# Successful treatment of *Dirofilaria repens* infections in dogs with melarsomine (Immiticide®, Merial) against adults and a combination of moxidectin 2.5%/imidacloprid 10% (Advocate®, Bayer) against microfilaria

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## Abstract

*Dirofilaria repens* occurs in Europe predominately in southern and south eastern countries. Transport of dogs from such endemic regions to areas free of *D. repens* bears the risk of introducing this zoonotic disease to non endemic areas. In this study 507 dogs transported from a Hungarian shelter to a shelter close to Cologne from August 2006-February 2009 were tested for presence of microfilaria (mf). 60 *D. repens* positive dogs were enrolled in a treatment program consisting of an adulticide treatment with melarsomine (Immiticide®, Merial, 2 injections 24 h apart), followed by a microfilaricidal treatment with monthly applications of moxidectin 2.5%/imidacloprid 10% (Advocate®, Bayer) at the standard dose over a period of three month. 36 dogs completed a surveillance period of 6 month following the treatment program. Macrofilaricidal treatment in dogs was tolerated well with few adverse reactions. Microfilaricidal treatment at monthly intervals did not show adverse reactions. All 36 dogs were screened for the presence of microfilaria, all but one stayed negative. It is known from *D. immitis* treatment, that melarsomine at the recommended dose will clear about 50-70% of the dogs from macrofilaria and is ineffective against L4 stages and early adult stages. This treatment protocol in conjunction with a follow up treatment of 3 monthly doses of moxidectin/imidacloprid is suitable to eliminate infections with *D. repens* almost completely and could be an important measure to avoid introduction of this zoonotic disease from endemic to non endemic areas.

## Introduction

In recent years it has been shown that vector borne parasitic diseases seem to be spread to areas previously considered to be free of such diseases. Among these, *D. repens* is of particular concern due to its zoonotic potential. More recently there have been case reports from Germany, Austria and the Netherlands from dogs without travel history, suggesting that environmental conditions would support transmission of the parasite. One risk to support establishment of such diseases is transport of dogs from endemic areas such as Hungary to non endemic areas. It is a common practice of animal welfare organizations to import stray and shelter dogs form Hungary to Germany to allow them access to improved lifestyle conditions. It is necessary to test these dogs for parasitic diseases and in positive cases to apply therapeutic measures and follow up control to restrict importation of such diseases not (yet) established in the target country.

## Methods

507 dogs from the region of Pecs (South Hungary) with a reported prevalence of *D. repens* up to 25% have been imported to Germany from August 2006-February 2009. All dogs have been tested for various parasites (Table 1). 61 dogs have been tested positive for microfilaria via modified Knott's test. Mf species diagnosis was performed by morphological and morphometric criteria, with acid phosphatase reaction and confirmed with multi species PCR. 60 dogs were included in the study consisting of a melarsomine treatment (Immiticide®, Merial, 2 injections 24 h apart) targeting adult *D. repens*, followed by a microfilaricidal treatment with 3 monthly applications of moxidectin 2.5%/imidacloprid 10% spot on formulation (Advocate®, Bayer). 6 month following the last treatment 36 dogs could be tested again for microfilaria (Table 2).

**Table 1** Methods used for parasitological screening

| Parasite | Methods |
|---|---|
| *Microfilaria* | Modified Knott's test, mf counts |
| *Microfilaria differentiation* | Morphology, Leucognost SP®-Testkit, PCR |
| *Dirofilaria immitis* | FASTest HW. Antigen; MegaCor Diagnostik GmbH |
| *Leishmania infantum* | Megascreen Fluoleish, MegaCor Diagnostik GmbH |
| *Ehrlichia canis* | Megascreen Fluoehrlichia c., MegaCor Diagnostik GmbH |
| *Anaplasma phagocytophilum* | Megascreen Fluoanaplasma ph, MegaCor Diagnostik GmbH |
| *Babesia canis* | Megascreen Fluobabesia c., MegaCor Diagnostik GmbH |

**Table 2** Procedures performed during the study

| Study day | -1 | 0 | 1 | 3 | 7 | 8 | 37 | 38 | 67 | 68 | 247 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mf count and differentiation | X | | | | | | | | | | X |
| Treatment melarsomine | | X | X | | | | | | | | |
| Treatment moxidectin/imidacloprid | | | | | X | | X | | X | | |
| Clinical assessment | X | X | X | X | X | X | X | X | X | X | |
| Registration of adv. events | | X | X | X | X | X | X | X | X | X | |

## Results

**Parasitological screening:** From 507 dogs 61 (12.0%) had microfilaria. No dog was positive for *Dirofilaria immits* antigen. No dog had antibodies against *Leishmania infantum*. 8 dogs (1.6%) showed an antibody titer >/= 1:200 against *Ehrlichia canis*. 97 dogs (19.1%) had an antibody titer >/= 1:80 against *Babesia canis*. 59 dogs (11.6%) showed an antibody titer >/= 1:200 against *Anaplasma phagocytophilum* (Fig. 1).

