Abstract. *Introduction:* Dirofilariasis is an emerging zoonosis (supported by climate change) in Central Europe. Human infections are usually caused by *Dirofilaria repens* and *Dirofilaria immitis* with mediation of mosquito vectors. Aims of this publication were to report our dirofilariasis cases, and to summarize Hungarian epidemiological data by reviewing literature.

Materials, methods and results: We present 5 (4 ophthalomological, 1 subcutaneous) cases observed within a two-year period in Southern Hungary. Ages of infected patients were between 31 and 74 years. First case during pregnancy is also reported. There was no travel history in the anamnesis of patients which could explain acquisition of the infection. Moving, intact worms eliminated by surgical interventions were identified on the basis of morphological features as *Dirofilaria repens*. Since the first report of human case, 115 further episodes (in addition to ours) were diagnosed in Hungary. Mean age of the patients was 47 years. Reviewing national reports, the ratio of subcutaneous infections was higher than that of the ocular ones (66 and 45, respectively). Evaluation of the territorial distribution of human episodes revealed that most infections occurred in patients living in the Danube-Tisza Interfluvial Region and eastern part of the country. However, sporadic cases were also found in western counties during 2014.

Conclusion: Most of the Hungarian dirofilariasis cases were autochthonous infections. Occurrence in the western counties may suggest the spreading of this emerging zoonosis to these areas. Comprehensive monitoring and data analysis are desirable, therefore reporting the epidemiologic data in the case of human infections should be made mandatory.

Keywords: *Dirofilaria repens*, epidemiology, case report, pregnancy, Hungary

Introduction

Dirofilariasis is an endemic zoonosis in the Mediterranean area and it seems to be an emerging disease in Central Europe as well [1,2]. Climate change plays a key role in this progress of distribution. Global warming influences the spreading of haematophagous arthropods acting as vectors of different parasitic infections, such as dirofilariasis [3]. The increasing temperature facilitates the reproduction of vectors and parasites by rising the number of days suitable for their development [4]. Another possible cause may be the migration of host animals. The main natural hosts of *Dirofilaria* species are dogs and wild caniforms, such as foxes, wolves and raccoons [3]. People handling or owning these animals are at higher risk of infections, although this phenomenon is not exclusive [5]. Vectors can carry infective forms (microfilariae) of these worms to larger distances. According to a recent study, potential vectors in Southern Hungary may be *Culex pipiens*, *C. modestus* and *Ochlerotatus caspius*. Although all of the reported human infections were caused by *D. repens*, the tested mosquitoes carried *D. immitis* at higher rate [6]. Tourism and migration can also contribute to the increase of incidence of human cases outside the endemic areas [7].

There are many species in the genus of *Dirofilaria*, but human infections are caused most commonly by *D. repens* and *D. immitis*. Humans become infected with these worms through mosquito bites. In humans, the disease manifests as a subcutaneous nodule, ocular or pulmonary dirofilariasis, but there are several reports about presentation in unusual body sites as well, including orbital area, liver, spermatic cord and abdominal cavity [8-11]. Ocular and subcutaneous infections are usually caused by *D. repens*, while *D. immitis* is involved in pulmonary dirofilariasis in humans [7,9]. Babes reported the first human case from Hungary in 1879 [12]. According to data of the Department of Parasitology, National Center for Epidemiology (NCE; Budapest), 13 cases of human dirofilariasis were reported in the literature between 1879 and 2000 from different parts of the country [13]. The tendency seems to be increasing based on 88 cases diagnosed in the NCE during the interval of 2001-2013 [14]. Based on these data, the most frequent form of human dirofilariasis in Hungary is subcutaneous infection (59 out of 101 episodes). The eyes were involved in 39 cases, and the worms were eliminated from other body sites (ligamentum gastrosplenicum, spermatic cord and excised lymph node) in the case 3 patients [13,14]. In this report we describe 5 cases of dirofilariasis diagnosed at different Departments of the University Hospital in Szeged, Hungary between October 2010 and May 2012.

Case descriptions

Case 1: A 31-year-old pregnant woman perceived a painless, migrating nodule on her scalp and forehead during the last few months. When the swelling appeared on her right eyelid (on her 29th gestation week), she was presented in the Outpatient Clinic of Ophthalmology (Szeged). Slit lamp examination revealed a 2x10 mm mass in the nasal part of right upper eyelid. There was no other abnormality in her ophthalmological status. The best corrected visual acuity was 1.0 in both eyes. After disinfection with povidone-iodine, an intact adult worm was eliminated through a 2x2 mm incision (Figure 1). There was no history of traveling abroad, and she had no animal contact.

