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Human dirofilariosis in Poland: the first cases of autochthonous infections with *Dirofilaria repens*

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Abstract

Dirofilaria (*Nochtiella*) *repens* Railliet et Henry, 1911 (Nematoda: Onchocercidae) is a subcutaneous parasite of dogs and other carnivorous animals, with human acting as incidental hosts. *D. repens* occurs endemically in warm climates on various continents, in Europe mainly in Mediterranean countries. The aim of this study was to summarize information on human dirofilariosis in Poland, taking into consideration parasitological and epidemiological data. Between April 2009 – December 2011, in the parasitological laboratories of Medical University in Warsaw and the National Institute of Public Health/National Institute of Hygiene, fragments of affected human tissues and parasite specimens were examined microscopically. Molecular methods were used to confirm the results from eight microscopic investigations. A literature review to summarize all data on dirofilarial infections in humans in Poland was conducted. In these investigations, autochthonous dirofilariosis was found in humans for the first time in Poland. During the last 3 years, 12 new cases of human *D. repens* dirofilariosis were recognized. Since 2007, a total of 18 *D. repens* infection have been found in humans in Poland. Parasitic changes were located in various parts of the body, in the form of subcutaneous nodules containing single nematodes surrounded by granulation tissue (15 cases). In 3 cases, a subconjuctival localization was found. Seventeen of the 18 described cases were noted in central Poland where dirofilariosis occured in dogs. In this area, autochtonous infection was identified in 3 women who had never left Poland in their lives; the others were probably infected outside the country while staying in endemic regions. Data on human and canine infection collected from central Poland during the last 5 years indicates that *Dirofilaria repens* has been introduced into our country, and that the infection is successfully spreading, with the border of the endemic area currently on 52°N, 21°E. To control the epidemiological situation it is necessary to identify *D. repens* hosts within local mosquito populations, and to monitor dogs. Because of the increasing number of cases of human infections, whether introduced or local, physicians should take dirofilariosis into consideration in differential diagnosis of skin and eye diseases.

Key words

Dirofilaria repens, human, Poland, autochthonous species

INTRODUCTION

The nematode from the Onchocercidae family *Dirofilaria* (*Nochtiella*) *repens* Railliet et Henry, 1911, is a subcutaneous parasite of dogs, cats and foxes, occurring endemically in the warm climate zone on various continents. The invasion is transmitted to humans by many species of zooanthropophilic mosquitoes. As a result of stinging by an infected mosquito, L3 larvae are introduced into the subcutaneous tissue where mature to adult forms reach from several to 15 cm in length, and about 0.5 mm in diameter. The migration of *D. repens* in tissue lasts from several weeks to several months, or even several years after infection have been described. The process may be accompanied by clinical symptoms in the form of local swellings, burning or pruritis. As a result of inflammatory reactions of subcutaneous tissue, dirofilaria is arrested and nodules containing parasites may occur in various parts of the body; sometimes, the nematode has a subconjuctival localization [1, 2, 3].

In Europe, over 1,500 cases of human dirofilariosis caused by *D. repens* have been described, most of them in Mediterranean countries – the greatest numbers in Italy (321) [2, 3, 4, 5, 6] and in Ukraine (932) [7]. During the last decade, autochthonous dirofilariosis have also been detected in European countries hitherto considered to be free from this invasion. The first cases of humans infected with *D. repens* in Poland were found in 2007 [8, 9, 10, 11]. There was, however, no evidence for their autochthonous origin, as all infected persons had visited areas with endemic dirofilariosis.

The aim of this study was to summarize information on human dirofilariosis in Poland, taking parasitological and epidemiological data into consideration.

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MATERIAL AND METHODS

Laboratory investigations were performed in the period April 2009 – December 2011 in 3 centres in Warsaw: the Department of Zoonoses and Tropical Diseases (9 cases) and Department of General Biology and Parasitology (2 cases) of the Medical University in Warsaw (MUW), and 1 case in the Department of Medical Parasitology, National Institute of Public Health – National Institute of Hygiene (NIPH-NIH). Microscopic evaluation of histopathological preparations from sections of affected tissues (3 cases) and from 9 specimens of isolated parasites was performed. The material from 6 cases described earlier was reanalyzed (Tab. 1).

