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HELMINTHOLOGY - ORIGINAL PAPER

Updates on the distribution and host spectrum of *Dirofilaria repens* in the Republic of Uzbekistan

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Abstract

Among the zoonotic mosquito-borne nematodes, *Dirofilaria repens* and *Dirofilaria immitis* (Spirurida, Onchocercidae) are highly significant from a public health perspective. While *D. immitis* is also of major veterinary concern, *D. repens* is regarded as less pathogenic for carnivores, but is the main causative agent of human dirofilariosis throughout the Old World. In the Republic of Uzbekistan, recent data refer exclusively to *D. immitis* infection in domestic and wild carnivores, while the current prevalence and distribution of *D. repens* remain unknown. Between 2015 and 2021, a total of 559 domestic and wild carnivore carcasses were collected and examined by necropsy. All subcutaneous nematodes were collected and identified morphologically. The overall prevalence of *D. repens* infection was of 11.03% in domestic dogs, *Canis familiaris*, and 9.29% in wildlife hosts: golden jackals, *Canis aureus* (11.76%), wolves, *Canis lupus* (9.09%), red foxes, *Vulpes vulpes* (9.23%), and jungle cats, *Felis chaus* (7.14%). Additionally, a human case of subcutaneous *D. repens* infection was also documented. The present study represents the first recent assessment of the occurrence of the zoonotic filarioid *D. repens* in the Republic of Uzbekistan. It indicates a wide distribution in domestic dogs and four species of wildlife hosts throughout the country, raising awareness on the public health risks associated with this parasite.

Keywords *Dirofilaria repens* · Distribution · Host · Zoonosis · Uzbekistan

Introduction

Among mosquito-borne zoonotic nematodes, *Dirofilaria repens* and *Dirofilaria immitis* (Spirurida, Onchocercidae) are highly significant from a public health perspective (Simón et al. 2012). Both species are able to infect a variety of carnivore species, but the typical host is represented by the domestic dog, *Canis familiaris*. The female nematodes are larviparous, releasing blood-circulating microfilariae, which can be ingested by numerous species of mosquito vectors, mainly within the genera *Culex*, *Aedes*, and *Anopheles* (Otranto et al. 2013). While *D. immitis* is of great veterinary concern due to the severe disease it causes in carnivores (heartworm disease) and can occasionally infect also humans (Mendoza-Roldan et al. 2021), *D. repens*, which resides in the subcutaneous tissues, is regarded as less pathogenic for carnivores, but is the main causative agent of human dirofilariosis throughout the Old World (Capelli et al. 2018).

The Republic of Uzbekistan includes three landscape zones: plains, foothills, and mountains; having a diverse vertebrate fauna. Wild and domestic mammals within the order Carnivora in modern Uzbekistan are represented by 34 species and subspecies belonging to five families: Felidae (13 species), Mustelidae (11 species), Canidae (7 species), Ursidae (2 species), and Hyenidae (1 species) (Shernazarov et al. 2006). Cases of infection with *Dirofilaria* spp. have been previously identified in some of these animal species. In particular, *D. immitis* was detected in domestic dogs *Canis lupus familaris* and golden jackals, *Canis aureus* (Delyanova 1958; Irgashev 1958). During N. Matchanov's research on the helminth fauna of dogs in the Tashkent region, cases of infection of dogs with *D. repens*

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were also identified (Matchanov 1959). *D. immitis* and *D. repens* have also been reported in golden jackals, *C. aureus,* and jungle cats, *Felis chaus,* along the Syrdarya River in Uzbekistan (Taryannikov 1983). Some human cases presenting with ocular infection have been recorded between 1915 and 1996, presumably caused by *D. repens*, but with unclear confirmation (reviewed by Pampiglione and Rivasi 2000). However, the great majority of these reports are outdated. Recent data refer exclusively to *D. immitis* (Azimov et al. 2019; Norkobilov et al. 2020; Berdibaev 2021), while the current prevalence and distribution of *D. repens* remain unknown. Therefore, the aims of the present study were to provide updates on the distribution and host spectrum of *D. repens* in carnivores from Uzbekistan, and to present a recent case of human infection.

