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Circulation of *Dirofilaria repens*, *Setaria tundra*, and *Onchocercidae* species in Hungary during the period 2011–2013

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ABSTRACT

Dirofilaria repens and recently *Dirofilaria immitis* are known to be endemic in Hungary. Since there is no related research on *Dirofilaria* carrier mosquito species from Hungary, we conducted a three-year mosquito surveillance study between 2011 and 2013. During the study period we examined 23,139 female mosquitoes with a generic filaria-specific TaqMan PCR assay, and characterized them by sequencing a 500 bp segment of 12S rRNA. An important result of our study was the detection of *Setaria tundra* and *D. repens* along with an unidentified *Onchocercidae* nematode. *D. repens* is known to be endemic in Hungary, however, the detection of *S. tundra* in all sampling sites throughout the study period indicates for the first time the endemicity of this parasite in Hungary. The *Onchocercidae* sp. nematode showed 95% nucleotide identity with previously detected unidentified nematodes from Germany, indicating a broader geographical distribution of this nematode in Europe. *D. immitis* specific DNA was not detected among the screened mosquitoes in this study. Here we report 11 mosquito species as potential vector organisms for local filarial infections, including *Aedes vexans*, *Ochlerotatus annulipes*, *Ochlerotatus sticticus*, *Coquillettidia richiardii*, *Anopheles hyrcanus* and *Ochlerotatus rusticus*. *Dirofilaria* development unit was calculated and the potential transmission period was estimated, which ranged between 65 and 113 days between sampling seasons. A relatively high infection rate (36.8%) was identified, which is a notable finding for veterinary and human health professionals. Moreover, the results of our study widen the group of possible mosquito vector species for *D. repens* and *S. tundra* in Central Europe.

1. Introduction

Filarial species of the order Spirurida represent a serious healthcare problem worldwide, mainly in the tropical and Mediterranean areas (Morales-Hojas, 2009). Lymphatic filariosis caused by *Wuchereria bancrofti* and onchocercosis (*Onchocerca volvulus*) continue to cause serious diseases in many parts of the world, with approximately 40 million people infected from 73 countries in South Asia and Africa (Simonsen et al., 2009). *Dirofilaria* species are considered as emerging zoonotic parasites in Europe. Their original distribution territory includes mainly the Mediterranean region (Morchón et al., 2007; Cancrini et al., 2007) and even further southern territories such as the Canary Islands (Morchón et al., 2011). The changing climatic conditions and other factors which facilitate the extrinsic incubation for *Dirofilaria* species extend the geographical area of these parasites towards Northern-Europe (Genchi et al., 2011). *Dirofilaria repens* and *Dirofilaria immitis* represent the most significant problem with both veterinary and human health relevance in Europe. Although dirofilariosis has been diagnosed in Hungary both in humans and dogs (Szénási et al., 2008; Fodor et al., 2009; Tolnai et al., 2014), data on natural carrier mosquito species are scanty (Zittra et al., 2015).

D. repens is the causative agent of canine and feline subcutaneous and ocular filariasis. Furthermore it can also cause serious infection in humans as subcutaneous or subconjuctival nodules (Tasić-Otašević et al., 2015). The first autochthonous case of *D. repens* was reported in 1998 from Hungary in a dog (Fok et al., 1998).

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Table 1
List of mosquito species collected in Southwestern Hungary between 2011 and 2013, along with IR (infection rate) values, positive pools and the total number of mosquitoes tested. Values represent the whole sampling period. CI (confidence intervals 95%) IR.

Species	IR	CI lower limit	CI upper limit	Number of pools	Positive pools	Number of specimens
Aedes cinereus	3.07	1.37	6.08	56	7	2399
Aedes rossicus	2.01	0.12	9.69	19	1	484
Aedes vexans	15.44	12.69	18.69	200	105	9545
Anopheles algeriensis	0.00	0.00	199.17	4	0	11
Anopheles claviger	0.00	0.00	134.36	3	0	13
Anopheles hyrcanus	14.91	7.77	27.15	24	10	876
Anopheles maculipennis	92.31	26.89	393.36	7	4	113
Anopheles plumbeus	0.00	0.00	88.49	6	0	27
Coquillettidia richiardii	15.61	8.15	28.39	26	10	835
Culex martinii	0.00	0.00	260.73	3	0	7
Culex modestus	5.83	1.17	18.60	13	2	323
Culex pipiens	21.42	5.00	73.08	5	2	87
Culex territans	0.00	0.00	270.53	1	0	5
Culex torrentium	0.00	0.00	178.93	1	0	8
Culiseta alaskaensis	0.00	0.00	793.45	1	0	1
Ochlerotatus annulipes	15.28	6.84	31.51	19	7	603
Ochlerotatus cantans	0.00	0.00	51.97	5	0	45
Ochlerotatus dorsalis	22.85	1.53	121.34	3	1	41
Ochlerotatus geniculatus	0.00	0.00	219.68	7	0	12
Ochlerotatus pulcripalpis	0.00	0.00	793.45	1	0	1
Ochlerotatus refiki	0.00	0.00	499.14	2	0	3
Ochlerotatus rusticus	3.26	0.59	10.93	21	2	642
Ochlerotatus sticticus	11.38	8.81	14.55	150	62	7024
Uranotaenia unguiculata	0.00	0.00	48.45	2	0	34

