

## Research Article

# Effect of Therapy by Using Advocate Spot-On Combination (Imidacloprid 10% and Moxidectin 2.5%) on Subcutaneous Dirofilariasis in Dogs

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Received 15 November 2010; Revised 18 February 2011; Accepted 2 April 2011

Academic Editor: Sven Poppert

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*Dirofilaria (Nochtiella) repens* is a filarioid parasite that causes subcutaneous dirofilariasis in dogs. Adults, while localized in subcutaneous tissues, lay embryos (microfilariae (mf)) into the blood stream of dogs, which constitute a reservoir for infection of other definitive or accidental hosts as humans. This study was carried out to assess the efficacy of spot-on combination of imidacloprid and moxidectin on microfilariaemia in naturally infected dogs. A group of 11 dogs was monthly examined for the presence of microfilariae in peripheral blood by modified Knott's test method. Treatment was administered monthly for 4 months. All dogs (i.e., 100%) became negative for microfilariaemia throughout the study. These results confirm the effect of the combination of imidacloprid and moxidectin on *D. (Nochtiella) repens*.

## 1. Introduction

*Dirofilaria (Nochtiella) repens* is a filarioid nematode of genus *Dirofilaria* that belongs to family Onchocercidae of the order Spirurida. Adults are mainly found in subcutaneous tissues where they may cause different clinical signs, such as dermal swelling, subcutaneous nodules, or pruritus [1].

Embryos are released into the blood stream by gravid female parasites and are ingested by mosquitoes during the blood meal. Over 60 mosquito species belonging to genus *Anopheles*, *Culex*, and *Aedes* could serve as vectors and produce infective (L3) larvae [2, 3]. Development of L3 is a temperate-dependent process, and climatic changes in last years enable the spread of canine subcutaneous dirofilariasis (CSD) from tropic and subtropics regions to temperate zone countries [4] such as Czech Republic [5], Slovakia [6], Hungary [7], and Austria [8].

Together with the spread of endemic CSD, there is an increase in transmission of *D. repens*, even to humans. People serve as occasional hosts in which aberrant migration of the larvae can cause different clinical signs [9]. Location of worms in the body can vary; nodules are found in subcutaneous tissue [10], lungs [9, 11] or conjunctival tissue [12].

Their importance in the case of differential diagnosis of human neoplasia is high, and several authors described human dirofilariasis as emerging disease [9, 11].

This implies that the management of CSD has high importance in the prevention of human infections by *D. repens*. Individual cases of CSD treatment were described [1, 13]. Field study for elimination of *D. repens* microfilariae in dogs using combination of moxidectin and imidacloprid was described [14]. The study describes specific protocol for *D. repens* infection treatment in dogs by commercial spot-on product (Advocate, Bayer).

## 2. Materials and Methods

**2.1. Animals and Treatment.** A total of 11 dogs were included in this study during the monitoring of *Dirofilaria* spp. infection in the southeastern area of the Czech Republic through years 2009/2010. The group was composed of outdoor-living dogs of various breeds, age, and gender, which were tested positive for *D. repens* mf.

*D. repens* mf positive dogs were diagnosed after first blood sampling (examination no. 1). Consequently, after a

TABLE 1: The influence of Advocate on the presence of *Dirofilaria repens* microfilariae in the blood of tested dogs.

Examination <sup>†</sup>	Treatment <sup>††</sup>																						
	Number of microfilariae/mL blood																						
1	—	—	—	—	—	—	—	—	—	—	—	175	29	265	135	2830	187	2950	215	580	725	246	
2	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	113	106	168	1838	2169	119	2105	184	412	633	730	
3	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	0	0	0	0	0	0	0	0	0	0	0	
4	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	0	0	0	0	0	0	0	0	0	0	0	
5	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	0	0	0	0	0	0	0	0	0	0	0	
6	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
7	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
8	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
9	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
10	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
11	—	—	—	—	—	—	—	—	—	—	—	0	0	0	0	0	0	0	0	0	0	0	
		Dog 1	Dog 2	Dog 3	Dog 4	Dog 5	Dog 6	Dog 7	Dog 8	Dog 9	Dog 10	Dog 11											

<sup>†</sup>Examinations were performed monthly (in one month interval).