Case 2: A 72-year-old woman arrived for ophthalmological investigation during the summer in 2011. Her main complaints were redness and itching in the left eye for a few days. The conjunctiva was hyperemic temporally, and a moving, thread-like parasite could be seen under the conjunctiva by slit lamp examination (Figure 2). The curly shadows of the worm could also be seen very well by the ultrasound biomicroscopy (UBM). There was no other abnormality in her ophthalmological status. The best corrected visual acuity was 1.0 in both eyes. After disinfection with povidone-iodine, the intact adult worm was eliminated through a small incision with the application of local anesthesia (0.4% w/v oxybuprocaine hydrochloride). There was no history of traveling abroad, but contact with animals (cats, dogs) was known.

Case 3: A 57-year-old woman presented in the Outpatient Clinic of Ophthalmology (Szeged) with swelling on her left eyelid, redness of the conjunctiva with itching and pain in her left eye. Slit lamp examination revealed enlargement of the lacrimal gland, suffusion and bulge of the conjunctiva. A moving, thread-like parasite could be seen in the nasal subconjunctival area by UBM. There was no other abnormality in her ophthalmological status. The best corrected visual acuity was 1.0 in both eyes. Eosinophilia was not detected in her laboratory test results. After povidone-iodine disinfection and local anesthesia (tetracaine 0.5% drops), a small incision was made on the conjunctiva, and a whitish, thin, intact adult worm was pulled out from the subconjunctival area. Traveling abroad and contact with potential host animals were not recorded in her medical history.

Case 4: A 36-year-old woman presented at the Outpatient Clinic of Ophthalmology (Szeged) with pain and redness in her right eye. Under the temporal conjunctiva, a thrum-like structure was seen in hyperemic surrounding. The worm was also detectable with UBM (Figure 3). The visual acuity was 1.0 with no other ophthalmologic abnormality and no signs of intraocular parasite after dilatation of the pupil with 1% atropin drops. Initial attempts of removing the parasite were unsuccessful, because the worm crawled away. Atropine drops as cholinerg antagonist did not paralyze the worm. Two days later the patient reported again, and the worm

was visible under her conjunctiva. She was kept under dark conditions, and 4% pilocarpin drops were applied in order to paralyze the parasite. After disinfection with 1% povidone-iodine, a moving, intact adult worm was removed through a small incision under 1% tetracain drop anesthesia (Figure 4). There was no history of traveling abroad in her anamnesis. The patient has been working in agriculture and had dogs in her household.

Case 5: A 74-year-old man presented in the Department of Dermatology and Allergology (Szeged). A single subcutaneous nodule was seen on his left arm with signs of inflammation. He remembered an insect (perhaps mosquito) biting on his left shoulder nearly 2 years ago. History of traveling abroad was not mentioned in his anamnesis. An itching, painful erythematous area developed acutely after the blood feeding of the mosquito. Several days later the symptoms became rather itching than painful and the erythema started to migrate distally on the arm. Subcutaneous nodules appeared and disappeared occasionally under the migrating area. Repeated inflammations related to physical effects were also detected near the nodules. This progression reached the proximal phalanx of the second finger of his left arm during a 2 months period. At his presentation the area was punctured after freezing. Minimal quantity of brown, turbid exudate and a white, thin adult worm were eliminated. On the next day the area above the granulated nodule was drained. The patient kept dogs in his household, but traveling abroad was not involved in his anamnesis.

Parasitological findings

The completely removed nematodes were sent for identification to the Parasitological Laboratory, Department of Clinical Microbiology (Szeged). All eliminated parasites were intact, thin and cylindrical, measured 12.6, 10.6, 7.7, 11.5 and 13.3 cm in length, respectively (Figure 5). The worm diameter interval was 0.37-0.47 mm. Anterior ends were rounded. Their thick, multi-layered cuticle was white with distinct longitudinal ridges and transverse striations. The ridges were separated by a distance greater than the width of the ridge (Figure 6). Based on the size and other morphological features, all intact, adult worms were identified as female *D. repens*.

Summary of human dirofilariasis cases recorded in Hungary

The first Hungarian case of dirofilariasis was reported in 1879 by Babes [12]. Since then, 28 further cases were described until 2006 [13]. According to another report, 88 human cases were diagnosed between 2001 and 2013 in the Department of Parasitology, NCE [14]. Besides cases mentioned in the reviews cited above, some further presentations are available from Hungary in the national and international literature. An abdominal occurrence was mentioned in a 61-year-old man by Révész *et al.* [15] Fodor *et al.* summarized 4 episodes of

