

Theses of doctoral (PhD) dissertation

**NEW POSSIBILITIES IN THE DIAGNOSIS AND
TREATMENT OF CANINE HEARTWORM
DISEASE**

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1. Background and aims of the doctoral thesis

Heartworm disease (HWD), caused by *Dirofilaria immitis* is a mosquito-borne disease in Carnivores, mainly in dogs, and could be found on most of the continents. HWD is an emerging problem also in Hungary, and several regions are already endemic, especially in the southern and eastern parts of our country. *Dirofilaria repens* causing mainly subcutan dirofilariosis is also present in Asia and Africa, but not in the Americas. This nematode can also be found in several middle European countries including Hungary, mainly in similar regions as *D. immitis*. The concomitant occurrence of these *Dirofilaria* species has special importance, due to their different diagnostic aspects and therapy. Proper diagnosis, treatment, and prevention of HWD is inevitable for protecting canine patients, and to inhibit human infections which might be transmitted by infected mosquitoes. In my thesis, I report on our research related to some new possibilities in the diagnosis and therapy of canine HWD.

The following goals have led to the present studies and to compile this dissertation.

1. In the first part of the thesis, I demonstrate the difficulties and the possibilities of serodiagnostic methods in infected dogs living in regions where concomitant infections by *D. immitis* and *D. repens* occur. The reason of this research was that we did not find information on the validation of *D. immitis* antigen tests in dogs from these regions. The VetScan antigen test was used, and the PCR assay served as a standard for the parasitological diagnosis. Sensitivity and specificity of this antigen test were studied under these conditions. As a second goal, we aimed to demonstrate if occult dirofilariosis could be the explanation for positive antigen tests despite of a negative PCR result of *D. immitis* what we experienced sometimes in our clinical praxis. In cases with occult dirofilariosis, several antigen tests of different manufacturers were applied for the diagnosis. These was based on the hypothesis that the chance for a cross-reaction among *D. immitis* and other helminths (including *D. repens*) is less

when more than one heartworm antigen tests provide positive results.

2. The standard therapy of HWD is recommended by the American Heartworm Society (AHS) which is effective and accepted internationally. This complex therapeutic protocol includes a macrocyclic lactone (ivermectin till nowadays) to destroy microfilariae as well as L3 and L4), doxycycline against the symbiotic *Wolbachia* bacteria, and the adulticide melarsomine dihydrochloride. However, side-effects and complications might occur of using these drugs. In our related research, we have modified and complemented the AHS protocol with some additional medications and therapeutic measures to decrease the possible side-effects and to further improve the efficacy and outcome of the complex treatment. For these purposes, moxidectin was used instead of ivermectin as part of the therapeutic protocol. Before our research, there has been only one preliminary report on the application of moxidectin in that respect, with the contribution

of one of my supervisors. These authors only partially used the three-dose alternate melarsomine protocol. In our study, probiotics were added to the application of doxycycline, in order to decrease the potential gastrointestinal side-effects of this antibacterial drug. The patients were sedated with butorphanol before the intramuscular application of melarsomine injection. The exact place of the needle was determined with the help of ultrasonography within the paralumbar musculature.

3. Occult dirofilariosis has not been published previously in Hungary. In a case report, we have described the diagnostic workup and the treatment of a severe case, for the Hungarian readers. This part of my thesis also demonstrates the clinical use of the scientific results of Chapter one and two.

2. New scientific results

2.1. Serodiagnostic difficulties and possibilities of heartworm disease in regions where both *Dirofilaria immitis* and *Dirofilaria repens* infections occur

The sensitivity of the antigen test performed on 71 *D. immitis* positive dog samples was excellent (97.7%), compared to the previous reports on this subject (Table 1). However, its specificity was lower (66.7%) in the examined dogs, which were infected with *D. repens* but negative for *D. immitis*, when compared to the related reports, all from the USA, where *D. repens* does not occur. In cases of positive *D. repens* and negative *D. immitis* PCR results, occult dirofilariosis could be the explanation for the positive *D. immitis* antigen tests. These observations highlight the importance of performing multiple antigen tests in those areas where both *Dirofilaria* species are present. This has special importance when a PCR-examination is not indicated due to a negative Knott test as well as when the PCR is negative because of the lack of microfilariae in the peripheral blood or when this technique reveals only the presence of *D. repens*.

