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Human Dirofilariosis: The report of subcutaneous *Dirofilaria repens* infection in the Slovak Republic

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Summary

Dirofilariosis is a vector-borne disease that is spreading in Europe from the southern endemic regions to the northern countries, including Slovakia. The dog parasites *Dirofilaria immitis* and *D. repens* are zoonotic agents, responsible for the development of human pulmonary and subcutaneous dirofilariosis, respectively. The present paper reports the third case of human dirofilariosis in Slovakia caused by *D. repens*. The patient, a 41-year-old woman, was referred with tumour process in the subcutaneous area of the right forearm. Within 14 days the USG confirmed the rapid increase of the nodule from 20 x 10 mm to 30 x 25 mm. The surgical extirpation of the tumour was indicated. Histological examination revealed the formation with eosinophilic rime and the presence of a worm in the centre, detected as *D. repens*.

Keywords: human dirofilariosis; *Dirofilaria repens*; Slovakia

Introduction

Climatic changes, together with an increase in the movement of dogs and cats across Europe, cause an increase in the geographical range of vector-borne parasites like *Dirofilaria*, and in the risk of infection for animals and humans (Genchi *et al.*, 2009). In addition to global warming, other important drivers for the emerging and spreading of vector-borne parasite include habitat changes, alterations in water storage and irrigation habits, development of insecticide and drug resistance, globalization and the increase in international trade, tourism and travel (Harrus & Baneth, 2005). Dirofilariosis is a vector-borne parasitic infection mainly of dogs and cats. In Europe, there are occurred two zoonotic species *Dirofilaria immitis* and *D. repens*. The life cycle consists of five larval stages developing both in

an intermediate host that also acts as vector and in a definitive vertebrate host. Male and female *D. immitis* adults occur in the pulmonary arteries and right heart chambers and cause heartworm (HW) disease in dogs and cats while *D. repens* is found mainly in subcutaneous tissues. Adult female *Dirofilaria* releases embryos (microfilariae) into the blood (Genchi *et al.*, 2009). Usually, the parasites do not develop to adult stages in humans. The intermediate hosts and vectors are nearly 70 species of mosquitoes of the genera *Aedes*, *Anopheles* and *Culex*. Transmission of dirofilariosis is dependent upon the presence of sufficient number of infected, microfilaraemic dogs, susceptible mosquitoes, and a suitable climate to permit extrinsic incubation of *Dirofilaria* in the mosquito intermediate host (Medlock *et al.*, 2007).

Human infections by *D. immitis* or *D. repens* are common, but only a part of these is clinically manifested as pulmonary or subcutaneous dirofilariosis. Human dirofilariosis is characterized by nodules that appear when preadult/adult worms are arrested in a pulmonary artery branch (*D. immitis*) or in subcutaneous tissue (*D. repens*), causing coagulation necrosis and inflammatory cell infiltration (Pampiglione & Rivasi, 2007). The nodules are benign, but they can be confused with lung or cutaneous cancer. Human cases reported that women are affected by dirofilariosis more often than men, age group distribution shows a higher incidence of cases after 40 years of age in both sexes (Muro *et al.*, 1999). At the last decade the number of human autochthonous dirofilariosis has been diagnosed out of the old endemic Mediterranean countries, in northern and eastern Europe (Kramer *et al.*, 2007; Szénási *et al.*, 2008; Genchi *et al.*, 2009). Slovakia is one of these new areas, where autochthonous canine and human dirofilariosis was confirmed (Svobodová *et al.*, 2005; Mitterpáková *et al.*, 2008; Bábal *et al.*, 2008; Ondriska *et al.*, 2010). In this

work the third case of human dirofilariosis in Slovakia is identified.

Case report

A 41 - years old woman in Slovak republic visited the hospital because of a formation in the inner site of right elbow that has been observed for one week. History of trauma or injury was negative. Travel history revealed the stay in Canada and Croatia 6 months before appearance of first signs of the disease. Objectively subcutaneous tangible small nodule at the site of right condyle of humerus measuring 20 x 10 mm, movable against the skin and base was described. The skin over the formation was without pathological findings or inflammation. Punction of the formation was diagnostically not significant. USG examination disclosed a formation over right carpal flexor muscles that measured 32 x 18 x 12 mm and was localized at the medial site of anterocubital area. The hypoechogenic part (13 x 18 mm) was identified in the centre of the formation without communication to joint. Because of progressive growth that lasted 3 weeks, the surgical extirpation of the formation was performed. The surgical conclusion confirmed the USG result. The tissue was sent for histological examination.



Fig. 1. A - Histological longitudinal and cross sections of worms with eosinophil rime in light microscope, magnification 240x.
B - The worm cross section. a - multilayered cuticle, b - external cuticular ridges, c - longitudinal muscles

Histological examination

The extirped material was fixed in formalin and routinely processed in paraffin. Histological sections of 5 μm were stained by hematoxylin-eosin.