ophthalmofilariasis [16]. Totally, 106 cases were published from Hungary so far and 10 furhter episodes were diagnosed at NCE in 2014. Thus, 116 dirofilariasis cases have been registered in Hungary since 1879. Based on available data, the gender distribution was 59 men and 56 women, so the difference was not significant. The mean age of the patients was 47.4 years. The youngest patient was a 10-year-old girl [16]. Subcutaneous dirofilariasis was diagnosed in 66 cases. Eyes were affected in 45 episodes, and the worms were eliminated from other body sites (spermatic cord, ligamentum gastrosplenicum, lymph node, abdominal cavity and lung granuloma) in 5 patients [12-24]. The territorial distribution of Hungarian cases is demonstrated in Figure 7. Natural water sources (rivers, channels, lakes, reservoirs) exist in the areas of all counties. Nonetheless, the occurrence of episodes is lower in Transdanubia (Baranya, Fejér, Somogy, Zala, Vas). There are counties without any reports of dirofilariasis cases in this part of the country (Veszprém, Győr-Moson-Sopron, Komárom-Esztergom, Tolna). The background of this phenomenon is not known. However, the cases presented in Vas and Zala counties were diagnosed in 2014 only, which may suggest the spreading of the infection to the western part of Hungary. This tendency should be followed and monitored in the future. Higher incidence was recorded in the Danube-Tisza Interfluvial Region and the eastern part of the country called Tiszántúl. Most infections were diagnosed in Pest county, where Budapest the capital and the most populated city of the country is located. Out of 44 cases detected in Pest county, 28 patients lived in Budapest.

Discussion

Dirofilariasis is becoming more important in Central Europe with a highly increasing tendency. There are several reports about autochthonous occurrence in humans and not only in endemic areas, but also in countries including Poland, Austria, Slovakia and Romania [3,25-27]. At our Departments we have diagnosed 5 dirofilariasis cases from 2010 to 2012. Because of the small number of cases this phenomenon may not be considered as a tendency, but an aggregation can be seen compared to the number of previous cases. The question is rising: was this a real aggregation, or just greater attention was given to this infection? Since 2012 no further cases occurred in our institution. Two out of 5 patients lived in rural areas, while 3 patients came from urban environment. Three patients lived in close proximity of rivers, but the residences of all patients were situated in the watershed of the river Tisza, where a higher density of mosquitoes is persistent (Figure 7). As one of the main risk factors of this zoonosis in Hungary, the presence of the infective agent and its final host(s) is also provided. Dogs and cats infected by *D. repens* were also found in this area during a one-year-survey [28]. Mosquitoes collected in the southern part of the country carried *D. immitis* at a higher rate, but vector competence of blood fed ones was not

mentioned in that study [6]. Nowadays, the size and quantity of wetlands are growing because of the effects of climate change (much more rain). This fact contributes to the increasing abundance of real and potential vectors.

Zoonotic dirofilariasis occurs among those people, who handle cats and dogs [5]. Among our patients, 3 had contact with potential host animals (dogs). We had no information about the possible infections of their animals. However, vectors can also carry larvae to other individuals. In two of our cases (1 and 3) there were no direct animal contacts. Visiting endemic areas can also be a factor predisposing to dirofilariasis. There was no travel history in the anamnesis of our patients, which can explain the acquisition of the infection either in time or in space. Thus we can suppose that – similarly to other Hungarian reports - our cases were autochthonous infections [13]. However, the most exact support of this statement can be based on research of vectors, which is recently in progress [6].

The age of the infected patients was between 31 and 74 (mean 54) years in our institution. The higher incidence among middle-aged and older people is in accordance with national and international literature data [29,30]. A greater prevalence in females (n=4 vs. n=1) can also be seen among our cases. Equal or nearly equal rates of infected men and women were reported in other Hungarian studies. Between 2001 and 2006, Szenasi et al. diagnosed dirofilariasis in 8 men and 8 women [13]. To the best of our knowledge, we report the first case of human dirofilariasis in a pregnant woman (Case 1). Seasonality cannot be seen in our episodes.

Four women suffered from ophthalmofilariasis, and one man had subcutaneous infection. However, the subcutaneous form occurs more frequently in humans in Hungary and other European countries (e.g. Poland, Italy) [14,25,31]. In our practice we have found a single case only, fewer than number of ocular episodes. People usually take medical care sooner in the case of more irritating ocular symptoms, than in the case of subcutaneous ones. The subcutaneous presence of the parasite causes local symptoms (swelling, inflammation and itching), which can intensify within weeks or months [3]. The first symptoms develop acutely near the penetration point, and subcutaneous nodules can be detected under the migrating erythematous area. Pre-adult or adult worms in subcutaneous nodules are often localized on the face, neck, hands, chest, eyelids, and can also found on the scrotum [3,29]. The worms can migrate to/from the eye. The percentage of reported cases of ocular dirofilariasis has been increasing: *D. repens* occurred in ocular regions in 30-35% of the cases [32]. According to the localization of the nematode, symptoms can vary from moderate discomfort sensation in the eye, redness, pain and itching to more serious complications (damaged vision, retinal detachment, glaucoma, opacity of the

vitreous humor, etc.) [32]. None of our patients with ocular infection had complications or any functional damages.

