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Case Report

Human ocular dirofilariosis in Slovakia, a case report

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Summary

A case of the human ocular dirofilariosis in 72-year-old man from southern Slovakia is documented. Two days before visiting a doctor he noticed a live worm in his right eye. The eye was inflamed and itching. The worm of 100 mm in length was isolated from the subconjunctival space. On the basis of morphological appearance, histological examination and PCR-based detection, it was identified as *Dirofilaria repens*. The patient was probably infected in the southern Slovakia, which is an enzootic area of dirofilariosis of dogs.

Key words: *Dirofilaria repens*; *Dirofilaria immitis*; ocular dirofilariosis; Slovakia

Introduction

Dirofilariosis is a zoonotic disease with growing incidence in the Mediterranean region of Europe (Genchi *et al*., 2005; Argy *et al*., 2011; Cielecka *et al.*, 2012; Salamatin *et al*., 2013). It is caused by the filarioid worms of genus *Dirofilaria*. There are two species circulating in Europe – more frequent *Dirofilaria* (*Nochtiella*) *repens*, a subcutaneous parasite of humans occurring also in the eye, and less frequent *D. immitis* causing lung dirofilariosis. Carnivores, especially dogs, foxes, and cats, are definitive hosts and also reservoirs of dirofilariosis. Infectious stage – third larval stage is transmitted by mosquitoes of genera *Aedes, Culex, Anopheles*, etc., that commonly occur in the Slovak region. The first diagnosed autochthonous canine dirofilariosis in Slovakia was caused by *D. repens* in 2005 (Svobodová *et al*., 2005), and in 2007 the first case of human subcutaneous dirofilariosis was identified in the patient from western Slovakia, who has never travelled abroad (Babál *et al*., 2008). Since the year 2007, 4 cases of human subcutaneous dirofilariosis caused by *D. repens* in Slovakia have been reported in total (Babál *et al*., 2008; Ondriska *et al*., 2010; Nováková *et al*., 2011; Hrčková *et al*., 2013). In Europe, the higher number of cases represented subcutaneous form, where cysts were frequently localized on head and around the eyes in comparison with the ocular form, when parasite was found in subconjuntival space of eye. The first documented case of ocular dirofilariosis in Slovakia was published in 1992 (Vasilková *et al*., 1992). In our work, we present the second case of human autochthonous ocular dirofilariosis caused by *D. repens*.

Case report

On July 4th, 2012, the worm removed from the patient's eye was delivered to the Department of Parasitology for species identification. The patient was a 72-year old man, who visited the Ophthalmology Clinic in Nitra on the July, 4th, 2012 having inflamed eye that was burning and itching. He started to have these symptoms a day before the doctor's visit. Bulbus was significantly irritated with striking hyperemia of blood vessels in bulbar conjunctiva. Live parasite was present subconjunctivaly (Fig. 1). In addition to this finding, the vision exhibited no abnor-

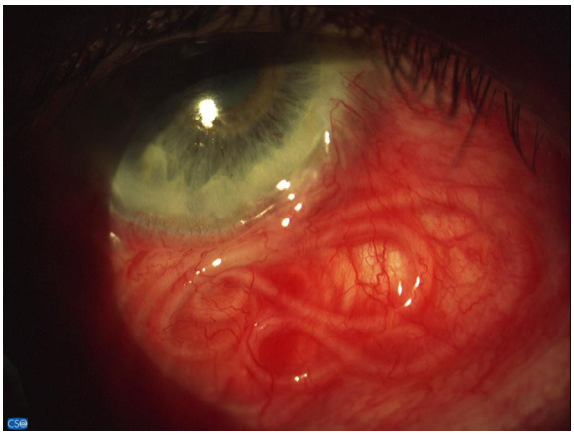

Fig. 1. *Dirofilaria repens* located subconjunctivaly in patient's eye

mality, visual acuity on both eyes was 20/20, with normal IOP (intraocular pressure). Fundus of the right eye showed a normal finding, localized pink optic disc of the optic nerve with age-appropriate vessels, macula was pink without foveolar reflex. The worm was surgically removed and the bulbus was much calmer on the first day after surgery. The focal hyperemia in the area of bulbar conjunctiva suture has remained. On the 7th day after the surgery, the bulbus was almost quiet with minimal injection around the stitch (Fig. 2).

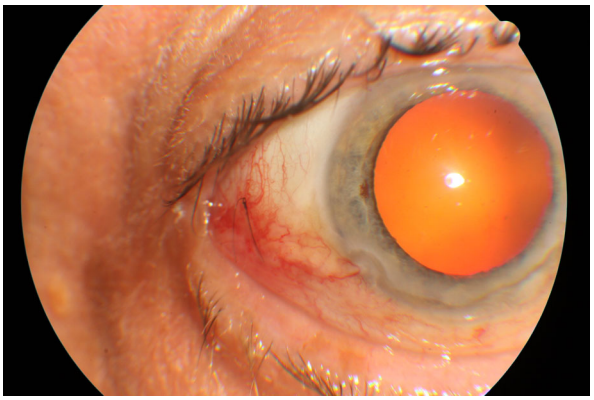

Fig. 2. Eye of patient one week after surgery. The eye bulbus was almost quiet with minimal injection around the stitch