Since its first detection, several human cases have been reported, a finding which not only reflects the importance of this parasite in veterinary health but also sheds light on the public health risks (Pampiglione et al., 1999; Elek et al., 2000; Pónyai et al., 2006; Szénási et al., 2008; Fodor et al., 2009). *D. immitis* as the causative agent of hearthworm filariosis is a major veterinary health threat in Europe. Since 2007, when the first autochthonous canine infection was confirmed, Hungary has been considered an endemic country for this parasite (Tolnai et al., 2014). *Setaria tundra* was recently detected in Hungary for the first time (Zittra et al., 2015). This parasite has a significant veterinary health importance in boreal regions of Europe as the causative agent of setariosis in cervids (*Rangifer tarandus*, *Capreolus capreolus*, *Alces alces* etc.) (Laaksonen et al., 2007). Its significance as a human parasite is still unknown. In Finland, *S. tundra* caused severe outbreaks among semi-domestic reindeer in the last couple of years (Laaksonen et al., 2009). As there are no veterinary health data available about the Hungarian cases of *S. tundra*, the most efficient method for estimating the risk for infection is the screening of mosquito vector population.

As a vector-borne infection, filarial parasites are transmitted by various haematophagous arthropod vectors, mostly mosquitoes (*Culicidae*) or arachnids (*Acari*) (Anderson, 2000). The main mosquito vector species in Europe belong to the *Aedes, Anopheles*, *Ochlerotatus*, and *Culex* genera (Czajka et al., 2012). Climatic and ecological factors may affect the life cycle of both the mosquito vector and the filarial parasites. Thus far a total of 50 mosquito species have been detected in Hungary, representing eight genera (*Anopheles, Aedes, Ochlerotatus, Coquillettidia, Culex, Culiseta, Orthopodomyia,* and *Uranotaenia*). Most frequent species are *Ochlerotatus annulipes, Culex pipiens pipiens, Aedes vexans, Coquillettidia richiardii, Ochlerotatus sticticus, Culiseta annulata* (Kenyeres and Tóth, 2012).

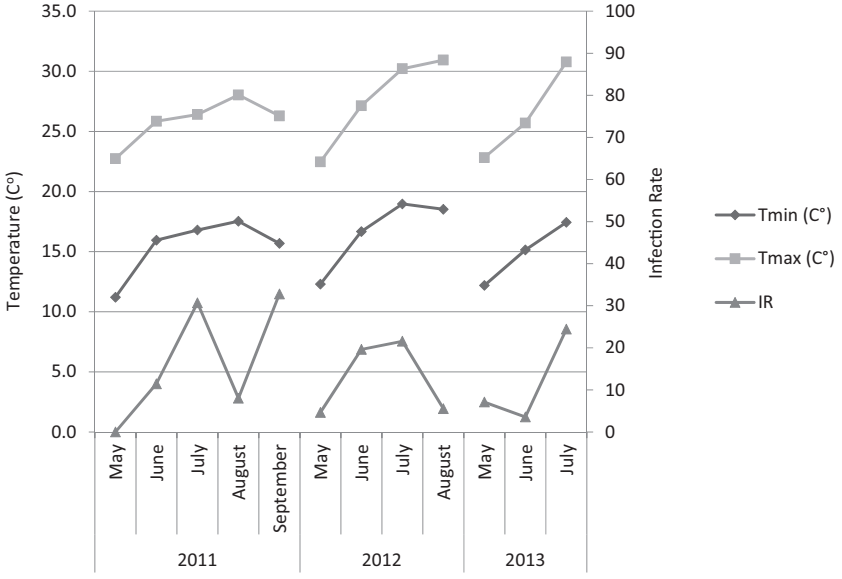

Fig. 1. IR (Infection Rate) value changes in parallel with temperature data along the whole sampling period. IR value represents all mosquito species.