Molecular analysis of the genetic material of 6 nematodes was performed at the NIPH-NIH. DNA isolation was performed according to procedures described earlier [12, 13, 14]. Dirofilaria DNA was amplified using DR COI-F1 and DR COI-R1 primers [15] using Real-Time PCR [13]. The fragment of the gene of the first subunit of cytochrome oxidase (COI) was amplified from *D. repens* (GenBank AJ271614). The positive control was DNA from a *D. repens* isolate from Italy.

Information was collected from infected persons about their domicile and about trips taken outside of Poland. Data were collected from the literature concerning dirofilariosis cases in Poland, from a review of Polish and foreign publications.

RESULTS AND DISCUSSION

In these investigations, autochthonous dirofilariosis was found in humans for the first time in Poland (Case Nos. 10, 16 and 17) (Table 1). In the period from April 2009 – December 2011 in Warsaw and the Mazovian province, 12 new cases of human dirofilariosis were found. Altogether, to date in Poland, infections with *D. repens* have been found in 18 persons – 17 from the area of Mazovia (Warsaw, Grójec, Legionowo, Nowy Dwór Mazowiecki and Białobrzegi) [8, 9, 10, 11, current data], and 1 from Wrocław in Silesia [16, 17].

Characteristics of dirofilariosis cases found in Poland, March 2007 – December 2011. The infection was present in 18 persons aged 20–78 years (average age 42.6), encompassing 12 women and 6 men. Most patients had previously visited areas of endemic occurrence of *D. repens*. The trips were mainly to European countries: Italy, Greece, Ukraine, Hungary and the Czech Republic. Visits to South America and South Africa were also reported. Three female patients had never traveled outside Poland.

The parasitic changes were mainly localized in the subcutaneous tissue of the corpus (stomach, back, side, clavicle) – 7 cases, head (forehead, chin, superciliary arch, occiput) – 6, on the thigh – 2. In 3 persons, the nematodes occurred subconjuctivally. The reason for visiting a physician (dermatologist, surgeon) was generally the appearance of a growing nodule. Sometimes pain symptoms with various degrees of intensity were present: tingling sensation, burning sensation, swelling and subcutaneous extravasations. In clinical diagnosis, cancer or furunculosis were suspected as the reason for the occurring symptoms. In 3 cases, the nematode was visible under the conjunctiva. Surgical interventions were used to remove only the nematode (11 persons) or the parasite with the affected tissue (7 persons).

The parasitic changes sent for histopathological analysis were in the form of nodules 1–2 cm in diameter. The nodules were filled with a mass composed of necrotically affected tissue, in which the parasite was centrally located. The necrotic material surrounding the nematode contained numerous granulocytes, mainly eosinophils. In 2 cases neutrophils were dominant, eosinophils were less numerous. Clusters of macrophages of the epithelioid type and single giant cells were also observed. The necrotic change was surrounded by fibrovascular granulation tissue, infiltrated by lymphocytes, plasmatic cells, single macrophages, and a large number of eosinophils; numerous strands of fibrous connective tissue were in the form of irregular clusters. In the fatty tissue surrounding the nodule there were also inflammatory infiltrations composed of numerous eosinophils, plasmatic cells and lymphocytes.

Morphological and molecular identification of *D. repens*. The completely removed nematodes were *D. repens* females, 95–115 mm long and at most 0.6 mm wide (Fig. 1A). A female removed from a patient's eye had slightly smaller dimensions 85.1 × 0.545 mm [17]. In the front part of the nematode, at a distance of 1.7–2.0 mm from the apically located mouth aperture, a muscular vaginal segment was visible, terminated

