Lista lucrărilor relevante

1. Gherman CM, Boros Z, Băieș MH, Cozma-Petruț A, Cozma V. 2022. A review of *Trichinella* species infection in wild animals in Romania. Food Waterborne Parasitol. 28:e00178.

   10.1016/j.fawpar.2022.e00178

2. Cotuțiu VD, Mihalca AD, Hołówka KA, Ionică AM, Cazan CD, Gherman CM. 2022. European Hares, *Lepus europaeus*, Represent a Reservoir Host for *Thelazia callipaeda* in Romania. Pathogens. 11(11):1225. 10.3390/pathogens11111225

3. Gherman CM, Kalmár Z, Györke A, Mircean V. 2018. Occurrence of *Giardia duodenalis* assemblages in farmed long-tailed chinchillas *Chinchilla lanigera* (Rodentia) from Romania. Parasit Vectors. 11(1):86.

   10.1186/s13071-018-2652-8.

4. Deak G, Mihalca AD, Hirzmann J, Colella V, Tăbăran FA, Cavalera MA, Brudașcă FG, Bauer C, Ionică AM, Alić A, Otranto D, Gherman CM. 2018. Validity of genus *Perostrongylus* Schlegel, 1934 with new data on Perostrongylus falciformis (Schlegel, 1933) in European badgers, *Meles meles* (Linnaeus, 1758): distribution, life-cycle and pathology. Parasit Vectors. 11(1):568. 10.1186/s13071-018-3124-x

5. Gherman CM, Mihalca AD. 2017. A synoptic overview of golden jackal parasites reveals high diversity of species. Parasit Vectors. 10(1):419.

   10.1186/s13071-017-2329-8

6. Gherman CM, Ionică AM, D'Amico G, Otranto D, Mihalca AD. 2016. *Angiostrongylus chabaudi* (Biocca, 1957) in wildcat (*Felis silvestris silvestris*, S) from Romania. Parasitol Res. 115(6):2511-7.

   10.1007/s00436-016-5032-3

7. Gherman CM, Deak G, Matei IA, Ionică AM, D'Amico G, Taulescu M, Barbu-Tudoran L, Sarmaşi A, Mihalca AD, Cozma V. 2016. A rare cardiopulmonary parasite of the European badger, *Meles meles*: first description of the larvae, ultrastructure, pathological changes and molecular identification of *Angiostrongylus daskalovi* Janchev & Genov 1988. Parasit Vectors. 9(1):423

   10.1186/s13071-016-1718-8

8. Gherman CM, Mihalca AD, Dumitrache MO, Györke A, Oroian I, Sandor M, Cozma V. 2012. CO2 flagging - an improved method for the collection of questing ticks. Parasit Vectors. 5:125

   10.1186/1756-3305-5-125

9. Gherman CM, Sándor AD, Kalmár Z, Marinov M, Mihalca AD. 2012. First report of *Borrelia burgdorferi* sensu lato in two threatened carnivores: the marbled polecat, *Vormela peregusna* and the European mink, *Mustela lutreola* (Mammalia: Mustelidae). BMC Vet Res. 8:137.

   10.1186/1746-6148-8-137

10. Mihalca AD, Dumitrache MO, Sándor AD, Magdaş C, Oltean M, Györke A, Matei IA, Ionică A, D'Amico G, Cozma V, Gherman CM. 2012. Tick parasites of rodents in Romania: host preferences, community structure and geographical distribution. Parasit Vectors. 5:266.