Extirpated worm was about 100 mm long (Fig. 3) and was subjected to the molecular and histological identification. Small pieces of the worm (approximately 1 cm) were used for DNA extraction. The fixative solution (non-buffered formalin) was removed from the portions of worm prior isolation of DNA by soaking in Tris/EDTA buffer (pH 8.2) for 4 hours and DNA was isolated using NucleoSpin Tissue DNA-extraction KIT (Macherrey-Nagel, Germany). In the PCR reactions the primers set specific for a conserved repetitive sequence in the cytochrome C oxidase subunit 1 (CO1)-encoding gene of *D. repens* (DIR3 / DIR4) was used (Vakalis *et al*., 1999). PCR amplifications were performed in 25 µl reactions containing 2.5 µl 10xPCR buffer, 3 µl of $MgCl_2$, 0.25 µl (1U) Hot Start Taq polymerase (from Roche, USA), 1 µl of dNTPs mix and 2 µl (10 µM concentration) of each primer. The positive control in PCR reaction represented DNA isolated from live *D. repens* adult worm obtained from the nodule of other patient (Ondriska *et al.,* 2010). Although we have modified the PCR cycling conditions to increase the stringency of reaction, the 246 bp long specific DNA fragment was not amplified on tested DNA only in positive control PCR reaction (Fig. 4A). Re-amplification reaction performed on 10 µl of the first PCR reaction using the same primer set and the conditions, yielded a band approximately 150 bp long

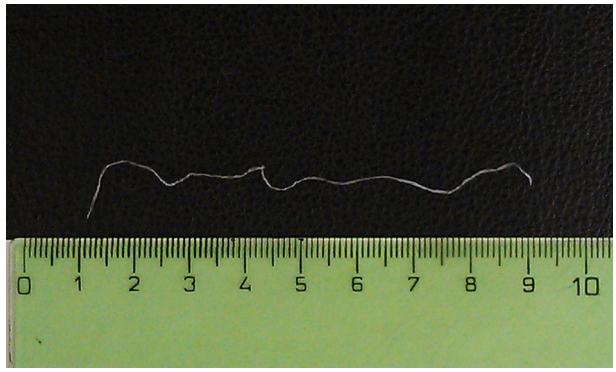

Fig. 3. The worm of *Dirofilaria repens* isolated from the subconjunctiva

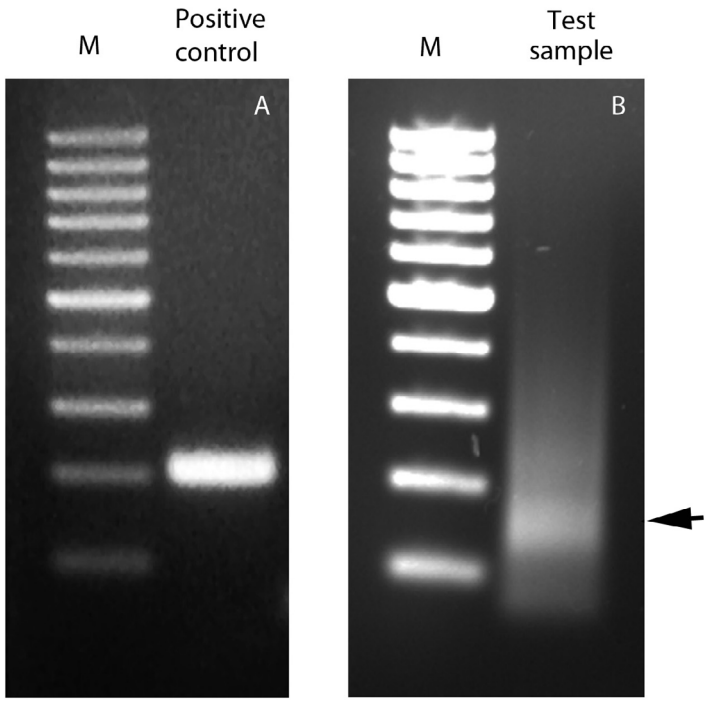

Fig. 4. Results from PCR amplification on: A, *Dirofilaria repens* positive control DNA showing intense band for COI endoding gene. B, DNA isolated from the examined worm, showing faint band of approximatelly 150bp long

(Fig. 4B), whereas no band was seen in the negative control PCR, in which worm DNA was omitted. This result indicates that due to the deleterious effect of fixation in un-buffered formalin for prolonged time period, the DNA integrity was significantly damaged, resulting in a very low signal in the form of truncated PCR product. Therefore the exact species identification was possible following histological examination. The other small parts of worm were immersed in PBS for 4h following the cryoprotection step and then were embedded upward in the cryo-embedding medium. The microtome stubs were immediately placed into freezer where the solid blocks were formed. The cross-sections of worm (20 µm thick) were cut on freezing microtome and placed on gellatin-coated slides. The fixation step was omitted due to the fact that worm was fixed immediately after excision from the patient. Sections were stained with Gill's haematoxylin for 10 min, washed in tap water and mounted into semi-permanent glycerol jelly mounting fluid. Microscopy examination revealed the characteristic morphological features, namely the longitudinal cuticular ridges and the sex organs. The species was identified as female of *D. repens* (Fig. 5A,B). Muscle layer was detached from the cuticule and the shape of body was irregular as result of unsuitable fixation.