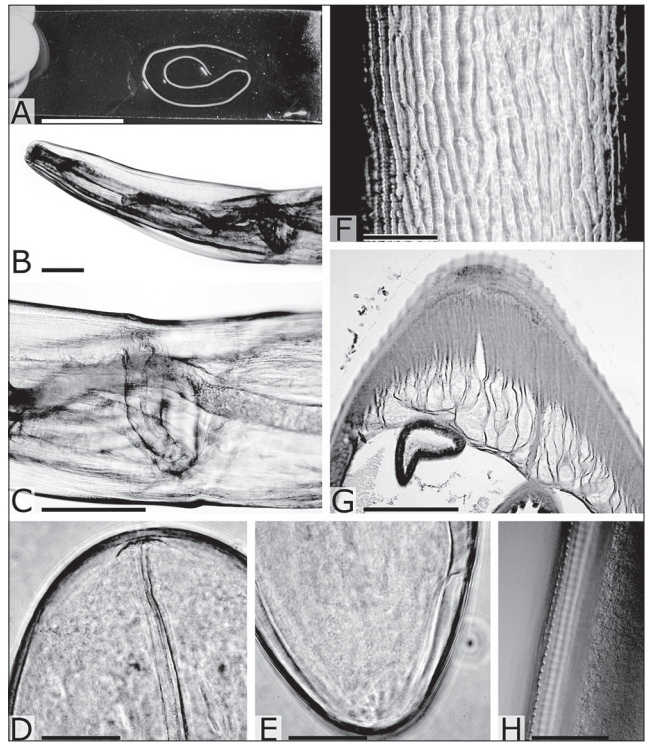

Figure 1. General view of *Dirofilaria repens* female; structure on the surface of the cuticle.
A. Female *D. repens*, intact nematode surgically removed from an ulcer on the thigh (Case No. 9).
B. Anterior end of the adult female removed from the conjunctiva of the eye through a surgical incision (Case No. 7).
C. Vagina and Vulva (Case No. 7).
D. Anterior end of the female; see the mouth and oesophagus (Hoyer's fluid, Case No. 13).
E. Posterior end of adult female showing the anus (Hoyer's fluid, case no. 13).
F. Longitudinal striations in the form of ridges on the surface of the cuticle (phase contrast microscopy, Case No. 13).
G. Slightly oblique section through a female, well visible longitudinal ridges (H&E, Case No. 10).
H. Longitudinal section through the wall of the nematode, visible small transverse stripes (phase contrast microscopy, Case No. 9).
Scale bars: A = 20 mm; B-C = 200 µm; D-G = 100 µm; H = 50 µm.

by an opening vulva (Fig. 1B–D). The anus was localized nearby – 100–160 µm, at the end of the body (Fig. 1E). The cuticle of the nematodes had characteristic structures in the form of longitudinal ridges and delicate transverse stripes (Fig. 1F–H). The number of longitudinal ridges in the central part of the nematode was about 100, and these structures disappeared in the anterior and posterior parts of the body.

In histological preparations (Fig. 2A, D), sections through the females were visible in the centre of the nodules; a male was present in only one case. The diameter of the females at the widest site was from 320–500 µm (average 430 µm), the diameter of the male was slightly less – 360 µm (Fig. 2B–G). The dimensions of *D. repens* specimens isolated from humans in Poland are similar in size to the dimensions of specimens isolated in Italy, in which the diameter of the female varied from 445–570 µm, and individuals from southern Russia, where the diameter of the females was 450 µm. The length of the Polish specimens of *D. repens* females was within the range described for Italian specimens, i.e. 95–140 mm. Adult nematodes from dogs, which are their final host, attain a length of 110–170 mm [18, 19, 20, 21, 22, 23].