    10.1186/1756-3305-5-266

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A review of *Trichinella* species infection in wild animals in Romania

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ABSTRACT

Nematodes of the genus *Trichinella* are important zoonotic parasites present throughout Romania. This study aimed to assess the status of *Trichinella species* in wild animals in Romania over the past 30 years. A literature review of original studies concerning the only two species (out of the four in Europe) of *Trichinella* (*T. spiralis* and *T. britovi*) confirmed in wildlife from Romania was conducted and corroborated with the results of our original research concerning the topic. This review article has shown that, in Romania, European minks were infected with *T. spiralis*, while wolves, European wild cats, Eurasian lynx, golden jackals, stone marten, and European badgers were infected with *T. britovi*, respectively. Both *Trichinella* species have been identified in foxes, bears, wild boars, and ermines, but mixed infections have been found only in European polecats. *Trichinella* infection is still significantly present in Romania, infecting several wild omnivorous and carnivorous species in an equal manner, with different prevalence rates over the years. Regarding the spatial distribution of *T. spiralis* and *T. britovi* in Romania, both species can be found all over the country, but in wild animals, *T. britovi* is the most prevalent.

1. Introduction

Romania is a southeastern European country located in the north of the Balkan Peninsula. The Country is characterized by a temperate-continental climate of transitional type, with four clearly defined seasons (Trușcă and Alecu, 2005). Romania's Carpathic-Danubian-Pontic geography is defined by the Carpathian Mountains, the Black Sea, the Danube river, and its Delta. These units are in a nearly balanced combination with the hills and plains, determined by the step-like arrangement of the relief (Ilieş et al., 2017). Due to the forested mountains, wild animals are found in large numbers and show high diversity (Tănase et al., 2019). Many wild omnivorous and carnivorous species can host *Trichinella* species in Romania, thus maintaining the parasite's sylvatic life cycle (Boros et al., 2020).

Nematodes of the genus *Trichinella* are zoonotic parasites, being among the most widespread parasites in domestic and wild omnivores and predatory animals (Campbell, 1988; Pozio et al., 2009; Șuteu and Cozma, 2012). Rodents can act as a source of infection

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with *Trichinella* spp. for domestic and wild animals (Pozio and Zarlenga, 2005). The infection develops after the ingestion of raw meat, harboring the infective larvae (Pozio, 2007).

In Romania, the first information regarding trichinellosis dates back to 1866, when the supreme medical authority introduced the control of all slaughtered pigs in the country, but without any infections identified. In 1868, Schreiber had diagnosed the first case of human trichinellosis in Colţea hospital in Bucharest. In the same year, the first case of swine trichinellosis was confirmed in the southeastern part of the Country (Lupu and Cironeanu, 1960). In 1913, the use of trichinelloscopy was officially introduced in all slaughterhouses from Bucharest. Afterwards, new laws and regulations have been implemented to help reduce the number of human infections (Cironeanu, 1961).

In Romania, a priority epidemiological study on *Trichinella* spp. in domestic and wildlife hosts was conducted in the year 1960 with the use of trichinoscopy (Lupaşcu et al., 1970). Since then, the knowledge regarding *Trichinella* spp. infections has significantly improved, due to the introduction of the artificial digestion method in the 1990s. This method was used initially in parallel with trichinoscopy, whereas later studies focused on the use of artificial digestion. The risk of *Trichinella* infection still remains a concern in Romania, because of local eating habits and customs (Blaga et al., 2007). Most human cases are caused by consuming undercooked meat of pigs infected with *T. spiralis* (Blaga et al., 2007). Additionally, wild boar meat consumed in several local dishes, sometimes infected with *T. britovi*, might represent another source of infestation for the local human population (Blaga et al., 2009a; Blaga et al., 2009b). According to the International Commission on Trichinellosis, Romania accounted for most cases of human trichinellosis reported worldwide in 2004 (Neghină et al., 2010a). Furthermore, an increase in the incidence of trichinellosis in Romania has been observed since the beginning of the 21st century. After the fall of communism in 1989, the annual incidence increased from 0.1 to 4.1 cases per 100,000 inhabitants (until 1989) to 6.2 cases per 100,000 inhabitants, with a range of 2–15.9 per 100,000 inhabitants between 1990 and 2007 (Neghină et al., 2009; Neghină et al., 2010b). In a more recent study from 2018, among 1347 blood donors from Timiş county, aged 18–63 years, *T. spiralis* IgG antibodies were detected only in 2.0%. However, with further development and implementation of sanitary education programs for pig farmers and meat consumers, the number of human infections is expected to further decrease in the future (Pavel et al., 2022).