Materials and methods

The present study was carried out between 2016 and 2021. A total of 559 domestic and wild carnivore carcasses were collected and examined at the Laboratory of General Parasitology of the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan, by full parasitological necropsy (Table 1). Wild carnivore carcasses were obtained during the legal hunting activity or were found dead at various locations in the Republic of Karakalpakistan (Northwestern Uzbekistan), Samarkand, and Surkhandarya regions (Northeastern Uzbekistan). The domestic dogs, screened during the study, died due to natural causes or were euthanized for medical reasons, in the city of Tashkent, or rural settings situated in Northwestern and Northeastern Uzbekistan. The carcasses were kept frozen at − 20 °C until processing. For each animal, data regarding species, sex, estimated or exact age in case of wild carnivores and dogs, respectively, and location of collection were recorded.

All nematodes residing in the subcutaneous tissues were collected in labeled vials containing 10% formalin. The collected nematodes were mounted on glass slides and examined under a light microscope (Microscope mbs-10. Modern counterpart. msp-2. Lomo). The morphological identification was carried out according to descriptions and keys from literature (Anderson and Bain 1976; Kozlov 1977; Demiaszkiewicz et al. 2011). The statistical analysis was performed using EpiInfo 7 software (CDC, USA). The prevalence and its 95% Confidence Interval (CI) were calculated, and differences among groups were assessed by chi square testing and considered significant for $p \leq 0.05$.

In the case of one golden jackal necropsied in February 2021, a single nematode was collected in 70% ethanol and processed by means of molecular tools. The DNA was isolated from a portion weighing approximately 20 mg, using a commercially available kit (ISOLATE II GENOMIC DNA kit, BioLine, UK), according to the manufacturer's instructions. An ~ 670-bp fragment of the *cox*1 gene of Spirurid nematodes was amplified by conventional PCR using the NTF/NTR primer pair, as previously described (Casiraghi et al. 2001). The PCR product was sequenced using an external service (performed at Macrogen Europe, The Netherlands) and then compared to other *D. repens* isolates from GenBank database by using Basic Local Alignment Tool (BLAST) analysis.

In 2019, a 32-year-old woman having no travel history, was admitted to the Syrdarya Regional Oncological Dispensary with complaints of the presence of a tumor in the left mammary gland. During ultrasound examination, a displaceable formation with a size of 8 × 5 mm was revealed in the left mammary gland, with a preliminary diagnosis of potential parasitic cyst. The regional lymph nodes were not enlarged. The formation was excised and found to contain a slender white nematode, which was collected in formalin and submitted for identification to The Laboratory of General Parasitology of the Institute of Zoology of the Academy of Sciences of the Republic of Uzbekistan.

Table 1 Carnivore species and number of specimens examined

Family	Species	Investigated specimens
Canidae	Domestic dog, *Canis familiaris*	290
	Golden jackal, *C. aureus*	102
	Grey wolf, *C. lupus*	44
	Red fox, *Vulpes vulpes*	65
Mustelidae	Eurasian badger, *Meles meles*	16
Felidae	Jungle cat, *Felis chaus*	42
Total		559

Results

Dirofilaria repens infection in domestic dogs

Out of 290 examined dogs, a total of 32 (11.03%; 95%CI 7.67–15.22) were found to harbor subcutaneous nematodes, all identified as *D repens*. The distribution of positive animals is presented in Table 2.

There were no statistically significant differences between Northeastern and Northwestern Uzbekistan ($p = 1$). However, the prevalence in Tashkent megalopolis was significantly lower as compared to rural sampling sited from both Northeastern and Northwestern Uzbekistan ($p < 0.0001$ in both cases). The prevalence of infection was significantly higher in rural sampling areas, in female dogs, and in dogs aged over 5 years (Table 3).

Table 2 The prevalence of *D. repens* infection in dogs from Uzbekistan

Region	Examined dogs	*D. repens* infection		
		N	%	95% CI
Tashkent	160	5	3.13	1.02–7.14
Northeast	65	13	20.00	11.10–31.77
Northwest	65	14	21.54	12.31–33.49
Total	290	32	11.03	7.67–15.22

The infection rate ranged between 1 and 11 nematodes/animal.