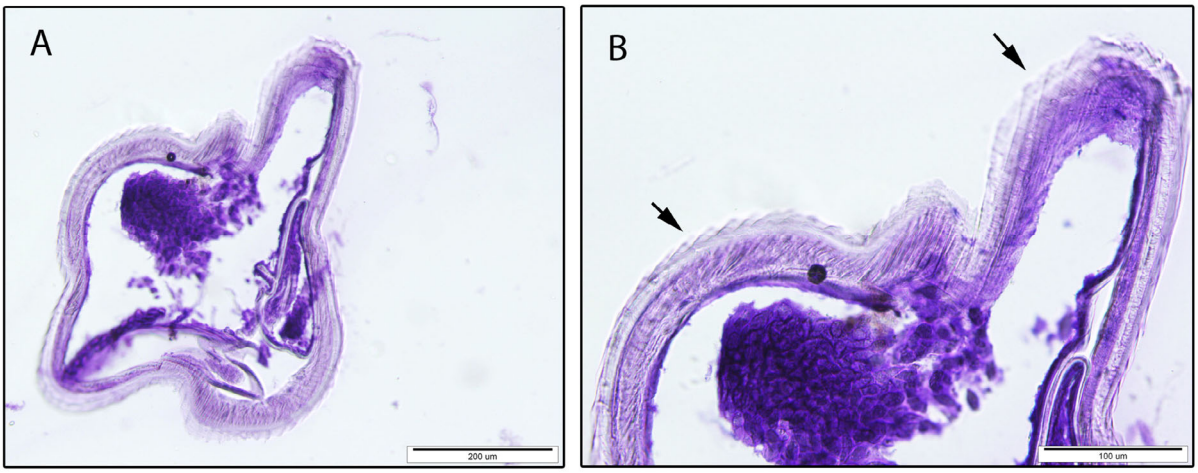

Fig. 5. Cross sections of *Dirofilaria repens* with typical longitudinal cuticular ridges (arrows) confirming *Dirofilaria repens* nematode

Discussion

Dirofilariosis is caused by filarioid helminths *Dirofilaria* sp. The infection is transmitted by mosquitoes from animals to humans. Humans are considered the occasional hosts for heartworm, in which the parasite generally completes developmental cycle. Gravid female worms have been described in subcutaneous nodules only rarely (Pampiglione *et al*., 1992). Subcutaneous dirofilariosis in humans is usually asymptomatic, acute symptoms are observed only in ocular infection, when living helminth enters the conjunctiva. Symptoms usually appear weeks after the infection with third larval stage. Pain in the eye, redness and swelling of eyelids represent symptoms that occurred in our patient and most patients with ocular dirofilariosis (Koltas *et al*., 2002; Yehudit *et al*., 2006; Raniel *et al*., 2006; Janjetović *et al*., 2010; Khoramnia & Wegner, 2010; Wesolowska *et al*., 2010). As observed by other authors, visual status usually remained intact after surgical removal of the worm (Koltas *et al*., 2002; Maraghi *et al*., 2006; Mittal *et al*., 2008; Argy *et al*., 2011; Otranto *et al*., 2011a; Otranto *et al*., 2011b). Diagnosis of human dirofilariosis is primarily based on histological evaluation of infected tissue and the macroscopic characteristics of the worm (Rouhani & Athari, 2003; Nath *et al*., 2010; Tafti *et al*., 2010; Argy *et al*., 2011; Otranto *et al*., 2011b). Confirmation of morphological identification and definite diagnosis of *D. repens* is possible by DNA analysis with polymerase chain reaction (Vakalis *et al*., 1999) and primers for a highly specific portion of COI gene. However, in our case, the molecular detection of *Dirofilaria* with a specific primers was not possible due to deleterious effect of fixation of worm in un-buffered formalin for prolonged period. The definitive diagnosis and the species identification was allowed only following histological examination of worm cross-sections based mainly on the presence of typical cuticular ridges as the inner morphology was destroyed. This human case is the second in new endemic region of Slovakia, where *D. repens* infection was confirmed by the complex diagnostic approach including macroscopic morphological characteristics, histological examination and PCR-based detection of *D. repens* (Hrčková *et al*., 2013). The incidence of human dirofilariosis generally correlates with the occurrence of canine dirofilariosis as a reservoir of infection (Taft *et al*., 2010, Otranto *et al*., 2011b). This is also confirmed in all documented cases of dirofilariosis from Slovakia. All patients, including ours, are likely to be infected in southern and south-western Slovakia. As microfilariemia was detected in 34.5 % of dogs, these areas are considered highly enzootic (Miterpáková *et al*., 2008; 2010).

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