The multilayer cuticle of the nematodes was 10-15 µm in thickness. Its surface in cross- sections through the nematodes had folds resembling ridges, whose number on the circumference of the nematode was 90–102 (Fig. 1G, 2E–G). The height of these ridges was in the range of 2.5–5 µm, and the distance between them reached 8–12 µm. In longitudinal sections (Fig. 1H), additionally narrow stripes were visible (5–6 µm) located perpendicular to the nematode. The thickness of the hypodermis layer was 3–5 µm. Lateral thickenings of the hypodermis in the form of flattened strands with granular cytoplasm and cell nuclei separated the muscle into dorsal and ventral part (Fig. 2F–G). Muscle cells were composed of contractile and non-contractile elements. The reproductive system in females had the form of 2 ducts filled with oocytes (Fig. 2B, E, F). No microfilaria were found in any of the analyzed preparations of females. The reproductive system of the male in a cross section had the form of a single duct (Fig. 2C, G). Even though the clinical manifestations in some infected persons in Poland were maintained for as long as one year, in none of the tissue changes were advanced processes of scarring, calcification, or parasite decomposition observed. The nematode had a low degree of damage visible as separation of muscle layers, destruction of muscle cells and of cells of the hypodermal lateral cords. Destructive cuticle changes (softening, dissolution of structures, entrance of inflammatory cells into the deeper layers) were found in only one case.

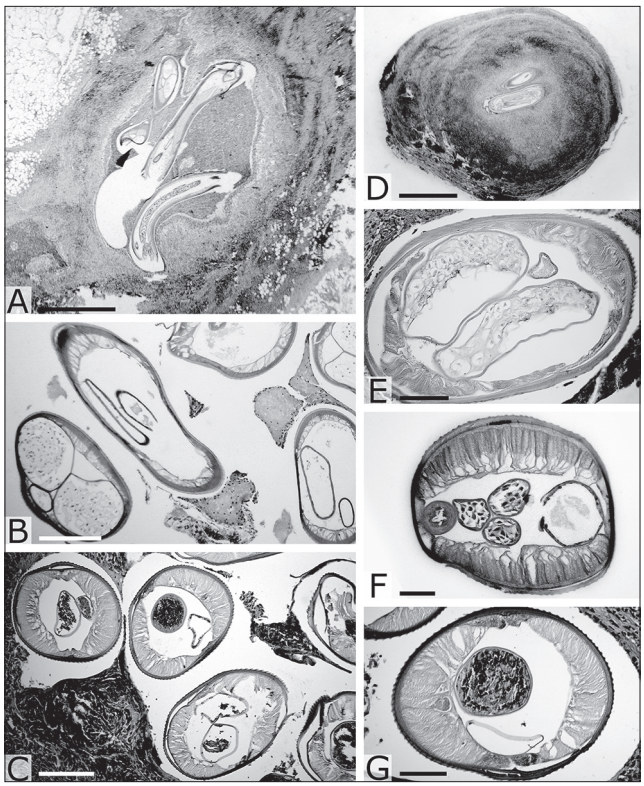

Figure 2. Sections of subcutaneous nodules with nematode *Dirofilaria repens*.
A. and D. Overall views of subcutaneous nodules showing *D. repens* (location – abdominal wall). Sections of the nematode were surrounded by an inflamatory granuloma (H&E, Case Nos. 1 and 4).
B. Sections of coiled *D. repens* female in subcutaneous nodule (location – forehead region) (H&E, Case No. 10).
C. Sections of coiled *D. repens* male of in subcutaneous nodule (location – lumbar region) (H&E, Case No. 14).
E. Transverse section of *D. repens* female, two uterine tubes are evident (H&E, Case No. 4).
F. Transverse section of *D. repens* female with visible: ovocytes visible inside the genital tubules; multilayered cuticle with external ridges, muscular cells, hypoderma, lateral chords, digestive tract (oesophagus) and genital tubes are well visible (H&E, Case No. 13).
G. Transverse section of an adult male; single genital tube contains spermatocytes (H&E, Case No. 14).
Scale bars: A, D = 2 mm; B, C = 200 µm; E–G = 100 µm.