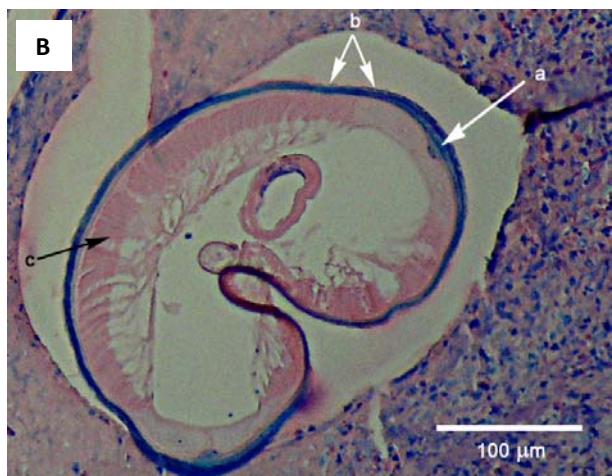
Light microscopy

Histological examination was focused on the parasite

identification by morphological data. Longitudinal and cross sections of unknown organism with cuticle and not stained inner area were observed and eosinophils surrounded the formations. The diameters of the parasites were 330 and 390 μm (Fig. 1. A). The external cuticular ridges (ECRs) were noted at the worms. The presence of ECRs is crucial element to differ *D. repens* from other subgenus *Dirofilaria*, which own a smooth cuticle. The worms were surrounded by eosinophilic inflammatory infiltration, they were identified as round worms v.s. *Dirofilaria* sp. The typical iridescence of multilayers cuticle was observed in polarised light, that is typical for *D. repens*. The multilayered cuticle with external lateral longitudinal ridges, thick hypodermis, and well-developed coelomyarial muscles are diagnostic signs of *D. repens* (hematoxylin and eosin stain) (Fig. 1. B).

Discussion

Many European countries have become new endemic areas for *Dirofilaria* infections due to climatic changes and global movement of animals and humans. This work reports the third case of subcutaneous dirofilariosis in Slovakia caused by *D. repens*. The previous two cases came from endemic areas for *Dirofilaria* spp. in dogs in the



southwestern Slovakia (Bábal *et al.*, 2008; Ondriska *et al.*, 2010; Miterpáková *et al.*, 2010). The diagnosis of our patient was based on clinical signs and morphological detection of the parasite. Woman came from the middle Slovakia, confirmed a stay in Croatia and Canada six months ago the nodule appearance, but a source of the infection was not revealed. An important and also autochthonous infection could be considered.

The detection and diagnosis of human dirofilariasis present two different aspects. In the case of subcutaneous nodules, it is usually the patient who seeks medical attention, while most pulmonary nodules are detected accidentally, by thoracic radiography. Given that both subcutaneous and pulmonary nodules can cause suspicion of a malignant tumour and other pathological conditions, surgery is usually recommended. Histological identification of worms in the nodules may be problematical because of similarities in morphological features between species and also because of the disruption of normal anatomy by the tissue response of the host (MacDougal *et al.*, 1992). As alternatives, serological and molecular biological techniques could be applied. Serological studies carried out in endemic areas showed a high seroprevalence (21 %) of *Dirofilaria* antibodies, what indicates that human contact with the parasite is more frequent than the number of reported clinical cases and that most individuals do not develop symptoms (Espinoza *et al.*, 1993).

In the literature, there is trend to relate subcutaneous and ocular dirofilariasis to *D. repens*, while pulmonary disease is usually considered to be caused by *D. immitis*. However, a critical report by Pampiglione *et al.* (2009) re-evaluated 28 cases of human pulmonary dirofilariasis in Europe attributed to *D. immitis* and corrected diagnoses that pulmonary dirofilariasis in Europe is associated with *D. repens* in contrast to pulmonary infection in the Americas caused by *D. immitis*. They proposed two hypotheses to explain this different pathogenic power of *D. immitis*: i) there are twin populations with different genotypes on two side of the Atlantic ii) the infective capacity to humans of the parasite could be modified in Europe by some factor (inherent to the vector) affecting the vector-parasite relationship decreasing the survival of the larvae. They also suppose that human organism in Europe seems to be unsuitable for the development of *D. immitis* larvae.

In spite of the trend to divide human dirofilariasis in two different clinical manifestations, existing data indicate a higher development capacity of *D. repens* than *D. immitis* in the human host: i) there exist higher incidence of subcutaneous than pulmonary cases, even in areas where *D. immitis* predominates over *D. repens* in dog population (Simón *et al.*, 2009) ii) *D. repens* intact live worms are recovered from many subcutaneous nodules (Pampiglione & Rivasi, 2007) iii) Subcutaneous cases contain both males and females of *D. repens* in the nodules, but *D. immitis* do not (Fernando *et al.*, 2000).

The difference between most human cases diagnosed as *D. repens* infection and a higher prevalence of *D. immitis* in dog populations could be explained by immunological study by González-Miguel *et al.* (2010) who identified immunoreactive proteins of *D. immitis* and *D. repens* from patient sera. Comparing their results with *D. immitis* canine infection by Olega *et al.* (2009) it seems that human host presents a greater reactivity than the dog against *D. immitis*, which is consistent with its limited capacity of development in humans when compared to the dogs.

In Slovakia, Miterpáková *et al.* (2008, 2010) detected

highly endemic areas in the Danubian Lowland in south-western Slovakia and also in Eastern Slovak Lowland, with mean 34.5 % prevalence of dirofilariasis in dogs. Our patient lives out of them, but she often travels not only across Slovakia, but throughout the world. Infected dogs, repeated floods, an increased abundance of mosquitoes represent a high risk of human infection. It would be supposed new cases of human dirofilariasis will appear in new areas of Europe.

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