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Original article

DIROFILARIA IMMITIS INFECTION IN CARNIVORES FROM BULGARIA: 2012–2013 UPDATE

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Summary

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Helminthological necropsies of 489 carnivores (325 golden jackals, 115 red foxes, 4 martens, 3 wolves, 27 domestic dogs and 15 cats) from Bulgaria were carried out in 2012–2013 aiming to establish infection with *Dirofilaria immitis* (Nematoda: Onchocercidae). The infection was found in the golden jackals, red foxes and domestic dogs. Adult worms were located in the heart (55.24%) or both in the heart and pulmonary arteries of the hosts (27.97%). Often in the place of localisation of the helminths macroscopic lesions as obturation of pulmonary arteries, thickness and granulation of the inner surface of pulmonary arteries, damaged atrioventricular valves were observed. The prevalence of infection was 37.54%, 25.22% and 33.33% in the golden jackals, red foxes and domestic dogs respectively. The infection intensity in the jackals varied between 1 and 19 specimens per animal (mean 4.1), in the foxes it was between 1 and 15 specimens (mean 4.79) and in the dogs – between 1 and 34 specimens (mean 14.43). Comparison of our data with those from literature showed a trend towards increased spread of infection in Bulgaria and South Europe in recent years. Morphometric description of *D. immitis* in materials from golden jackals, red foxes and domestic dogs was performed too. This is the first report in materials from golden jackals and red foxes.

Key words: Bulgaria, *Dirofilaria immitis,* domestic dogs, golden jackals, morphometric description, prevalence, red foxes

INTRODUCTION

Dirofilaria immitis (Nematoda: Onchocercidae) is a parasitic worm with an indirect life cycle. Its final hosts are mammals, different species of the orders Monotermata, Pilosa, Rodentia, Artiodactyla, Carnivora, Pinnipedia, Primates, and intermediate hosts – invertebrates, usually various species of mosquitoes. The species is spread all over the continents except the Antarctic and is of zoonotic importance. The parasite occurs in the lung arteries and heart and causes the so-called heartworm disease in dogs and cats.

Dirofilaria infections may develop into serious diseases. This fact puts dirofilariasis among the relevant parasitic

zoonoses. The wide spreading of dirofilariae and their importance for health explains the increasing interest in their study throughout the world (Marks & Bloomfield, 1998; Schwan & Durand, 2002; Simón *et al*., 2005; Lee *et al.,* 2007; Genchi *et al*., 2011; Brown *et al*., 2012; Morchón *et al*., 2012).

In Bulgaria, investigations for natural infection of dogs, some wild animals and humans with dirofilariids have been conducted by our research team (Mirchev & Trifonova, 2012a,b; Mirchev *et al.*, 2013; Panayotova-Pencheva *et al*., 2013; 2014) as well as other authors (Kanev *et al*., 1996; Georgieva *et al*., 2001; Kirkova *et al*., 2008; Kostadinov, 2013; Radev *et al.*, 2015). The results show that the spread of dirofilariae in Bulgaria increased gradually during the years. This unfavourable trend gave us reason to perform the present study. Our goal was to update the epidemiological data regarding *D. immitis* infection in animals. On the other hand there are no morphometric data about the etiological agent of the disease in materials from golden jackals and red foxes. Bearing in mind the latter we have described the morphometric characteristics of *D. immitis* in specimens from aforementioned species as well as from domestic dogs and compared them.

MATERIALS AND METHODS

During the period 2012–2013 helminthological necropsies of hearts and lungs of 489 carnivores were performed, 325 of them were from golden jackals (*Canis aureus* L.), 115 from red foxes (*Vulpes vulpes* L.), 27 from domestic dogs (*Canis familiaris* L.) – most of them were stray dogs, 15 from stray cats (*Felis silvestris catus* L.), 4 from martens (*Martes foina* L.) and 3 from wolves (*Canis lupus* L.). The wild animals were hunted according to the Law of hunting and preserve of the game. The dogs and cats were found dead or given dead by their owners. The animals originated from 19 regions of the country – Burgas, Dobrich, Gabrovo, Haskovo, Kyustendil, Montana, Pazardzhik, Pernik, Pleven, Plovdiv, Silistra, Sliven, Smolyan, Sofia city, Sofia district, Stara Zagora, Shumen, Vratsa and Yambol.

