Polish Journal of Veterinary Sciences Vol. 14, No. 3 (2011), 485-487

DOI 10.2478/v10181-011-0073-0

Short communication

The first report on detection of canine *Acantocheilonema reconditum* in Poland and the associated diagnostic problems

A. Masny[1], T. Lewin[2], R. Salamatin[1,3], E. Golab[1]

[1] National Institute of Public Health – National Institute of Hygiene, Department of Medical Parasitology, Chocimska 24, 00-791 Warszawa, Poland
[2] VISVET Veterinary Practice, Chopina 66/70, 05-800 Pruszków, Poland
[3] Department of General Biology and Parasitology, Medical University of Warsaw, Chałubińskiego 24, 02-004 Warsaw, Poland

Abstract

Acanthoheilonema reconditum was found during monitoring dogs living in the vicinity of Warsaw, for *Dirofilaria* spp. infection. The microfilaremia in blood was at first detected by microscopy and then molecular tests for distinct filarial markers were performed. PCR product sequencing confirmed that the microfilaria detected in two dogs were *A. reconditum*. These are the first two cases of canine acanthocheilonemiasis detected in Poland.

Key words: canine filariosis, *Acanthocheilonema reconditum*, PCR diagnostics, microfilaremia detection, *Dirofilaria*, Poland

Introduction

Climatic changes and the ease of transporting dogs within EU may influence the spread of canine filarial diseases into the regions of Europe considered non-endemic (Menn et al. 2010). In Poland the first cases of canine microfilaremia were detected in 2007 in the vicinity of Warsaw (20°48'E, 52°10'N) (Tomasz Lewin – unpublished data). Two years later the first report on *Dirofilaria repens* invasions was published (Demiaszkiewicz et al. 2009).

The aim of our work was to determine the prevalence of *Dirofilaria spp.* invasion among dogs living in the Warsaw area.

Materials and Methods

The dogs examined were patients of the VISVET Veterinary Clinic between September 2009 and October 2010. Knott's test and direct thick smears were used for detection of microfilaremia in peripheral blood samples. From the microfilaraemic blood samples DNA was isolated according to the modified protocol of Boom et al. (1999): lysis buffer was supplemented with EDTA to 40 mM and two extra washing steps with lysis buffer were added. PCR with primers pair DR COI-F1 / DR COI-R1 specific for *D. repens* was performed. Samples negative in the first PCR were tested with *D. immitis*-specific primers

Correspondence to: E. Gołąb, e-mail: egolab@pzh.gov.pl, tel.: +48 22 542 1220

– DI COI-F1 / DI COI-R1 (Rishniw et al. 2006). The samples negative in both *Dirofilaria* specific reactions were tested with universal primers specific for rDNA regions of at least 10 filarial species DIDR-F1 / DIDR-R1, followed by *Acanthocheilonema reconditum* specific marker amplification with primers A.rec-F1 / A.rec-R1 and AR COI-F1 / AR COI-R1 (Rishinw et al. 2006). DNA isolated from the blood of a healthy dog was used as a negative control and DNA samples of: *D. repens*, *D. immitis* and *A. reconditum* were used as positive controls. The products of two PCRs performed with DIDR-F1 / DIDR-R1 primers were sequenced, and the obtained DNA sequences were aligned (McGinnis and Madden 2004, Waterhouse et al. 2009) to the *A. reconditum* sequences deposited in GenBank™ (AF217801.2, GU593976.1, GU593978.1).

Results and Discussion

Detection of *A. reconditum* in Polish dogs was unexpected and occurred during screening the animals for *Dirofilaria* spp. invasions. It was assumed that species identification of *Dirofilaria* using molecular methods would be less laborious and more reliable than microscopy and therefore the PCRs were performed on blood samples containing microfilaria. In two out of 28 samples examined by PCR method amplification of both *D. repens* and *D. immitis* specific DNA markers failed. Therefore, it was decided to amplify DNA of the samples with universal primers applicable to detection of a wide range of filarial parasites (Rishniw et al. 2006). The size of the obtained PCR products matched the size of the products from control samples of *A. reconditum* DNA. The sequences of the PCR products showed a high level of homology to *A. reconditum* sequences deposited in GenBank (Fig. 1).