The present review of studies conducted between 1991 and 2021 aimed to assess the presence of *T. spiralis* and *T. britovi* (the only two species currently present in Romania) in Romania over the past 30 years.

2. Prevalence of *Trichinella* spp. infections in wild animals in Romania

One of the earliest studies aiming to broaden the epidemiological knowledge on *Trichinella* spp. in Romania, was conducted in 1991 in bears (*Ursus arctos*), wolves (*Canis lupus*), foxes (*Vulpes vulpes*), wild cats (*Felis silvestris*), badgers (*Meles meles*), wild boars (*Sus scrofa*), and polecats (*Mustela putorius*) (Tables 1, 2, 3, 4; Figs. 1, 2, 3) (Nesterov et al., 1991). A further study was conducted between 1991 and 1994 in a restricted area of the Carpathian Mountains (Jiu valley), in red foxes and wild boars (Table 2, Table 3, Fig. 1, Fig. 2) (Cristea and Şuteu, 1996), indicating that these animal species play a limited role in the sylvatic cycle in that area. Afterwards, several studies focused on the detection of *Trichinella* spp. infection in wild animals from different regions of Romania. Between 1992 and 1997, wild boars and bears from Transylvania were subjected to larvae detection methods and the results are provided in Tables 1 and 2, and in Fig. 1, respectively (Gherman, 1998). The low prevalence rates detected in wild boars compared to bears show that, in the

Table 1
Trichinella spp. infections in bears (*Ursus arctos*) from Romania between 1991 and 2021.

Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
1991	Central Romania	50	Trichinelloscopy	18.5%		Nesterov et al., 1991
1992–1997	Transylvania	503	Trichinelloscopy Artificial digestion	12.1%		Gherman, 1998
1997–2004	Transylvania Other counties	1062	Trichinelloscopy	12.4%		Blaga et al., 2009b
2000–2005	Cluj county Mureş county	2	Artificial digestion PCR	100.0%	*T. spiralis*	Blaga et al., 2009a
2000	Covasna county	6	Trichinelloscopy	66.6%		Oprescu et al., 2007
1997–2007	Covasna county	60	Trichinelloscopy Artificial digestion	38.3%		Oprescu et al., 2007
2010–2015	Eastern Romania	49	Artificial digestion PCR	6.5% 15.6%	*T. spiralis* *T. britovi*	Iacob, 2017
2011-2015	Eastern Transylvania	37	Trichinelloscopy	5.4%		Borka-Vitális et al., 2017
2015	North-Eastern, North-Western, Central regions, Western, South, and South-Eastern regions of Romania	147	Artificial digestion PCR	6.1% 4.7%	*T. spiralis* *T. britovi*	Nicorescu et al., 2015

Table 2
Trichinella spp. infections in wild boars (*Sus scrofa*) from Romania between 1991 and 2021.

Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
1991	Central Romania	38,908	Trichinelloscopy	0.1%		Nesterov et al., 1991
1991–1994	Jiu Valley	1210	Trichinelloscopy Artificial digestion	23.5%		Cristea and Șuteu, 1996
1992–1997	Transylvania	17,053	Trichinelloscopy Artificial digestion	0.3%		Gherman, 1998
1997–2007	Covasna county	210	Trichinelloscopy Artificial digestion	9.5%		Oprescu et al., 2007
1990–1999	Constanța county	340	Trichinelloscopy	0.1%		Olteanu, 2001
1997–2004	Transylvania Other counties	29,825	Trichinelloscopy	8.7%		Blaga et al., 2009b
1998–2011	Timiș county	823	Trichinelloscopy	0.5%		Borza et al., 2012
2010–2014	Hunedoara county	973	Trichinelloscopy Artificial digestion	1.3%		Ciobotă et al., 2015
2000–2005	Cluj county Mureș county	5	Artificial digestion PCR	30.0% 70.0%	*T. spiralis* *T. britovi*	Blaga et al., 2009a
2015	North-Eastern, North-Western, Central, Western, South-Western, Southern, and South-Eastern regions of Romania	5596	Artificial digestion PCR	0.8% 0.6% *T. spiralis* + *T. britovi* / 0.0%	*T. spiralis* *T. britovi* *T. spiralis* + *T. britovi*	Nicorescu et al., 2015
2010–2015	Eastern Romania	8024	Artificial digestion PCR	6.5% 0.4%	*T. spiralis* *T. britovi*	Iacob, 2017