In the differentiation of zoonotic nematodes isolated from humans in Europe, different species from the family Onchocercidae should be taken into consideration which cause tissue, subcutaneous or subconjuctival form of filariosis. The most important morphological characteristics allowing their differentiation is the structure of the cuticle surface and the diameter of the body [18, 19]. Of lesser diagnostic importance is the structure of the digestive and reproductive systems, the hypodermal lateral cords and the number of muscle cells. The *D. immitis* species found in Europe has a smooth cuticle surface. A cuticle structure similar to *D. repens* is found in species of zoonotic *Dirofilaria* occurring outside Europe: *D. tenius, D. ursi* and *D. striata*. However, they differ in respect to the body dimensions and a smaller number of ridges. Other nematodes with a similar subcutaneous or suconjuctival localization in humans *Loa loa* (smooth cuticle with knob-like structures) or *Onchocerca volvulus* (cuticle with circular stripes), are rarely brought to Poland from tropical countries. In Europe, single cases of *Onchocerca jakutensis* infections have also been described [24], *Onchocerca lupi* in Hungary [25], *Setaria labiatopapillosa* in Romania [26]. These species, however, are characterized by the presence on the cuticle surface of a configuration with a circular and not longitudinal structure, as is found in *Dirofilaria*.

Positive results of the amplification of the gene of the first subunit of cytochrome oxidase and the analysis of melting curves of PCR products confirmed that out of 9 nematodes analyzed using this method, all belonged to the species *D. repens* (Tab. 1).

Epidemiology of *D. repens* invasions. Nematodes from the genus *Dirofilaria* are currently considered as one of the most expansive parasites in humans and animals, and subcutaneous dirofilariosis is considered as the most rapidly spreading transmitted disease in Europe, which poses a high risk of invasion for both animals and humans [6]. Until recently, the geographical range of *D. repens* in Europe

Table 1. Characteristics of *Dirofilaria repens* samples analyzed in Warsaw* in the period March 2007 – December 2011

Case No.	Date analyzed	Type of sample	Results of microscopic analysis	Results of DNA analysis	Infected persons	
					Domicile	Travel outside Poland
1	March 2007	sections from nodule	*D. repens* ♀ [8,9,10,11]	not done	MV, Warsaw	Greece
2	May 2007	sections from nodule	*D. repens* ♀ [10,11]	not done	MV, Warsaw	South Africa
3	September 2007	sections from nematode	*D. repens* ♀ [10,11]	not done	MV, Warsaw	Italy, Greece, Ukraine, Hungary
4	February 2008	sections from nodule	*D. repens* ♀ [10,11]	not done	MV, Pruszków	Czech Republic
5	April 2008	sections from nodule	*D. repens* ♀ [10]	*D. repens*	MV, Zalew Zegrzyński	Hungary
6	March 2009	whole nematode**	*D. repens* ♀ [16,17]	*D. repens*	LSV, Wrocław	Greece
7	April 2009	whole nematode**	*D. repens* ♀	*D. repens* [13,14]	MV	Europe
8	August 2009	sections from nematode	*D. repens* ♀	*D. repens* [13,14]	MV	South America
9	September 2009	whole nematode	*D. repens* ♀	*D. repens* [13,14]	MV	Europe
10	**May 2010**	**sections from nodule**	**D. repens ♀**	**not done**	**MV, Grójec**	**did not travel outside Poland**
11	July 2010	whole nematode	*D. repens* ♀	not done	MV, Legionowo	Europe
12	September 2010	whole nematode	*D. repens* ♀	not done	MV	Europe
13	March 2011	whole nematode, sections from nematode	*D. repens* ♀	*D. repens*	MV, Nowy Dwór Mazowiecki	no data
14	June 2011	sections from nodule	*D. repens* ♂	*D. repens*	MV	Italy
15	August 2011	whole nematode**	*D. repens* ♀	*D. repens*	MV, Warsaw	no data
16	**November 2011**	**sections from nodule**	**D. repens ♀**	**not done**	**MV, Białobrzegi**	**did not travel outside Poland**
17	**December 2011**	**sections from nematode**	**D. repens ♀**	**not done**	**MV, Warsaw**	**did not travel outside Poland**
18	December 2011	whole nematode	*D. repens* ♀	*D. repens*	MV	no data