The identification of the established helminths was carried out on the basis of their morphological characteristics (Sonin, 1975). Morphometric data of *D. immitis* were obtained on the basis of specimens isolated from the pulmonary arteries from three animals: an adult male golden jackal and a stray dog originating from the region of Pazardzhik and an adult female red fox from the region of Plovdiv. Morphological structures of the adult helminths were measured using the classic methods of parasitology. Larvae were obtained directly from the uterus of the parasites. Their length and width were evaluated by the image analysing computer programme Image-Pro Plus-Version 6 as described by Panayotova-Pencheva & Alexandrov (2008). Pictures were taken using a light microscope "Leica DM5000 B", supplied with a camera and software (Leica Application Suite LAS v. 3.1). The adult specimens were deposited in the collection of the Institute of Experimental Morphology, Pathology and Anthropology with Museum, Bulgarian Academy of Sciences, Sofia, Bulgaria.

RESULTS

Epidemiological and pathological data

During the necropsies we found adult *D. immitis* in 160 of the animals i.e. the overall prevalence was 32.72%. In the jackals the prevalence was 37.54%, in the

Table 1. Prevalence of *Dirofilaria immitis* in carnivores from Bulgaria

Region	Golden jackals			Red foxes			Domestic dogs		
	N	N+	Pr	N	N+	Pr	N	N+	Pr
Burgas	79	23	29.11	15	4	26.67	8	4	50
Vratsa	6	0	0	8	0	0	–	–	–
Gabrovo	7	0	0	3	0	0	3	0	0
Dobrich	5	0	0	1	0	0	1	0	0
Kyustendil	1	0	0	1	0	0	–	–	–
Montana	2	0	0	4	0	0	–	–	–
Pazardzhik	63	37	58.73	18	8	44.44	5	3	60
Pernik	1	0	0	–	–	–	–	–	–
Pleven	18	5	27.78	11	0	0	3	0	0
Plovdiv	79	38	48.10	22	9	40.91	–	–	–
Silistra	18	8	44.44	5	2	40	1	1	100
Sliven	11	2	18.18	9	3	33.33	3	0	0
Smolyan	–	–	–	2	0	0	–	–	–
Sofia city	1	0	0	–	–	–	2	1	50
Sofia region	7	0	0	5	0	0	1	0	0
Stara Zagora	5	3	60	8	2	25	–	–	–
Haskovo	4	4	100	1	1	100	–	–	–
Shumen	4	0	0	–	–	–	–	–	–
Yambol	14	2	14.29	2	0	0	–	–	–
Total	325	122	37.54	115	29	25.22	27	9	33.33

N: number of necropsied animals; N+: number of animals infected with *D. immitis*; Pr: prevalence.

Fig. 1. Localisation of *Dirofilaria immitis.* **A.** In the right heart ventricle of a golden jackal. **B.** In the pulmonary arteries of a red fox.

foxes 25.22% and in the dogs – 33.33%. The infection intensity in the jackals varied between 1 and 19 specimens in animal (4.1 mean), in the foxes it was between 1 and 15 specimens (4.79 mean) and in the dogs – between 1 and 34 speci-

mens (14.43 mean). The cats, martens, and wolves were uninfected with dirofilariids. More detailed results about the spread of the parasite are shown in Table 1.

Most of the infected wild animals were over one year old. The age of the infected dogs varied between 6 and 10 years, 3 of them were stray dogs and the remaining 6 – domestic (dogs living in the yard or hunting dogs).