mentioned region, bears have a more important role in the maintenance of the sylvatic cycle than wild boars. Furthermore, of two ten-year studies, the first one (1990–1999) focused on wild boars (Olteanu, 2001), while the other study took into consideration the prevalence of *Trichinella* infection in bears and wild boars from Covasna county in central Romania (Table 1, Table 2, Fig. 1) (Oprescu et al., 2007), highlighting the importance of these animal species for trichinellosis in Romania. The presence of *Trichinella* spp. infection in three wild carnivore species from Romania (fox, wolf, and wild cat) was assessed between October 1999 and March 2002, which brought updates regarding the epidemiology of trichinellosis in these wild carnivore species (Table 3, Fig. 2) (Gherman et al., 2002). Based on the results obtained, wild carnivores represent the most important hosts in the sylvatic cycle of *Trichinella* spp. in Romania. Routine *Trichinella* test (trichinelloscopy) was conducted with game species (wild boars, bears), between 1997 and 2004, to investigate the extent of the infection in hunted animals in Romania (Table 1, Table 2, Fig. 1). Apart from their role in the sylvatic cycle, they could represent a source of inter-foci transmission of *Trichinella* spp. due to different feeding habits compared to domestic species (Blaga et al., 2009b). An epidemiological study of *Trichinella* infection in wild boars in Timiș county was done between 1998 and 2011 (Table 2). The data were collected from the Veterinary Public Health Department of Timiș County and show a low prevalence rate in wild boars, meaning this species of animal exhibit a minor role in the local sylvatic life cycle of *Trichinella* (Fig. 1) (Borza et al., 2012).

Another study was conducted between 2010 and 2014 on the epidemiology of *Trichinella* infection in wild boars from Hunedoara county in western Romania. The highest prevalence of infection was established in 2012 (1.3%), followed by 2013 (1.1%), 2010 (0.8%), and then 2014 (0.7%), whereas all animals examined in 2011 were negative (Table 2, Fig. 1). The results indicated that wild boars from this county had a low infection rate with *Trichinella* spp. (Ciobotă et al., 2015) and that, over the years, infected animals became less and less common. Brown bears in eastern Transylvania were also tested for infection between 2011 and 2015 and the results confirmed that bears from this area contribute to the maintenance of the sylvatic life cycle of parasites (Table 1, Fig. 1) (Borka-Vitális et al., 2017). Marian et al. (2015) assessed the prevalence of *Trichinella* spp. infection in large wild carnivores from Romania between 2014 and 2015. The highest prevalence was identified in Eurasian lynx, followed by wolves, golden jackals, and wildcats, as seen in Table 3. The methods used in the detection of *Trichinella* spp. in these studies are presented in Tables 1, 2, and 3.

The present review, which included research conducted over the last 30 years, performed an analysis of the presence of *Trichinella* infection in more than 80% of Romania's territory (33 out of 41 counties), as it can be observed in the figures. Infections of wild animals were found in all studied areas during this period. As the number of examined animals and the detection methods varied drastically among the different studies, the comparative contribution of different host species to the parasite's maintenance is difficult to assess. Looking at the studies with large sample sizes, prevalences of wild canids and felids are at the higher end of the range, while bears and mustelids are less frequently affected. *Trichinella* infection in wild boars seems to be least frequent and may reflect a low proportion of mammalian carcasses in the diet (Tabels 1–4 and Oltean et al., 2014; Boros et al., 2020; Boros et al., 2021b).

Table 3
Trichinella spp. infections in wild carnivores from Romania between 1991 and 2021.