Legend
* Parasitological laboratories: Department of Zoonoses and Tropical Diseases, MUW; Department of General Biology and Parasitology, MUW; Department of Medical Parasitology, NIPH-NIH.
** subconjunctival localization
bold – autochtonous cases
MV – Masovian Voivodeship; LSV – Lower Silesian Voivodeship

was limited to endemic Mediterranean regions. Climatic changes, frequent floods, and the increase in the numbers of mosquitoes have led to a rise in the number of autochthonous cases and the spreading of the boundaries of this invasion. Analysis of European meteorological data has shown that in summer the thermal conditions allow dirofilaria to develop in areas located at 56°N latitude and 39°E longitude [27]. The ability of *D. repens* to attain the invasive L3 stage under conditions similar to those occurring in summer in Poland have been demonstrated by laboratory analyses performed in Kiev (Ukraine). Microfilaria reached the L3 stage in common mosquito species (*Culex pipiens* and *Anopheles maculipenis)* during 13-14 days at a temperature of 18-28°C [28]. It seems, therefore, that detection of vectors among the local mosquito population in Poland is just a question of time.

In Poland, cases of dirofilariosis in dogs have been observed since 2007 in Warsaw (52°35'N, 21°05'E) and in surrounding counties: Pruszków, Piaseczno i Żyrardów. Recently, microfilariosis has been detected in 2 dogs in the Lublin province (capital, city of Lublin at 51°14'N, 22°34'E). Most animals infected with *D. repens* have never left Poland [23, 29, 30, 31, 32, 33]. Information has appeared about detection of dirofilariosis in 2 dogs imported from Poland to Germany: the animals were from the surroundings of Warsaw and from the Baltic coast [34].

Two reports on human dirofilariosis in Poland [10, 17] were erroneously cited as describing autochthonous infections [6]. The authors of the original articles identified those Polish cases as being imported [17] or possibly imported [10]. The first cases of an autochthonous invasion by *D. repens* in humans were found in the area of dirofilariosis occurrence in dogs. In May 2010, the infection was detected in a 37-year old woman living near Grójec (51°51'N, 20°52'E), in November 2011, in a female 50-year old inhabitant of Białobrzegi (51°39'N, 20°52'E), and in December 2011, in a 56 year old woman living in Warsaw, who spent last summer in the Lublin province. As these women never left Poland, it can be assumed that the infection occurred in Poland. The remaining 15 invasions because of earlier trips abroad to endemic areas by infected persons or because of the lack of information about such trips, may be considered as imported. However, it can neither be confirmed nor excluded that at least some of these infections also took place in Poland. Most of the investigated persons (17 out of 18) live in an area where dirofilariosis has been detected in dogs, and all had in the past been exposed to mosquito bites.

Data from central Poland concerning dirofilaria invasion in animals and humans are in agreement with observations from other European countries. Cases of autochthonous dirofilariosis in humans are becoming increasingly common, among others in Serbia [35], Hungary [36], Austria [37] and in Slovakia [38, 39, 40]. In recent years, the boundary of dirofilariosis occurrence has moved a few degrees latitude in the northern direction. Currently, the northernmost area of dirofilariosis occurrence is the Moscow region in Russia (56°N), in which *D. repens* infections are noted both in animals and in humans [41, 42]. In western Europe, the *D. repens* has crossed the 48°N parallel – an infection was observed in a dog in the northern part of Baden-Wurttemberg in Germany [43, 44]. In the Netherlands, dirofilariosis has crossed 52°N parallel – an infection was observed in a dog [45].

The 3 new cases of autochthonous dirofilariosis in humans in Poland indicate that at present in central Europe the

52°N parallel is the northern boundary of occurrence of autochthonous *D. repens* dirofilariosis, both in animals and humans.

CONCLUSIONS

In these investigations, subcutaneous autochthonous dirofilariosis was found in humans for the first time in Poland. Collected data indicate that *D. repens* has been introduced into our country and the infection is successfully spreading. To control the epidemiological situation it is necessary to identify *D. repens* hosts within local mosquito population and to monitor dogs. Because of the increasing number cases of human infections, whether introduced or local, physicians should take dirofilariosis into consideration in differential diagnosis of skin and eye diseases.

List of abbreviations used:

MUW – Medical University of Warsaw
NIPH-NIH – National Institute of Public Health – National Institute of Hygiene
MV – Masovian Voivodeship
LSV – Lower Silesian Voivodeship
H&E – hematoxylin and eosin stain

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