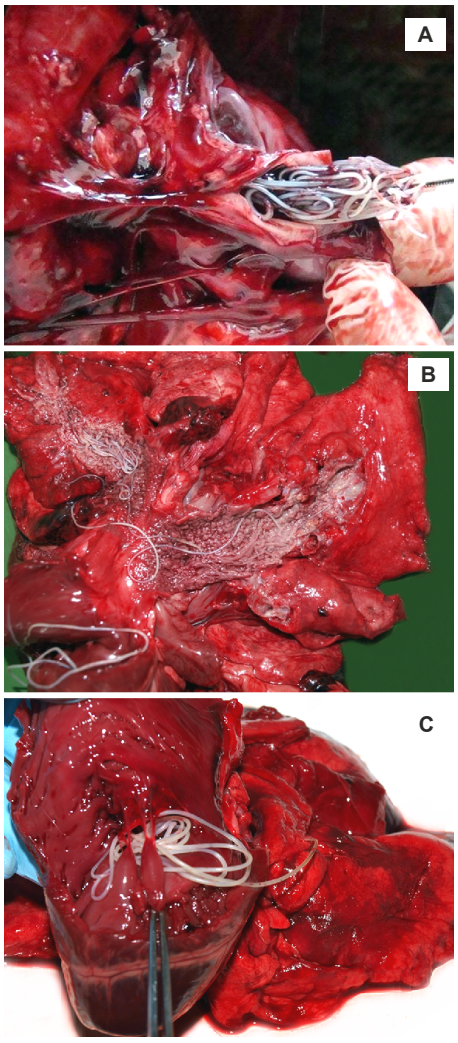

Fig. 2. Common macroscopic lesions by adult *Dirofilaria immitis*: **A.** Pulmonary artery obturation in a golden jackal. **B.** Granular appearance of the inner surface of pulmonary arteries in a red fox. **C.** Affected right atrioventricular valve in a golden jackal.

The worms were localised mainly in the heart (55.24%) of the hosts (Fig. 1A). In 27.97% of the cases they were found both in the heart and pulmonary arteries (Fig. 1B). Often in the loci with adult helminths, macroscopic lesions as obturation of pulmonary arteries, thickness and granulation of the inner surface of pulmonary arteries, affected atrioventricular valves were observed (Fig. 2).

Morphometric data

Adult *D. immitis* were large, elongated, whitish worms with a body length between 14 and 17 cm for the males and 19 and 29 cm for the females. The posterior end of the females was upright and that of males – spirally-curved. The oral opening was terminally located and surrounded by some pairs of cephalic papillae (Fig. 3A). The oesophagus was tube-like with a gradual extension of its shape from the anterior to the posterior end, the nerve ring was situated around its anterior part (Fig. 3A). The spicules (Fig. 3B) were of different size, the one being nearly twice longer than the other. The spicule stems were duct-like of a spongiform structure. Their proximal ends were broadened and distally the spicule stems were gradually getting narrower.

The distal ends of the spicules were also different (Fig. 4A). The final parts of the spicule stems turned into sharp growths which were rather marked in the large spicule. Furthermore, small spicule wings were observed in the distal end of the spicules differing in shape. The small spicule wing was oval and this of the large spicula was sharpened (Fig. 4A). Four pairs of large pre-cloacal papillae were

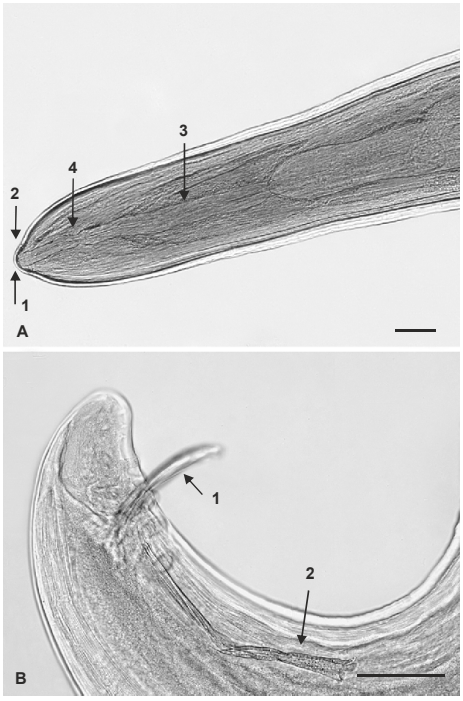

Fig. 3. Adult male *Dirofilaria immitis* found in a golden jackal from Bulgaria: **A.** Anterior end of the body – oral opening (1), cephalic papillae (2), oesophagus (3), nerve ring (4); bar=200 µm. **B.** Spicules – small spicule (1), large spicule (2); bar=100 µm.