**Mf positive dogs:** 61 dogs were positive for microfilaria. 60 dogs (98.4%) were infected with *Dirofilaria repens* (Fig. 2). One dog (1.6%) with *Acanthocheilonema (Dipetalonema) reconditum*. The average mf count was 2,864 mf/ml EDTA blood (min. 12, max. 49,033) (Fig. 3).

**Treatment:** From 60 dogs 36 were available for a follow up diagnosis 6 month after the last mf treatment. 35 dogs (97%) were free of mf, one dog showed mf counts of 100 mf/ml (Fig. 4). 23 dogs did not show up for the final diagnosis, one dog died following melarsomine therapy. Mf count reduction was 99.85%.

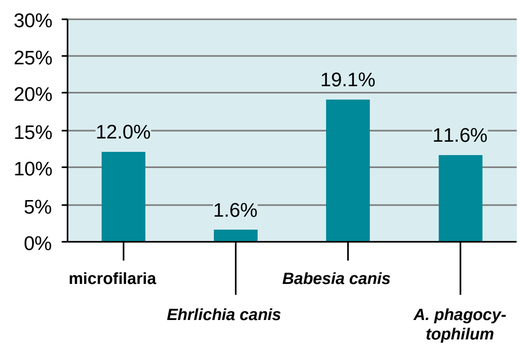


**Fig. 1** Results parasitological screening of 507 dogs imported from Hungary

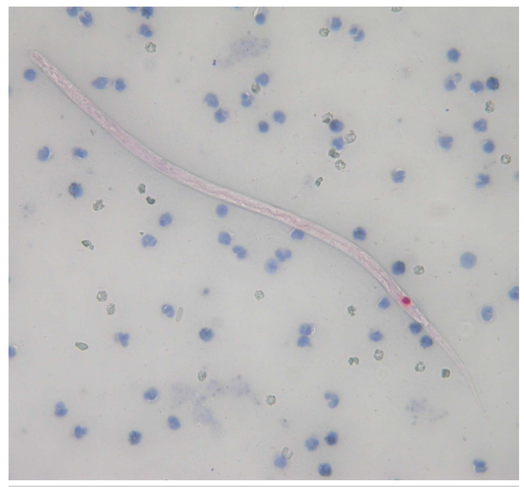

**Fig. 2** Microfilaria of *D. repens* stained with Leucognost SP®-Testkit

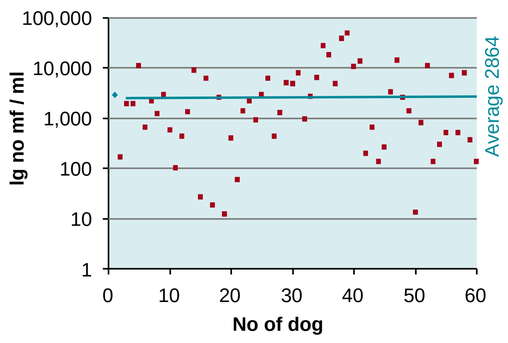


**Fig. 3** Mf counts of 60 dogs completing the study at day -1

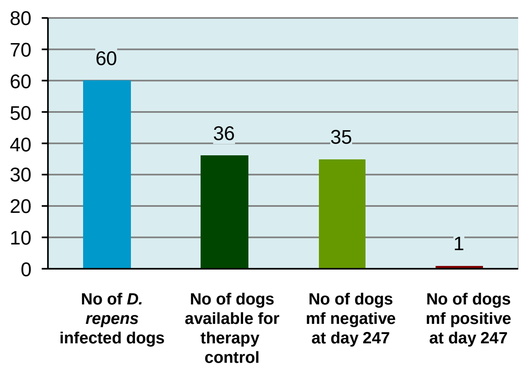


**Fig. 4** Results of dogs included in the therapy study

## Discussion and Conclusion

- Transport of dogs from Hungary to Germany bears the risk of importing parasites so far not (yet) endemic.
- All dogs imported from countries with such parasitic diseases should undergo diagnostic and therapeutic procedures and follow up control to restrict importation of such parasitic diseases especially if suitable conditions (vectors and climate) exist for transmission.
- In case licensed treatment is not available, published protocols based on scientific evidence shall be used.
- In this study a combination treatment of melarsomine (Immiticide®, Merial) against adults and moxidectin/imidacloprid (Advocate®, Bayer) against microfilaria was applied, and mf could be eliminated almost completely.
- The applied protocol of 2 injections of melarsomine is known to clear 50-70% of *D. immitis* positive dogs and has limited efficacy against juvenile stages. If a similar efficacy is assumed against *D. repens*, several worms may have survived initial therapy and restarted or continued to produce microfilaria.
- The subsequent treatment with 3 monthly applications of moxidectin/imidacloprid effectively eliminates existing microfilaria in 35 out of 36 dogs and thus seems to have efficacy also against surviving adult worms. In one dog still harbouring mf, a longer treatment period may be suggested to clear mf and potential surviving adult worms.