Animal species	Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
Wolves (*Canis lupus*)	1991	Central Romania	399	Trichinelloscopy	30.5%		Nesterov et al., 1991
	1999–2002	Transylvania	7	Artificial digestion	71.4%		Gherman et al., 2002
	2014–2015	Transylvania	3	Artificial digestion	66.7%		Marian et al., 2015
Foxes (*Vulpes vulpes*)	1991	Central Romania	972	Trichinelloscopy	15.8%		Nesterov et al., 1991
	1991–1994	Jiu Valley	163	Trichinelloscopy Artificial digestion	23.5%		Cristea and Șuteu, 1996
	1999–2002	Transylvania	50	Artificial digestion	16.0%		Gherman et al., 2002
	2000–2005	Cluj, Covasna and Harghita counties	71	Artificial digestion PCR	14.0% 57.1%	*T. spiralis* *T. britovi*	Blaga et al., 2009a
	2015	Arad, Hunedoara, and Timiş counties	121	Artificial digestion PCR	96.0% 4.0%	*T. britovi* *T. spiralis*	Imre et al., 2015
Wild cats (*Felis silvestris*)	1991	Central Romania	158	Trichinelloscopy	31.5%		Nesterov et al., 1991
	1999–2002	Transylvania	6	Artificial digestion	16.6%		Gherman et al., 2002
	2014–2015	Buzău, Tulcea, and Maramureș counties	3	Artificial digestion	66.7%		Marian et al., 2015
Eurasian lynx (*Lynx lynx*)	2014–2015	Transylvania	3	Artificial digestion	66.7%		Marian et al., 2015
Golden jackals (*Canis aureus*)	2006	Tulcea county	1	Artificial digestion PCR	100.0%	*T. britovi*	Blaga et al., 2008
	2014–2015	Botoșani, Buzău, Brăila, Tulcea, Ialomița, Ilfov, Giurgiu, Teleorman, Olt, Vâlcea, Dolj, Gorj, and Timiș counties	54	Artificial digestion	53.7%		Marian et al., 2015

3. Species of *Trichinella* circulating in wild animals

Identifying the species of *Trichinella* in Romania is important due to the fact that *Trichinella spiralis* is more often found in domestic animals. However, this species can also appear in wild animals. Infection in humans is most often caused by *T. spiralis*. Therefore, identifying the exact species of *Trichinella* present in wild animals in Romania represented an important step in this field (Cozma et al., 2013; Cozma et al., 2016). Several studies have been conducted over the last 15 years and PCR-based methods confirmed the presence of *T. spiralis* and *T. britovi* in wild species in Romania.

3.1. *Trichinella spiralis infections in wild animals in Romania*

Trichinella spiralis infections were found in bears, wild boars, red foxes (Blaga et al., 2009a; Nicorescu et al., 2015; Imre et al., 2015), European minks (*Mustela lutreola*) (Oltean et al., 2014), and European polecats (*Mustela putorius*) (Boros et al., 2021b), as seen in Tables 1, 2, 3, and 4.

The results show that this parasite species was identified more frequently in bears and less frequently in foxes. However, there are few studies using PCR to determine the parasite species in wild animals, so final conclusions regarding this topic still remain to be drawn.

3.2. *Trichinella britovi infections in wild animals in Romania*

Trichinella britovi infections were found in golden jackals (Blaga et al., 2008;), wild cats, wolves, Eurasian lynx (Blaga et al., 2009a), beech martens (*Martes foina*), short-tailed weasels (*Mustela erminea*) (Oltean et al., 2014), foxes (Imre et al., 2015), wild boars, bears (Blaga et al., 2009a; Nicorescu et al., 2015; Iacob, 2017), European badgers (*Meles meles*) (Boros et al., 2021a), as seen in Tables 1, 2, 3, and 4. Mixed infections with *T. britovi* and *T. spiralis* were found in wild boars (Nicorescu et al., 2015) and polecats (Boros et al., 2021b).

The results show that *T. britovi* was identified more frequently in wild boars (Table 2) and mustelids (Table 4) but less frequently in

Table 4
Trichinella spp. infections in mustelids from Romania between 1991 and 2021.