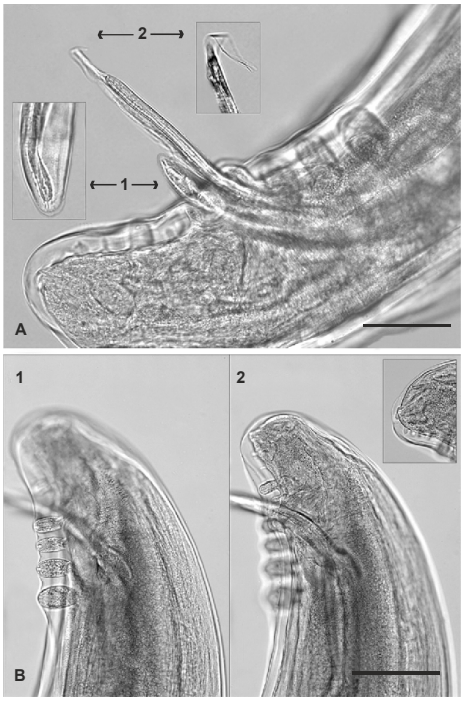

Fig. 4. Adult male *Dirofilaria immitis* found in a golden jackal from Bulgaria: **A.** Distal parts of the small (1) and large (2) spicules; bar=50 µm. **B.** Cloacal papillae – pre-cloacal papillae (1) and post-cloacal papillae (2); bar=100 µm.

observed (Fig. 4B). Post-cloacal papillae were of different size: one pair of middle size, one large pair and several pairs of small papillae (Fig. 4B). The number of the small ones varied between 4 and 10.

The vulva was opened in the anterior end of the body without any cuticular formations around it (Fig. 5A). The anus was near the tail tip also without cuticular formations around (Fig. 5B). The larvae were with an oval anterior end and a sharpened posterior one without specific cuticular formations.

The metric data of parasite species in materials from the different hosts are presented in Table 2.

DISCUSSION

During the survey for natural infections of dogs, some wild animals and humans with dirofilariids in Bulgaria, Kanev *et al*. (1996) have found adult *D. immitis* in 2.4% of the dogs. *Dirofilaria* infection has not been registered in the wild Canidae and Mustelidae. Later studies, however, showed that dirofilariasis was a common nematodosis in both dogs and wild canids. Georgieva *et al*. (2001) detected microfilariae of *D. immitis* in 7.4% of the investigated dogs. The authors found adult worms at necropsies in 12.5% of the stray dogs, in 5.1% of the foxes, in 4.4% of the jackals and in 5.5% of the wolves. Kirkova

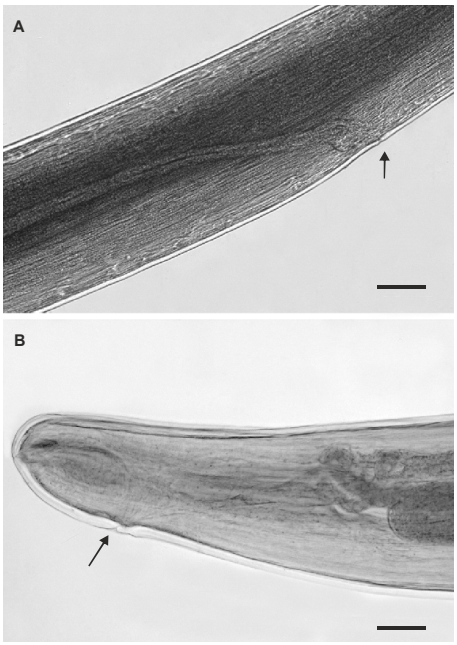

Fig. 5. Adult female specimen of *Dirofilaria immitis* found in a golden jackal from Bulgaria: **A.** Vulva (bar=200 μm). **B.** Anus (bar=100 μm).