	Present study	Atkins 2003	Aron et al. 2012**	Henry et al. 2018
Sensitivity % (95% CI)	97.7 (89.0-99.9) n=47	78.0 (72.0-84.0) n=208	92.0 n=25	98.5 (95.7-99.7) n=200
Specificity % (95% CI)	66.7 (45.6-83.1)* n=24	97.0 (84.0-100) n=32	100 n=24	94.0 (83.4-98.7) n=50

Table 1. Comparison of the sensitivity and specificity values of the VetScan antigen test obtained in our study with those published by others. CI: Blaker 95% confidence interval; n: number of tested animals; *: Applied to patients found infected with *D. repens* and negative for *D. immitis* by PCR. **: CI was not provided.

2.2. Application of moxidectin and ultrasound-aided injection of melarsomine during the American Heartworm Society (AHS) recommended treatment protocol in *Dirofilaria immitis* infected dogs

During the treatment of heartworm disease of 44 *D. immitis* infected dogs (Table 2), moxidectin was used instead of ivermectin, probiotics were added during the admission of doxycycline, and the patients were sedated

with butorphanol before the application of melarsomine injection. The exact place of the needle was determined by the help of ultrasonography. With the applied therapeutic modifications and complementary measures compared to the AHS 2014 protocol, favorable results were achieved regarding the outcome and the mainly mild post-therapeutic systemic and local complications. All 44 patients were symptomless one month after the 3rd melarsomine injection and healed parasitologically at the control examination on the 271st day regarding the 33/44 dogs examined with antigen tests.

Day	AHS recommended management protocol*	Modifications and additional therapy of the present study**
Day 0	Begin exercise restriction. If the dog is symptomatic: stabilize with appropriate therapy and nursing care. Prednisone prescribed orally from day 1, at 0.5 mg/kg BID 1 st week, 0.5 mg/kg SID 2 nd week, 0.5 mg/kg EOD 3 rd and 4 th weeks.	<i>To prevent anaphylaxis if mf detected:</i> prednisolone prescribed orally from one week before day 1, applying the same therapeutic regimen for 4 weeks. <i>To prevent thromboembolism possibly</i>

		<p>caused by mf: clopidogrel 2-4mg/kg/SID orally.</p> <p>To prevent GI side effects of prednisolone: famotidine 0.5-2.0 mg/kg SID (BID) po. or omeprazole 0.5-1.0 mg/kg SID (BID) po.</p>
Day 1	<p>Administer heartworm preventive***</p> <p>If mf detected, pretreat with antihistamine and glucocorticoid if not already on prednisone.</p> <p>Observe for at least 8 hours for signs of anaphylaxis.</p>	<p>Heartworm preventive: moxidectin topically, applied as per package insert.</p> <p><i>To prevent anaphylaxis if mf detected:</i> dexamethasone 0.1-0.2 mg/kg im. just before moxidectin application; chloropyramine 0.5-1.0 mg/kg im. 4 hours after moxidectin application.</p>
Days 1-28	<p>Administer doxycycline 10 mg/kg BID for 4 weeks.</p>	<p><i>To prevent GI side effects of prednisolone and doxycycline during day 1 to day 28:</i> famotidine or omeprazole as written above.</p>

		<i>To prevent GI side effects of doxycycline: probiotics made for veterinary use during day 1 to day 28 as per package insert.</i>
Day 30	Administer heartworm preventive.	Heartworm preventive: moxidectin topically.
Day 60	Administer heartworm preventive. First melarsomine injection 2.5 mg/kg im. Prednisone for 4 weeks as above.	Days 60, 90, and 91: Heartworm preventive: moxidectin topically. <i>Before melarsomine injection:</i> <i>Sedation with butorphanol</i>
Day 90	Administer heartworm preventive. Second melarsomine injection 2.5 mg/kg im.	0.2-0.4 mg/kg im.; <i>Ultrasound examination of the epaxial (lumbar) muscles to determine the exact location of melarsomine injection.</i>
Day 91	Third melarsomine injection 2.5 mg/kg im. Prednisone for 4 weeks as above.	<i>Keeping the injection needle in place for 5 minutes during injection to prevent leakage of melarsomine.</i> <i>To prevent thromboembolism:</i>

		dexamethasone 0.1 (0.2) mg/kg im. before melarsomine inj. During the 60 th to 69 th days: dalteparin sodium 100-150 IU/kg/SID sc. for 10 days.
Day 120	Test for presence of mf. If positive, treat with a microfilaricide and retest in 4 weeks.	No modifications or additional measures.
Day 271	Antigen test 6 months after completion; screen for microfilariae.	No modifications or additional measures.