Animal species	Year	Location (areas or counties)	Number of animals	Methods	Prevalence	*Trichinella* species (PCR)	Reference
European badgers (*Meles meles*)	1991	Central Romania	166	Trichinelloscopy	6.0%		Nesterov et al., 1991
	2015–2019	Timiș, Bihor, Sălaj, Maramureș, Cluj, Alba, Mureș, Sibiu, Brașov, Harghita, Ilfov, Giurgiu, Constanța, and Tulcea counties	61	Trichinelloscopy Artificial digestion PCR	1.6%	*T. britovi*	Boros et al., 2021a
Polecats (*Mustela putorius*)	1991	Central Romania	157	Trichinelloscopy	5.2%		Nesterov et al., 1991
	2016–2020	Arad, Brașov, Constanța, Brăila, Călărași, Ialomița, Giurgiu, Teleorman, and Olt counties	75	Trichinelloscopy Artificial digestion PCR	1.3%	*T. spiralis*	Boros et al., 2021b
European mink (*Mustela lutreola*)	2009–2013	Danube Delta	3	Artificial digestion PCR	33.3%	*T. spiralis*	Oltean et al., 2014
Beech martens (*Martes foina*)	2009–2013	Danube Delta	4	Artificial digestion PCR	50.0%	*T. britovi*	Oltean et al., 2014
Short-tailed weasels (*Mustela erminea*)	2009–2013	Danube Delta	4	Artificial digestion PCR	50.0%	*T. britovi*	Oltean et al., 2014

Fig. 1. The map of Romania showing the collection sites of bears and wild boars. Black circles: bear samples; Black stars: wild boar samples; Big circles and stars: general areas; Small circles and stars: counties.

golden jackals (Table 3). Regarding the mixed infection with *T. britovi* and *T. spiralis*, it was identified only two times (Table 2, Table 3), indicating that its occurrence is a rare phenomenon.

3.3. Serology in wild animals with Trichinella spp. infections

The serology approach regarding *Trichinella* infection in wild and domestic animals has been used less frequently in Romania. Nevertheless, a study from 2018 reported the seroprevalence of *Trichinella* spp. in wild boars (84 plasma samples) from Bihor county, located in western Romania. These animal samples were tested by ELISA and Western blot, although the artificial digestions of the tissue samples ($n = 84$) were negative. At analysis by indirect ELISA, 65.4% ($n = 55$) were positive, 7.1% ($n = 6$) were doubtful, and 27.38% ($n = 23$) were negative. On analysis by Western blot, from 26 samples, only 23.7% (n = 6) were positive, whereas 76.9% ($n = 20$) were negative, thus indicating the presence of anti-*Trichinella* antibodies in these animals (Boros et al., 2020). This study is important because it shows that antibodies can be found in animals that are negative in the golden standard method, thus indicating these animals probably had a very small infection or the samples (tissue) weren't taken correctly. The same situation might occur in other similar contexts and by this exposing the local population to this parasitic infection.

Fig. 2. The map of Romania showing the collection sites of wolves, foxes, wild cats, lynxes and golden jackals. Black circles: wolf samples; Black rectangles: fox samples; Black stars: wild cat samples; Black triangle: lynx samples; Black diamonds: golden jackal samples; Big circles, rectangles, stars, triangles, and diamonds: general areas; Small circles stars, rectangles, triangles and diamonds: counties.

Fig. 3. The map of Romania showing the collection sites of badgers, polecats, European minks, beech materns, and short-tailed weasels. Black circles: badger samples; Black stars: polecat samples; Black rectangles: beech matern samples; Black triangle: European minks samples; Black diamonds: short-tailed weasel samples; Big circles, rectangles, stars, triangles, and diamonds: general areas; Small circles and stars: counties; The rectangle, triangle and diamond: Danube Delta.

The results referenced above regarding *Trichinella* infection in wild species from Romania seem to reconfirm the combined statements of Campbell (1983) and Neghină et al. (2012) according to which „the saga of the helminth, destined to remain with us, both in nature and in the laboratory, will still haunt and fascinate scientists at the same time!" from both developing and developed countries, as they try to answer new questions regarding the parasite's evil nature.