et al. (2008) demonstrated microfilariae in 10.17% of the investigated dogs and adult *D. immitis* in 8.9% of the jackals and in 3% of the foxes. Studies by Kostadinov (2013) in stray dogs proved still higher spread of *D. immitis* – 12.5%. Adding our results in this line of investigations (prevalence of 37.54%, 25.22% and 33.33% in golden jackals, red foxes and dogs respectively) it is realised that the spread of *D. immitis* among canids in Bulgaria increased gradually over the last 20 years. Moreover, our data reveal prevalence of infection which was several times higher compared to those of the previous Bulgarian studies.

The comparison of our results with those of the foreign literature shows that *D. immitis* among the canids in the country was more widespread than in Romania, where the prevalence of infection in domestic dogs was between 2.17 and 3.3% (Ciocan *et al*., 2010; Mircean *et al*., 2012). These data are logical in view of the fact that Romania is located to the north of Bulgaria and there the conditions for the development of intermediate hosts of dirofilariids are less favourable. However, the prevalence of infection established by us was also higher to that reported for other countries of the Balkan Peninsula. For instance, the prevalence of *D. immitis* among domestic dogs in Greece was 10% *(*Papazahariadou *et al.,* 1994), in Albania – 13.5% (Rapti & Rehbein, 2010), in Serbia – between 7.2% and 17.2% (Tasić *et al*., 2008; 2012), in Turkey – 26% (Yaman *et al*., 2009). The prevalence of infection, established by us in foxes was even higher than that reported years ago in other Southern European countries – 11% in Spain (Gortazar *et al*., 1994) and 6.2% in Italy (Magi *et al*., 2009). These data give us grounds to assume a general trend towards increase of the spread of the infection in Southern Europe in the recent years.

The analysis of our data shows that dirofilariasis was widely distributed mainly in southern and lowland parts of Bulgaria. In the present study high parameters of infection were established in the Upper Thracian Plain. The fact that there were places in the Danubian Plain where canids were affected at a significant extent by the infection should not be overlooked. Such areas were most often situated near wet places, favourable for the reproduction of the intermediate hosts. We consider that recent changes in the land use and agricultural practices in Bulgaria are also causes for the wider distribution of dirofilariasis. The recommencement of the rice cultivation during the last 4–5 years in the southern parts of the country, the

Table 2. Metric data on *Dirofilaria immitis* in materials from different species of carnivores