Dirofilaria repens infection in wild carnivores

Out of the 269 wild carnivores examined, 25 (9.29%; 95% CI 6.10–13.41) were found to harbor *D. repens* infection. Excepting the Eurasian badger, *Meles meles*, adult *D. repens* were found in the subcutaneous tissues of all the examined wild carnivore species (Table 4).

There were no statistically significant differences in the overall prevalence of infection between regions, neither globally, nor according to host species (Table 5).

The sequence analysis of the *D. repens* isolate from the golden jackal revealed a 100% nucleotide identity with five other *D. repens* isolates: three from human cases investigated in Europe (KR998257; KX265049; MW017212), one from a mosquito in Austria (MF695085), and one from a dog from Lower Austria (MW590257). The sequence was deposited in GenBank under the Accession Number MZ081850.

Human case

The nematode removed from the patient's breast had a body length of 52 mm, with a maximum width of 3.9 mm. The cuticle presented longitudinal striations on the entire body length. The anterior extremity was rounded, having a circular oral opening surrounded by four pairs of cephalic papillae. The posterior extremity was spirally twisted, and two unequal spicules were visible. Based on these characteristics, it was concluded that the nematode was a male of *D. repens*.

Table 4 The prevalence of *D. repens* infection in wild carnivores from Uzbekistan

Species	Examined	*D. repens* infection			p
		n	%	95% CI	
Canis aureus	102	12	11.76	6.23–19.65	0.624
Canis lupus	44	4	9.09	2.53–21.67	
Vulpes vulpes	65	6	9.23	3.46–19.02	
Meles meles	16	0	0	0–20.59	
Felis chaus	42	3	7.14	1.5–19.48	
Total	269	25	9.29	6.10–13.41	-

Discussion

The occurrence of *D. repens* was reported on various occasions in the Republic of Uzbekistan, but no data from the twenty-first century was available prior to the present study. Similarly, in neighboring countries, in Kazakhstan, during the years 1953–1956, infection with *D. repens* was found in 42.6% of 970 dogs examined from the town Kzyl Orda, and in 4.17% of 30,700 *Aedes maculipennis* sacharovi mosquitoes (Chun-syun 1959). However, this information is outdated and there is no available data on research conducted in recent years in Central Asia. The first molecular survey and confirmation of *D. repens* nematode in the Kyrgyz Republic was carried out recently, in the Bishkek region, with just one dog (0.29%) being positive (Aydın et al. 2020). In Iran, Tehran province, the molecular prevalence of *D. repens* was 26% in 2017 (Pedram et al. 2019). In Uzbekistan, according to the centralized data published by Sultanov et al. (1975), and Muminov (1976), the prevalence of dirofilariosis in the studied dogs from Uzbekistan showed great variation, according to environmental conditions: 0.66–2.9% for *D. immitis* and 1.4–20% for *D. repens*. In the present

Table 3 The distribution of *D. repens* infection in domestic dogs from Uzbekistan

Parameter		Examined	*D. repens*			p
			N	%	95% CI	
Environment	Urban	160	5	3.13	1.02–7.14	< 0.0001
	Rural	130	27	20.77	14.16–28.76	
Sex	M	187	13	6.95	3.75–11.59	0.005
	F	103	19	18.45	11.49–27.30	
Age	≤ 2	42	0	0	0–8.41	0.0001
	2–5	210	21	10.0	6.30–14.88	
	> 5	38	11	28.95	15.42–45.90	

Table 5 The distribution of *D. repens* infection in wild carnivore species from Uzbekistan

Species	Region	Examined	*D. repens* infection			p
			N	%	95% CI	
Canis aureus	Karakalpakistan	51	7	13.73	5.70–26.26	0.235
	Samarkand	37	2	5.41	0.66–18.19	
	Surkhandarya	14	3	21.43	4.66–50.80	
Canis lupus	Karakalpakistan	28	2	7.14	0.88–23.50	0.818
	Samarkand	9	1	11.11	0.28–48.25	
	Surkhandarya	7	1	14.29	0.36–57.87	
Vulpes vulpes	Karakalpakistan	18	2	11.11	1.38–34.71	0.906
	Samarkand	27	2	7.41	0.91–24.29	
	Surkhandarya	20	2	10.00	1.23–31.70	
Meles meles	Karakalpakistan	6	0	0	0–45.93	1
	Samarkand	3	0	0	0–70.76	
	Surkhandarya	7	0	0	0–40.96	
Felis chaus	Karakalpakistan	4	1	25	0.63–80.59	0.254
	Samarkand	29	1	3.45	0.09–17.76	
	Surkhandarya	9	1	11.11	0.28–48.25	
Total	Karakalpakistan	107	12	11.21	5.93–18.77	0.263
	Samarkand	105	6	5.71	2.13–12.02	
	Surkhandarya	57	7	12.28	5.08–23.68	
	Northwest	107	12	11.21	5.93–18.77	0.397
	Northeast	162	13	8.02	4.34–13.33	