4. Conclusions

Trichinella infection is still significantly present in Romania, infecting several wild omnivorous and carnivorous species in an equal manner, with different prevalence rates over the years, thus maintaining the sylvatic focus of the parasites. Two species of *Trichinella*, namely *T. spiralis* and *T. britovi*, were identified in wild animals. Although the relative frequency of the two parasite species and the contribution of different host species are difficult to assess given the heterogenous data available, it is clear that dietary habits of the carnivores and omnivores play a major role, which needs to be addressed in future studies.

Declaration of Competing Interest

All authors declare no conflicts of interest regarding the content in this article.

References

Blaga, R., Durand, B., Antoniu, S., Gherman, C., Creţu, C.M., Cozma, V., Boireau, P., 2007. A dramatic increase in the incidence of human trichinellosis in Romania over the past 25 years: impact of political changes and regional food habits. Am. J. Trop. Med. Hyg. 76, 983–986. https://doi.org/10.4269/ajtmh.2007.76.983.

Blaga, R., Gherman, C., Seucom, D., Cozma, V., Boireau, P., 2008. First identification of *Trichinella* sp. in Golden jackal (*Canis aureus*) in Romania. J. Wildl. Dis. 44, 457–459. https://doi.org/10.7589/0090-3558-44.2.457.

Blaga, R., Gherman, C., Cozma, V., Zocevic, A., Pozio, E., Boireau, P., 2009a. *Trichinella* species circulating among wild and domestic animals in Romania. Vet. Parasitol. 159, 218–221. https://doi.org/10.1016/j.vetpar.2008.10.034.

Blaga, R., Durand, B., Stoichici, A., Gherman, C., Ştefan, N., Cozma, V., Boireau, P., 2009b. Animal *Trichinella* infection in Romania: geographical heterogeneity for the last 8 years. Vet. Parasitol. 159, 290–294. https://doi.org/10.1016/j.vetpar.2008.10.059.

Borka-Vitális, L., Domokos, C., Földvári, G., Majoros, G., 2017. Endoparasites of brown bears in eastern Transylvania, Romania. Ursus 28, 20–30. https://doi.org/10.2192/URSU-D-16-00015.1.

Boros, Z., Vallée, I., Panait, L.C., Gherman, M.C., Chevillot, A., Boireau, P., Cozma, V., 2020. Seroprevalance of *Trichinella* spp. in wild boars (*Sus scrofa*) from Bihor county, western Romania. Helminthologia 57, 235–240.

Boros, Z., Ionică, A.M., Deak, G., Mihalca, A.D., Chişamera, G.B., Gyorke, A., Gherman, C., Cozma, V., 2021a. The European badger, *Meles meles*, as a new host for *Trichinella britovi* in Romania. Vet. Parasitol. 297, 109545 https://doi.org/10.1016/j.vetpar.2021.109545.

Boros, Z., Ionică, A.M., Deak, G., Mihalca, A.D., Chişamera, G.B., Constantinescu, I.C., Adam, C., Gherman, C., Cozma, V., 2021b. *Trichinella* spp. infection in European polecats (Linnaeus, 1758) from Romania. Helminthologia 58, 323–327. https://doi.org/10.2478/helm-2021-0032.

Borza, C., Neghină, A.M., Dumitraşcu, V., Tirnea, L., Calma, C.L., Neghină, R., 2012. Epizootiology of trichinellosis in pigs and wild boars in western Romania, 1998–2011. Vect. Borne Zoonotic Dis. 12, 712–713. https://doi.org/10.1089/vbz.2011.0955.

Campbell, W.C., 1983. Historical introduction. In: Campbell, W.C. (Ed.), *Trichinella* and Trichinosis. Plenum, New York, pp. 1–30.

Campbell, W.C., 1988. Trichinosis revisited, another look at modes of transmission. Parasitol. Today 4, 83–86. https://doi.org/10.1016/0169-4758(88)90203-7.