	Structure	2012–2013 data from Bulgaria			Data from other authors		
		Golden Jackal (*Canis aureus* L.)	Red Fox (*Vulpes vulpes* L.)	Domestic Dog (*Canis familiaris* L.)			
		Mean±SD (Min–Max)	Mean±SD (Min–Max)	Mean±SD (Min–Max)	Sonin (1975) (Domestic Dog)	Sonin (1975) (American coati)	Furtado *et al.,* (2010) (Domestic Dog)
Male	BL, cm	15.3±0.9 (14–16.5)	15±0.4 (14.5–15.5)	16.7±0.3 (16.5–17)	12–18	10–11	11.9-16.2
	BWe, µm	529±69.9 (400–620)	506±24.1 (480–540)	528±58.5 (440–560)	877	–	–
	BWk, µm	122±13.9 (110–140)	141±7.4 (130–150)	135±5.8 (130–140)	192	–	–
	EL, µm	1266±59.2 (1200–1400)	1265±44.3 (1200–1300)	1328±18.9 (1300–1340)	1462	–	1080–1460
	MEW, µm	128±12.0 (110–140	88±4.5 (80–90)	97.5±15.0 (80–110)	124	–	90–130
	NR-AE, µm	374±38.4 (300–420)	390±18.3 (370–410)	362.5±25.0 (350–400)	300–400	306–378	350–470
	K-PE, µm	110±11.2 (90–120)	123±8.4 (110–130)	105±5.8 (100–110)	136	–	–
	LSL, µm	311±28.5 (280–350)	324±18.2 (300–340)	347.5±5.0 (340–350)	216–318	344–374	310–390
	SSL, µm	185.6 ±5.3 (180–190)	184±5.5 (180–190)	195± 5.8 (190–200)	188–200	168–180	150–190
Female	BL, cm	23.3±2.5 (20–27)	23.3±2.8 (19–26)	26.6±2.4 (22.5–29)	25–30	18–19	17.7–27.2
	BWe, µm	542.2±44.9 (450–600)	524±33.6 (470–560)	601.4±59.6 (480–660)	482–913	–	–
	BWa, µm	246.7±28.0 (220–300)	230±14.1 (220–250)	251.4±18.6 (240–290)	–	–	–
	EL, µm	1560±210.6 (1300–1900)	1416±107.0 (1240–1520)	1634±264.5 (1280–2120)	1084–1600	1606–1625	1050–1570
	MEW, µm	108.6±20.4 (90–150)	122±8.4 (110–130)	118.6±10.7 (110–140)	116–140	–	90–170
	NR-AE, µm	376.7±20.7 (350–400)	380±15.8 (360–400)	394.3±41.6 (360–480)	–	252–281	330–520
	V-AE, µm	2633±320.4 (2400–3200)	3228±791.2 (2600–4500)	2920±560.7 (2280–3600)	1651–2272	2381	2230–3260
	A-PE, µm	230±28.3 (200–260)	197.5±5.0 (190–200)	211.4±36.3 (170–260)	–	–	150–270
L	Length, µm	314±14.2 (289–336)	292.3±14.5 (264–320)	291.6±10.4 (258–308)	220–290	255–265	–
	Width, µm	7.5±0.8 (6–8.9)	6.8±0.7 (5.7–8.2)	6.2±0.5 (4.8–7)	5–7	4.5	–

BL – body length; BWe – body width at the end of the oesophagus; BWk – body width at the cloaca; EL – oesophagus length; MEW – maximum oesophagus width; NR-AE – distance from the nervous ring to the anterior end of the body; K-PE – distance from the cloaca to the posterior end of the body; LSL – large spicule length; SSL – small spicule length; BWa – body width at the anus; V-AE – distance from the vulva to the anterior end of the body; A-PE – distance from the anus to the posterior end of the body; L – larvae

large number of warm ponds, canals, puddles etc. provide excellent conditions for reproduction of mosquitoes. The lack of routine measures for disinsection additionally supports the increase in their number. We also suggest that the climate changes, together with the enhanced movement of dogs and people across Europe, have a direct relation to the increased geographical spread of dirofilariasis in the last years.

The analysis of morphometric data of *D. immitis* obtained from three different host species (Table 2) demonstrated that adult dirofilariae from dogs were the largest followed by those from the jackal and the fox. With regard to the length of the larvae, another fact was established – the microfilariae obtained from the jackal were the largest followed by those from the fox and the dog. No relationship could be identified as the other parameters in the different hosts were concerned. Comparing our results with those of other authors (Sonin, 1975; Furtado *et al*., 2010) it was found that the morphometric data for the different structures either matched the reported numerical values or were comparable to them.

In conclusion, the present study updated the epidemiological data on *D. immitis* infection in Bulgaria and in a larger context – in the Balkans. Besides, it provides the first morphometric description of the species in specimens from our country and the first one ever in materials from golden jackals and red foxes.

REFERENCES

Brown, H., L. Harrington, P. Kaufman, T. McKay, D. Bowman, C. Nelson, D. Wang & R. Lund, 2012. Key factors influencing canine heartworm, *Dirofilaria immitis*, in the United States. *Parasites & Vectors*, **5**, 245.

Ciocan, R., G. Darabus, M. S. Ilie, M. Imre & I. Hotea, 2010. Prevalence of dirofilariosis in dogs in Timis County. *Lucrari Stiintifice – Universitatea de Stiinte Agricole a Banatului Timisoara, Medicina Veterinara*, **43**, 36–40.

