

DOI 10.2478/v10181-011-0098-4

Short communication

# Autochthonous canine *Dirofilaria repens* in the vicinity of Warsaw

A. Masny<sup>1</sup>, T. Lewin<sup>2</sup>, R. Salamatin<sup>1,3</sup>, E. Golab<sup>1</sup>

<sup>1</sup> National Institute of Public Health – National Institute of Hygiene, Department of Medical Parasitology, Chocimska 24, 00-791 Warsaw, Poland

<sup>2</sup> VISVET – Tomasz Lewin, Chopina 66/70, 05-800 Pruszków, Poland

<sup>3</sup> Department of General Biology and Parasitology, Medical University of Warsaw, Chałubińskiego 5, 02-004 Warsaw, Poland

## Abstract

In 2009 microfilaremia was recognized in ten dogs living in five distinct districts located near Warsaw, the capital of Poland. Based on PCR results, all the animals were found to be infected with *Dirofilaria repens*. Nine of the infected dogs have never travelled outside the country and it was assumed that the cases were native. Monitoring the infection in the European countries should be introduced to establish the actual geographic range of dirofilariasis.

**Key words:** canine dirofilariasis, *D. repens*, PCR, DNA isolation

## Introduction

*Dirofilaria repens* is an etiological agent of canine subcutaneous dirofilariosis, a vector-borne disease. Dirofilariosis can be asymptomatic or cause mild clinical symptoms e.g. subcutaneous nodules, dermal swelling and itching (Simón et al. 2009). Infected dogs are the source of *D. repens* but the dissemination of the disease depends on the presence of a mosquito – vector and temperatures suitable for development of the infective larvae in the vector (Genchi et al. 2005, Cancrini et al. 2007). The aim of the present study was to determine the prevalence of autochthonous canine *Dirofilaria* spp. infection in the Warsaw area.

## Materials and Methods

The dogs examined lived in small towns in the Mazowieckie province: Komorów, Kanie, Podkowa Leśna, Płochocin and in four suburban districts of the city of Pruszków. In 2009, microscopy screening of blood samples was carried out. From the samples containing microfilariae, DNA was isolated (Boom et al. 1999) and then PCRs were performed. To amplify *Dirofilaria* genomic fragments, four primer pairs were used: DIDR-F1/DIDR-R1, D.rep-F1/D.rep-R1, DR COI-F1/DR COI-F1, D.imm-F1a/D.imm-R1 (Rishniw et al. 2006). The amplification products were separated on 2% agarose gels with ethidium bromide.

