 877. 878. 879. 880. 881. 882. 883. 884. 885. 886. 887. 888. 889. 890. 891. 892. 893. 894. 895. 896. 897. 898. 899. 900. 901. 902. 903. 904. 905. 906. 907. 908. 909. 910. 911. 912. 913. 914. 915. 916. 917. 918. 919. 920. 921. 922. 923. 924. 925. 926. 927. 928. 929. 930. 931. 932. 933. 934. 935. 936. 937. 938. 939. 940. 941. 942. 943. 944. 945. 946. 947. 948. 949. 950. 951. 952. 953. 954. 955. 956. 957. 958. 959. 960. 961. 962. 963. 964. 965. 966. 967. 968. 969. 970. 971. 972. 973. 974. 975. 976. 977. 978. 979. 980. 981. 982. 983. 984. 985. 986. 987. 988. 989. 990. 991. 992. 993. 994. 995. 996. 997. 998. 999. 1000.

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. Dr. Howard, S.D., Arthur, R., Spent, T. Laboratory evaluation of the efficacy of 100% but were not adopted in the 2.5% ivermectin topical solutions (Advantage® Multi, Advocate®) for the treatment

of *Dirofilaria immitis* circulating microfilariae in dogs. *Parasitol. Res.* 2015, 114 (Suppl. S1), S165–S174. 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.

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

6. Prichard, R.K. Is resistance a concern for year-round control? What can we learn from the heartworm control programs? *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

compliance 2004–2011. *Vet. Parasitol.* 2014, 200, 1–10.

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 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].

11. Bourguinat, C.; Keller, K.; Blagburn, B.; Senenker, R.; Geary, T.G.; Prichard, R.K. Correlation between loss of efficacy of macrocyclic lactone heartworm anthelmintics and P-glycoprotein

genotype. *Vet. Parasitol.* 2011, 176, 374–381.

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

preventative purchase history and testing, nor [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

potential macrocyclic lactone-resistant heartworm. *Parasites Vectors* 2017, 10 (Suppl. S2), 479. found to be currently the best available diagnostic tool for the confirmation of clinically suspected cases [16].

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

validation of molecular markers of macrocyclic lactone resistance in *Dirofilaria immitis*. *Int. J. Parasitol. Drugs Drug Resist.* 2018, 8, 596–606.

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. 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

17. Drake, A.; Koutinas, C.; Besungrat, C.; Ballesteros, C.; Diez, D.; Chalkias, V.; Baran, M.; Traversa, D.; Prichard, R. Heartworm infection in military dogs under preventive treatment of *D. immitis* is not suggested as a risk. In Proceedings of the 6th European Dirofilaria Party, 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 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.

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22. McCall, J.W. *Dirofilaria immitis* and *Wolbachia pipiens*: A thorough investigation of the symptoms responsible for canine heartworm disease. *Parasitol. Res.* **2012**, *110*, 499–502.

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24. Maier, T.; Six, R.; Pullins, A.; Chapin, S.; Kryda, K.; Mahabir, S.P.; Woods, D.J.; Maeder, S.J.

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

25. Kryda, K.; Six, R.H.; Walsh, K.F.; Holzer, S.J.; Chapin, S.; Mahabir, S.P.; Myers, M.; Inskip, T.; Rugg, J.; Cundiff, B. Laboratory and field studies to investigate the efficacy of a novel, orally administered combination product containing moxidectin, sarolaner and pyrantel for the removal of the filarial endosymbiont *Wolbachia pipiens* 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

26. Bowman, D.D. Heartworms, macrocyclic lactones, and the specter of resistance to prevention in the United States. *Parasites Vectors* **2012**, *5*, 138.

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.

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 [\[15\]](#).