Furtado, A., F. Melo, E. Giese & J. Dos Santos, 2010. Morphological redescription of *Dirofilaria immitis*. *Journal of Parasitology*, **96**, 499–504.

Genchi, C., L. H. Kramer & F. Rivasi, 2011. Dirofilarial infections in Europe. *Vector-Borne and Zoonotic Diseases*, **11**, 1307–1317.

Georgieva, D., Z. Kirkova & A. Ivanov, 2001. A study on the incidence and diagnostics of dirofilariosis (heartworm disease) in carnivores. *Bulgarian Journal of Veterinary Medicine*, **4**, 231–236.

Gortazar, C., J. A. Castillo, J. Lucientes, J. C. Blanco, A. Arriolabengoa & C. Calvete, 1994. Factors affecting *Dirofilaria immitis* prevalence in red foxes in Northeastern Spain. *Journal of Wildlife Diseases*, **30**, 545–547.

Kanev, I., I. Kamenov, G. Ganchev, P. Prelezov, Y. Tzvetkov, V. Toncheva, M. Halacheva, D. Vuchev, T. Tanchev & G. Slavova, 1996. *Dirofilaria repens* and *Dirofilaria immitis* in animals and humans in Bulgaria. *Parasitologia*, **38**, 358.

Kirkova, Z., A. Ivanov, D. Georgieva & P. Prelezov, 2008. An update on dirofilariosis in dogs and wild canines in Bulgaria. In: *Proceedings of the Science Conference with International Participation Traditions and Contemporaneity in Veterinary Medicine*, Sofia, Bulgaria. University of Forestry Publishing House, Sofia, pp. 247–252. (BG).

Kostadinov, M., 2013. Prevalence of *Dirofilaria immitis* and *Dirofilaria repens* in 56 stray dogs from Burgas. In: *Proceedings of the IX National Conference of Parasitology* (Abstracts), Plovdiv, Bulgaria, p. 32–33 (BG).

Lee, S., H. Kim, S. Chong, T. A. Klein & W. Lee, 2007. Molecular survey of *Dirofilaria*

immitis and *Dirofilaria repens* by direct PCR for wild caught mosquitoes in the Republic of Korea. *Veterinary Parasitology*, **148**, 149–155.

Marks, C. A. & T. E. Bloomfield, 1998. Canine heartworm (*Dirofilaria immitis*) detected in red foxes (*Vulpes vulpes*) in urban Melbourne. *Veterinary Parasitology*, **78**, 147–154.

Mircean, V., M. Dumitrache, A. Györke, N. Pantchev, R. Jodies, A. Mihalca & V. Cozma, 2012. Seroprevalence and geographic distribution of *Dirofilaria immitis* and tick-borne infections (*Anaplasma phagocytophilum*, *Borrelia burgdorferi* sensu lato, and *Ehrlichia canis*) in dogs from Romania. *Vector-Borne and Zoonotic Diseases*, **12**, 595–604.

Magi, M., F. Macchioni, M. Dell'Omodarme, M. C. Prati, P. Calderini, S. Gabrielli, A. Iori & G. Cancrini, 2009. Endoparasites of red fox (*Vulpes vulpes*) in central Italy. *Journal of Wildlife Diseases*, **45**, 881–885.

Mirchev, R. & A. Trifonova, 2012a. The prevalence of *Dirofilarla immitis* (Leidy, 1856) in jackals (*Canis aureus* L., 1758) from Pazardzik region, Bulgaria. In: *Proceedings of the Seminar of Ecology – 2012*, Sofia, Bulgaria. Union of the Bulgarian Scientists, pp. 250–251.

Mirchev, R. & A. Trifonova, 2012b. *Dirofilaria immitis* in jackals from the West part of the Upper Thracian Plain. In: *Proceedings of the International Conference "Ecology – Interdiciplinary Science and Practice"*, Sofia, Bulgaria, PublishScie Set-Eco Ltd, pp. 208–214.