<sup>††</sup>Treatment with Advocate, Bayer (imidacloprid at 10–25 mg/kg and moxidectin at 2.5–6.5 mg/kg) monthly for four months (— no treatment, ✓ treatment).

second blood sampling, dogs were treated following manufacturer's instructions by combination of imidacloprid (minimum dosage 10 mg/kg, max. 25 mg/kg) and moxidectin (min. dosage 2.5, max. 6.25 mg/kg) in spot-on (Advocate, Bayer). Treatment was given monthly for 4 consecutive months. After the end of treatment, blood sampling and mf examination continued for 6 months. In total, this clinical study lasted 11 months. According to the owners, no other drug was given 5 months before and through the entire study.

**2.2. Sampling and Examination.** Peripheral venous blood (3 mL) was collected every month from each dog (*vena cephalica antebrachii*) into tubes with EDTA and transported into the laboratory on cooler blocks at about 4–5°C. Blood samples were examined by a modified Knott's test [7] for detection of circulating mf. After centrifugation, all the sediment was collected, mf checked by light microscope (10x magnification), and the number of mf/1 mL of blood calculated.

Commercial kit (Leucognost-SP) was used for mf identification by histochemical staining of acid phosphatase activity [15].

### 3. Results

Before treatment all dogs included in the study were mf positive (examination no. 1) in the range of 29–2950 mf/mL.

All dogs were found positive for *D. repens* circulating mf. One month after first treatment, no dog was found microfilaricidal and lasted negative until the end of the study, that is, 6 months after the last treatment (see Table 1).

### 4. Discussion

The aim of the clinical study was to assess the efficacy of imidacloprid and moxidectin combination (Advocate, Bayer) on microfilariaemia level under field conditions. Dirofilariosis caused by *D. repens* is considered to be an emerging zoonoses [9], and it is spreading from tropical and subtropical countries to temperate zone. The main source of human infection is infected mosquitoes which had a blood meal on *Dirofilaria*-positive animals, mainly dogs [16]. The prevalence of human dirofilariosis rapidly increased in the last decades, even in areas where the infection was not expected [16–19]. Humans serve as dead-end hosts, where usually the parasites do not develop to adult stage [20] though few cases of circulating mf have been reported in humans [16, 21].

The prepatency of *D. repens* infection lasts 6 to 9 months [22, 23]. Six months of sampling and examination after treatment were carried out to verify both the microfilaricidal effect and the preventive efficacy of the treatment. One hundred percent of dogs lasted negative 6 months after last treatment.

This study was carried out in Czech Republic, a country that is in a temperate zone, where the first autochthonous case of canine dirofilariosis was found in 2006 [5]. During the following years, *D. repens* infection spread rapidly in wider areas, particularly along the main rivers [24].

Dogs can serve as source of infection regardless of circulating mf amount. On the other hand, longevity of *D. repens* leads to accumulation of parasites over years, and it could increase microfilariaemia. Therapy and prevention of subcutaneous dirofilariosis in dogs minimize the frequency of mf in their blood and dramatically reduce the risk of transmission. Reduction of infection source for vector decreases the risk of transmission and the further spread in dog population. Simultaneously, it poses the only way to control and inhibit transmission to human. Thus, therapy of *D. repens* positive dogs is recommended.

Until now, information about therapy and prevention of *D. repens* infection in dogs has been sporadic, and only few studies have been carried out with a combination of injectable melarsomine and oral administration of macrocyclic lactones, though the actual efficacy was questionable because of the death of the patient [1, 22].

## 5. Conclusions

The results of this study showed that a combination of imidacloprid (10–25 mg/kg) and moxidectin (2,5–6,25 mg/kg) in spot-on formulation (Advocate, Bayer) was safe and 100% effective in clearing circulating mf from the blood of naturally infected dogs one month after a single treatment. The treatment was repeated monthly for 4 consecutive months, and dogs lasted negative both throughout the treatment and during the further 6 months of the study.

## Acknowledgments

Special thanks belong to the Bayer Animal Health GmbH, Leverkusen, Germany that supported this study from the beginning. At the same time the authors would like to thank all the owners of dogs for their cooperation and patience during the eleven-month duration of the study.

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