The appropriate treatment of each form of dirofilariasis is usually the surgical intervention [29]. Antiparasitic treatment is generally not needed. After disinfection and local anesthesia, the intact worm can be eliminated through a small incision. In one of our cases (case 4) the worm migrated from the eye, which was possibly due to the light of the microscope (negative phototaxis). However, we did not see this phenomenon in other ocular cases. It is a fact, that the light of slit lamp has an impact on the worm: an intense moving is detectable. Some parasitologists studied the mechanisms of periodicity of microfilariae in relation to the circadian rhythm of the host, while others examined the response of microfilariae to external stimuli, however no information is available about the adult forms [33,34]. Neither atropine, nor pilocarpin was effective in paralyzing the worms, they continued the moving after the application of both drugs. In the case of the patient with subcutaneous infection drainage was also needed. After removal of the infective agent, all patients were recovered without any complications and permanent damages. No further worms were found during their control medical observations.

The laboratory diagnosis of dirofilariasis can be based on macroscopic and microscopic examinations of the intact worm. Adult female *D. repens* worms are 10-15 cm, while males are 5-7 cm long. Their diameter is near 0.5 mm [14,29]. The parasites are cylindrical and have white cuticles with transverse striations and longitudinal ridges. This property can also aid the differentiation of *D. repens* from *D. immitis*. Although *Dirofilaria* worms cannot fully develop into adult forms in humans according to the commonly accepted theory, there are several contradictory data. The sizes of eliminated worms in different (and also our) case reports correspond to adult forms. According to the life cycle of *Dirofilaria* sp., humans are infected with L3 larva, which passes through the mosquito's proboscis. (Measurement data: 667-1005 μm long and 22-25 μm wide [35].) In the definitive hosts, L3 larvae molt to L4 form and develop into adults after 6-7 months. The incubation period (from mosquitobite to the appearance of the symptoms) is similar in humans and the definitive hosts. Microfilariae were also detected in human blood [36]. These data suggest development into the adult form and fertilization of this worm in humans [32].

In Hungary all human cases published until now were caused by *D. repens*. Based on anamnestic data of our patients and epidemiological data for Hungary, further laboratory examinations for the differential diagnosis of *D. repens* from other *Dirofilaria* sp. was not required. In the majority of cases the diagnosis is based on histological examination of the specimen, which enables identification of the worm at species-level, and in

many cases also the detection of its gender. Circadian rhythm is an important attributie in microbiological (and clinical) diagnosis of filariasis. The chances to find microfilariae in stained peripherial blood smears are generally higher, if the samples were taken in the evening (at about 8.00 p.m.). However, the rate of positivity of the Knott concentration method still remained low in humans: 1 positive result was achieved from 49 tested samples at NCE [13,30]. Serological methods for the detection of specific antibodies are useful for screening of human infections. Furthermore molecular techniques also have an increasing importance in reliable species-level identification of the parasite.

Conclusion

Many risk and predisposing factors of dirfilariasis exist in the southern and/or eastern part of Hungary. The effects of climate change which favor reproduction of potential or real vectors, are noticable. Host animals can also be found. Thus the conditions are given to infect humans, and not only those who handle host animals. Nowadays, dirofilariasis is an emerging zoonosis in Hungary, however, the exact prevalence of this infection in humans and host animals is not well known yet. Comprehensive monitoring and data analysis could give a much clearer picture about the situation. Therefore the epidemiological data of human infections should be reported to the National Center for Epidemiology. Prevention should also be emphasized by the distribution of informations among the inhabitants.

Figure Legends

Figure 1: Elimination of an intact, 12.6-cm-long worm from Case 1.

Figure 2: Thread-like worm could be seen under the conjunctiva by slit lamp examination in Case 2.

Figure 3: Coiled worm could be seen by UBM in Case 4.

Figure 4: Intact worm was eliminated from the eye in Case 4.

Figure 5: All worms were thin, cylindrical and white. Their length varied between 7.7 and 13.3 cm.

Figure 6: Cross section of *D. repens* stained with methylene blue. CR: Cuticular ridges; LC: Lateral cord; MF: Muscle fibers.

Figure 7: Territorial distribution of dirofilariasis cases diagnosed in Hungary from 1879 to 2014, based on the residence of patients. Localization of our episodes (n=5) are marked with circled numbers according to the case numbers in the text.

Conflict of interest

Ilona Dóczi, László Bereczki, Tamás Gyetvai, Imre Fejes, Ákos Skribek, Áron Szabó, Szilvia Berkes, László Tiszlavicz, Noémi Bartha, Balázs Bende, Erika Kis, and István Kucsera declare that they have no conflict to interest.

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