Mirchev, R., A. Trifonova & M. Panayotova-Pencheva, 2013. *Dirofilaria immitis* in foxes (*Vulpes Vulpes,* LINNAEUS, 1758) in different areas from Bulgaria. *International Scientific Online Journal Science & Technologies,* **3**, 38–42, Union of Scientists – Stara Zagora (BG).

Morchón, R., E. Carretón, J. González-Miguel & I. Mellado-Hernández, 2012. Heartworm disease (*Dirofilaria immitis*) and their vectors in Europe – new distribution trends. *Frontiers in Physiology*, **3**, 196.

Panayotova-Pencheva, M. & M. Alexandrov, 2008. Morphometric characteristics of first stage *Elaphostrongylus cervi* (Nematoda: Protostrongylidae) larvae from Bulgaria. *European Journal of Wildlife Research*, **54**, 771–774.

Panayotova-Pencheva, M., A. Trifonova, R. Mirchev & D. Salkova, 2013. Screening study for microfilaremia in canids from different regions of Bulgaria. In: *Proceedings of the Science Conference with International Participation "Traditions and Contemporaneity in Veterinary Medicine"*, Yundola, Bulgaria. 29.11–01.12 2013 (in press) (BG).

Panayotova-Pencheva, M., A. Trifonova, R. Mirchev & S. O. Movsesyan, 2014. Diversity and morphometric data of blood filarial larvae in carnivorous from Bulgaria. *Russian Parasitological Journal*, **1**, 14–22.

Papazahariadou, M. G., A. F. Koutinas, T. S. Rallis & S. T. Haralabidis, 1994. Prevalence of microfilaraemia in episodic weakness and clinically normal dogs belonging to hunting breeds. *Journal of Helminthology*, **68**, 243–245.

Radev, V., N. Lalkovski, P. Zhelyazkov, T. Kostova, P. Sabev, N. Nedelchev & R. Vassileva, 2015. Prevalence of gastrointestinal parasites and *Dirofilaria* spp. in stray dogs from some regions in Bulgaria. *Bulgarian Journal of Veterinary Medicine*, DOI: 10.15547/bjvm.872, http://tru.uni-sz.bg/bjvm/V%20Radev%20OnFirst.pdf (29 July date last accessed).

Rapti, D. & S. Rehbein, 2010. Seroprevalence of canine heartworm (*Dirofilaria immitis*) infection in Albania. *Parasitology Research*, **107**, 481–485.

Schwan, E. V. & D. T. Durand, 2002. Canine filariosis caused by *Dirofilaria immitis* in Mozambique: a small survey based on the identification of microfilariae: Research communication. *Journal of the South African Veterinary Association*, **73**, 124–126.

Simón, F., J. López-Belmonte, C. Marcos-Atxutegi, R. Morchón & J. R. Martín-Pacho, 2005. What is happening outside North America regarding human dirofilariasis? *Veterinary Parasitology*, **133**, 181–189.

Sonin, M. D., 1975. Filyariaty zhivotnyh i cheloveka i vyzyvaemye imi zabolevaniya. In: *Osnovy nematodologii*, ed. K. Rijikov, Moskva, 396 pp (RU).

Tasić, A., L. Rossi, S. Tasić, N. Miladinović-Tasić, T. Ilić & S. Dimitrijević, 2008. Survey of canine dirofilariasis in Vojvodina, Serbia. *Parasitology Research*, **103**, 1297–1302.

Tasić, A., S. Tasić-Otašević, S. Gabrielli, N. Miladinović-Tasić, A. Ignjatović, J. Dorđević, S. Dimitrijević & G. Cancrini, 2012. Canine dirofilaria infections in two uninvestigated areas of Serbia: Epidemiological and genetic aspects. *Vector-Borne and Zoonotic Diseases*, **12**, 1031–1035.

Yaman, M., M. Guzel, I. S. Koltas, M. Demirkazik & H. Aktas, 2009. Prevalence of *Dirofilaria immitis* in dogs from Hatay province, Turkey. *Journal of Helminthology*, **83**, 255–260.

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