study, we report similar values, with the prevalence of *D. repens* infection in domestic dogs ranging between 3.13% and 21.54%. However, the infection rate was significantly higher in rural dogs as compared to those from an urban environment. This is probably due to the owner's increased awareness and the extensive use of anthelmintics for urban pet dogs (Panarese et al. 2021). In contrast, in rural areas, the dogs are often neglected by their owners and are generally regarded as service animals, therefore they don't regularly receive deworming treatments. Although sex is generally not considered as a risk factor for contracting the filarial infection (Montoya et al. 2006; Rapti and Rehbein 2010) and in some cases, a higher prevalence is described in male dogs (Montoya et al. 1998; Scaramozzino et al. 2005; Yildirim et al. 2007), in the present study, the proportion of females infected by *D. repens* was significantly higher. The highest prevalence was found in dogs over five years of age. This is most probably a consequence of a longer period of exposure to competent vectors, as also in the case of the heartworm, *D. immitis* (Rhee et al. 1998). A recent study performed on domestic dogs from various regions of Uzbekistan revealed an overall prevalence of 9.6% for *D. immitis* infection, with regional variations from 5% in urban dogs to 13.2% in rural ones (Norkobilov et al. 2020). The overall prevalence of *D. repens* exceeds that of *D. immitis*, as also observed in several European countries (Genchi et al. 2011), and also in Iran (Pedram et al. 2019). Nevertheless, infected microfilariaemic dogs serve as the main reservoir of infection, both for other carnivores and humans, representing a public health hazard (Capelli et al. 2018; Brianti et al. 2021).

Previous data indicated the occurrence of *D. repens* in two species of wild carnivores in Uzbekistan, namely the golden jackal, *C. aureus*, and the jungle cat, *F. chaus* (Irgashev 1958; Murtazaev 1964a, b, 1975; Matchanov 1968; Muminov 1968; Sultanov et al. 1969; Taryannikov 1983). Herein, we report two new host-parasite associations in the country, the gray wolves, *C. lupus*, and red foxes, *V. vulpes*. In the assessed wild carnivores, the prevalence of *D. repens* infection did not vary significantly between host species, indicating a similar susceptibility to infection. Although infection of wild carnivores is generally regarded as an epi-phenomenon of dog infection in overlapping territories (Otranto et al. 2015), the relatively high prevalence (9.29%) suggests that wildlife species could also play a role in the maintenance and dissemination of infection. Furthermore, the involvement of wild carnivores as sources of human dirofilariosis has never been properly investigated, but it is estimated that they could play a major epidemiological role, due to the complete lack of preventative control strategies (Otranto and Deplasez 2020).

The recently documented human case of *D. repens* infection was autochthonous, as revealed by the lack of travel history of the affected patient. The clinical presentation was subcutaneous, initially regarded as a tumor. This case represents an alarm, highlighting the necessity of development

and implementation of solid control strategies, based on thorough investigation of the infection status of the parasite's natural hosts (i.e. domestic dogs) and consequent mass administration of deworming treatment in the affected regions. Further studies focused on the identification of potential vector populations, and implementation of mosquito control actions would also be necessary.

Conclusion

The present study represents the first recent assessment of the occurrence of the zoonotic filarioid *D. repens* in the Republic of Uzbekistan and the first molecular confirmation. It indicates a wide distribution in domestic dogs and four species of wildlife hosts throughout the country, raising awareness on the public health risks associated with this parasite.

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Data availability All relevant data is enclosed within the manuscript.

Code availability Not applicable.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Conflict of interest The authors declare no competing interests.

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