*Table 2. Comparison of the three-dose alternate melarsomine AHS management protocol with the therapeutic scheme of the present study. *: Nelson et al. 2014; **Dosages of prednisolone, doxycycline, and melarsomine were the same as indicated in the AHS protocol; ***: Only ivermectin as part of the AHS treatment was published earlier; mf: microfilariae; im.: intramuscularly; GI: gastrointestinal; po.: per oral; sc.: subcutaneously*

2.3. Description of the diagnosis and treatment of a dog with occult heartworm disease

In this part of my thesis, I present a report on a dog with true occult dirofilariosis which caused severe clinical symptoms. Occult dirofilariosis was not published in Hungary before this case report. It is also an example for the diagnostic work-up and therapeutic management of

severe HWD caused by occult dirofilariosis with special regard for applying several Ag tests in these cases to confirm the diagnosis. The latter has never been published as to our search through the international literature.

2.4. Summary of the new scientific results

1. Where concomitant infections by *D. immitis* and *D. repens* occur, the sensitivity of the VetScan antigen test was similar, and its specificity was lower compared to other studies carried out in the USA where only *D. immitis* occurs.
2. In cases of positive *D. repens* and negative *D. immitis* PCR results, occult dirofilariosis can be responsible for the positive *D. immitis* antigen tests as well as for their lower specificity compared to other studies.
3. In the lack of microfilaraemia, like in occult dirofilariosis, multiple antigen tests from the same blood samples should be performed, to increase the diagnostic accuracy of HWD.

4. As part of the complex treatment, moxidectin is not only effective against microfilariae as well as L3 and L4 stages but its partial adulticid effect can contribute to the effectivity of HW therapy.

5. Gastrointestinal side effects of doxycycline therapy can be decreased by probiotics supplementation as an additive to the complex treatment of HWD.

6. Sedation with butorphanol and ultrasonographic determination of the location of the injection needle can increase the safety of the melarsomine injection and decrease the severity of its local side effects.

7. Clinical signs, diagnosis and therapy of canine occult heartworm disease was firstly described in Hungary.

3. Publications the dissertation is based on

Becker, Zs., Holló, N., Farkas, R., Gyurkovszky, M., Reiczigel, J., Olasz, K., Vári, Z. and Vörös, K. (2022a): Serodiagnostic difficulties and possibilities of heartworm disease in regions where both *Dirofilaria immitis* and *Dirofilaria repens* infections occur. Acta Vet. Hung. **70**, 92-99.

Becker, Zs., Vörös, K., Arany-Tóth, A. and Jerzsele, Á. (2022b): A kutyák súlyos szívférgességének klinikai jellemzői és gyógykezelése. Irodalmi összefoglaló és saját tapasztalatok. Clinical characteristics and treatment of severe heartworm disease in dogs. Literature review and own experiences. Magy. Állatorvosok Lapja. **144**, 473-492.

Vörös, K., **Becker, Zs.**, Dudás-Györki, Z., Gronover, B.S. and Szalay, F. (2022a): Ultrasonography of the paralumbar muscles as a new aid during melarsomine treatment in canine heartworm disease. Acta Vet. Hung. **70**, 263-268.

Vörös, K., **Becker, Zs.**, Kónya, R., Arany-Tóth, A. and Farkas, R. (2022b): Application of moxidectin and ultrasound-aided injection of melarsomine during the American Heartworm Society recommended treatment protocol in *Dirofilaria immitis* infected dogs. Vector Borne Zoonotic Dis. **22**, 382-390.

Vörös, K., **Becker, Zs.**, Arany-Tóth, A., Gyurkovszky, M. and Farkas, R. (2017): Okkult *Dirofilaria immitis* szívférgesség kutyában. Esetismertetés és irodalmi áttekintés. Occult *D. immitis* heartworm disease in a dog. Case report and literature review. Magy. Állatorvosok Lapja. **139**, 675-685.

4. Presentations related to the topic of the dissertation

Becker, Zs., Vörös, K., Dudás-Györki, Z., Gyurkovszky, M. and Farkas, R.: Complex therapy of heartworm disease in dogs: preliminary experiences. Annual Conference of the Veterinary Scientific Committee of the Hungarian Academy of Science, 2018, Budapest.

Becker, Zs., Vörös, K., Holló, N., Dudás-Györki, Z, Reiczigel, J. Takács, N. and Farkas, R.: Sensitivity and

specificity of VetScan antigen test, in the individual or mixed infection of *D. immitis* and *D. repens* in dogs. Annual Conference of the Veterinary Scientific Committee of the Hungarian Academy of Science, 2019, Budapest.

Szalay, F., Vörös, K., Gronover, B. S., **Becker, Zs.** and Dudás-Györki, Z.: Anatomic and ultrasonographic examination of the paralumbar musculature after the application of melarsomine during the treatment of canine heartworm disease. Annual Conference of the Veterinary Scientific Committee of the Hungarian Academy of Science, 2020, Budapest.