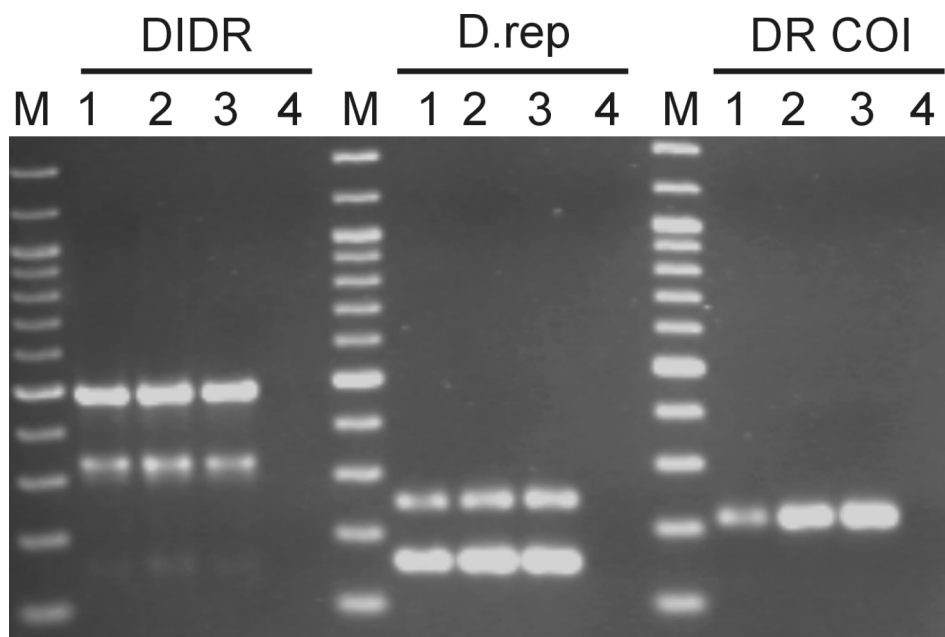


Fig. 1. PCR results. Primers: **DIDR** (5SrRNA), **D.rep** (5.8S-ITS2-28S), **DR COI** (mitochondrial COI partial gene), **M** – molecular weight marker, the first 10 bands in 100 bp increments. Lanes: **1-3** – infected dogs, **4** – negative control.

The products of PCR performed with primers DIDR-F1 / DIDR-R1 (major band – 484 bp) and DR COI-F1 / DR COI-R1 (band – 209 bp) were sequenced and analyzed using NCBI BLAST.

## Results and Discussion

Microfilaremia was recognized in 10 dogs; subcutaneous nodules were observed in four of them. The microfilariae present in the analyzed samples were identified as *D. repens* based on the size of the PCR products (Fig. 1). The results of DNA sequencing showed a high level of homology of the PCR products to *D. repens* 5SrRNA gene regions **AY693808** and to the mitochondrial partial gene for the cytochrome oxidase subunit I (COI) **AJ271614**. Only one of the animals examined in our work has travelled abroad and therefore, we excluded the imported character of the disease in nine out of ten cases. The infected animals lived within a radius of around 10 kilometers from the center of Pruszków (20°48'E, 52°10'N) which indicated that the disease has already spread in the local canine population. The detection of the first microfilaremia cases took place in 2007 (unpublished data – Tomasz Lewin), however, the parasite species was unknown. The first cases of *D. repens* in Polish dogs, confirmed by molecular methods, were described in 2010 (Sapierzyński et al. 2010). One year earlier, data on dirofilariosis outbreaks in three breeding kennels near Warsaw was published (Demiasz-

kiewicz et al. 2009). The prevalence of the disease in these kennels was surprisingly high, even compared to the endemic regions in Southern Europe, and reached up to 60% of the dogs examined. Infected pure breed dogs might pose a high risk of further dissemination of the disease since such animals may be sold to remote locations or travel to dog shows. Infectious larvae of *D. repens* develop in *Culex pipiens molestus* mosquitoes within 14 days, at the temperature range 18-28°C (Kuzmin et al. 2005). The analysis of temperature in the Warsaw region revealed that during summer months at least one generation of *Dirofilaria immitis* would reach the infective stage in mosquitoes (Genchi et al. 2005), however, unfortunately, a similar analysis for *D. repens* has not been performed. Theoretically, a local vector of *D. repens* could be *C. pipiens* which is one of the most prevalent species within mosquitoes in the vicinity of Warsaw (Kubica-Biernat et al. 2009) and its role as a vector for subcutaneous dirofilariosis in natural environments was described (Cancrini et al. 2007). Our results and the published data (Demiaszkiewicz et al. 2009) show that *D. repens* spreads among dog populations in the Warsaw area. Furthermore, multiple cases of canine dirofilariosis in the vicinity of Warsaw support the suspicion (Żarnowska-Prymek et al. 2008) that human *D. repens* infections detected in the same region were not imported. Thus, new data from Poland suggest that a revision of the views on *Dirofilaria* infection pattern in Europe is required (Masny et al. 2011). Monitoring canine dirofilariosis in the EU countries would allow

establishing its actual prevalence and might help to undertake measures to prevent further dissemination of the infection.

### Acknowledgments

The research was supported by National Science Centre (Poland) – grant No. N404 256840.

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