Acantocheilonema spp. are usually described as non-pathogenic, but still little is known about their effect on animal health and well-being. Infected animals create a risk for infection in humans and cases of human acanthocheilonemosis have been noted in endemic areas (Huynh et al. 2001). *A. reconditum* could easily spread among dogs because one of its vectors are fleas, active most of the year in the temperate climate. Further studies are required to establish the prevalence of distinct filarial species among the Polish dog population. For molecular screening purposes, DNA markers universal for a number of filarial species should be used.

Acknowledgments

The authors thank Dr. Mark Rishniw for the *A. reconditum* DNA samples and prof. Claudio Genchi for *D. repens* and *D. immitis* DNA samples. The research was supported by National Science Centre (Poland) – grant No. N404 256840.

References

Boom R, Sol C, Beld M, Weel J, Goudsmit J, Wertheim-van Dillen P (**1999**) Improved silica-guanidiniumthiocyanate DNA isolation procedure based on selective binding of bovine alpha-casein to silica particles. J Clin Microbiol 37: 615-619.

Demiaszkiewicz AW, Polańczyk G, Pyziel AM, Kuligowska I, Lachowicz J (**2009**) The first foci of dirofilariosis of dogs evoked by *Dirofilaria repens* Railliet et Henry, 1911 in central Poland. Wiadomości Parazytologiczne 55: 367-370.

Huynh T, Thean J, Maini R (**2001**) *Dipetalonema reconditum* in the human eye. Br J Ophthalmol 85: 1391-1392.

McGinnis S, Madden TL (**2004**) BLAST at the core of a powerful and diverse set of sequence analysis tools. Nucleic Acids Res 1: 32.

```
                10        20        30        40        50        60        70
Taiwan    TTGCACTATCGGGTTGATTCCCGGTAGTACGTCTGGTTGAGGGTCAATGAAAAAAGTAAACATTGTTTTTATTCGTCAG 79
DIDR-F1   TTGCACTATCGGGTTGATTCCCGGTAGTACGTCTGGTTGAGGGTCAATGAAAAAAGTAAACATTGTTTTTATTCGTCAG 79
Delhi                       CCGGTAGTACGTCTGGTTGAGGGTCAATGAAAAAAGTAAACATTGTTTTTATTCGTCAG 59
Mumbai                      CCGGTA-TACGTCTGGTTGAaGGTCcATGAAAAAAGTAA-CgTTGTTTTTATTCGTCAG 57

          80        90        100       110       120       130       140       150
Taiwan    GTGATGGTTTGATGTGCTGCTGTCATTACATGATCCCCTGATAAGTAAATGAAAAAGCAGTATACAGTTGGCACACAAT 158
DIDR-F1   GTGATGGTTTGATGTGCTGCTGTCATTACATGATCCCCTGATAAGTAAATGAAAAAGCAGTATACAGTTGGCACACAAT 158
Delhi     GTGATGGTTTGATGTGCTGCTGTCATTACATGATCCCCTGATAAGTAAATGAAAAAGCAGTATACAGTTGGCACACAAT 138
Mumbai    GTGATGGTTTGATGTGCTGCTGTCATTACATGATCCCCTGATAAGTAAATGAAAAAGCAGTATACAGTTGGCACACAAT 136
```

Fig. 1. Alignment of the sequencing products and *Acanthocheilonema reconditum* sequences deposited in GeneBank. **DIDR-F1** – fragment of the sequence (identical for both isolates) of PCR products obtained with DIDR-F1 primer. **Taiwan** – Taiwanese canine *A. reconditum* isolate (GenBank AF217801.2) bases 468-625. **Delhi** and **Mumabai** – canine *A. reconditum* isolates from Delhi (GenBank: GU593976.1) bases 1-138 and Mumabai (GenBank GU593978.1) 1-136. **Grey bars** – polymorphic regions; **"–"** – deletions; **lower case** – substitutions.

Menn B, Lorentz S, Naucke TJ (**2010**) Imported and travelling dogs as carriers of canine vector-borne pathogens in Germany. Parasit Vectors 3: 34.

Rishniw M, Barr SC, Simpson KW, Frongillo MF, Franz M, Dominguez Alpizar JL (**2006**) Discrimination between six species of canine microfilariae by a single polymerase chain reaction. Vet Parasitol 135: 303-314.

Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ (**2009**) Jalview Version 2 – a multiple sequence alignment and analysis workbench. Bioinformatics 25: 1189-1191.