Ciobotă, F.O., Cristea, Gh., Ioniţă, M., Mitrea, I.L., 2015. Epidemiological study on *Trichinella* infection in pigs and wild boars in Hunedoara county (Romania), during of 2010-2014 period. Proc. Rom. Acad. B 45–47.

Cironeanu, I., 1961. Istoricul şi combaterea trichinelozei în ţara noastră (the history and control of trichinelosis in our country). Microbiol. Parasitol. Epidemiol. 5, 397–407.

Cozma, V., Iovu, A.M., Onac, D., Oltean, M., 2013. The genetic variability within different food parasites strains and genotypes, in geo-climate conditions from Romania. In: Book of Abstracts of the Conference "Actual Problems of Protection and Sustainable Use of the Animal World Diversity", 10–12 October 2013, Chişinău, Moldavia, 8th ed., pp. 13–15 https://ibn.idsi.md/sites/default/files/imag_file/13-15_0.pdf.

Cozma, V., Gyorke, A., Gherman, C., Oleleu, A.M., Jarca, A., Barburaş, D., Cozma-Petruţ, A., 2016. Major meat parasites in Romania - species, strains, and genotypes. In: Book of Abstracts of the Conference "Sustainable Use, Protection of Animal World and Forest Management in the Context of Climate Change", 12–13 October 2016, Chişinău, Moldavia, 9th ed., pp. 16–17 https://ibn.idsi.md/sites/default/files/imag_file/16-17_2.pdf.

Cristea, G., Şuteu, I., 1996. Măsuri de supraveghere, combatere şi prevenire a trichinelozei în Valea Jiului – cel mai mare focar din România (1987-2001) (surveillance, control and prevention measures of trichinosis in the Jiu Valley - the largest outbreak in Romania (1987-2001)). Sci. Parasitol. 2, 22–27.

Gherman, C., 1998. Epizootologia Trichinelozei in Transilvania (Epizootology of Trichinosis in Transylvania). PhD thesis. Cluj-Napoca, Romania, p. 238.

Gherman, C., Cozma, V., Mircean, V., Brudaşcă, F., Rus, N., Detesan, A., 2002. Zoonoze helmintice la specii de carnivore sălbatice din fauna României (helminthic zoonosis in species of wild carnivores from the Romanian fauna). Sci. Parasitol. 3, 17–21.

Iacob, O., 2017. Epidemiological surveillance of *Trichinella* spp. infection in animals in the eastern part of Romania (Moldova) and the potential risk of human infection. In: Book of Abstracts of the Symposium "Actual Problems of Zoology and Parasitology: Achievements and Prospects", 13 October 2017, Chişinău, Moldavia, p. 161. https://ibn.idsi.md/sites/default/files/imag_file/161_0.pdf.

Ilieş, A., Ilieş, D.C., Tătar, C., Ilieş, M., 2017. Geography of tourism in Romania. In: Widawski, K., Wyrzykowski, J. (Eds.), The Geography of Tourism of Central and Eastern European Countries, 2nd ed. Springer, Cham, pp. 329–374. https://doi.org/10.1007/978-3-319-42205-3_9.

Imre, K., Pozio, E., Tonanzi, D., Sala, C., Ilie, M.S., Imre, M., Morar, A., 2015. The red fox (*Vulpes vulpes*) plays a minor role in the epidemiology of the domestic cycle of *Trichinella* in Romania. Vet. Parasitol. 212, 448–450. https://doi.org/10.1016/j.vetpar.2015.06.032.

Lupaşcu, G., Cironeanu, I., Hacig, A., Pambuccian, G., Simionescu, O., Solomon, P., Ţânţăreanu, J., 1970. Trichineloza (Trichinelosis). Editura Academiei Republicii Socialiste, Bucharesti, p. 246.

Lupu, A., Cironeanu, I., 1960. Anchetă asupra frecvenţei şi răspândirii parazitului *Trichinella spiralis* la animale domestice şi sălbatice din R.P.R. Consideraţii epizootologice şi de combatere a trichinelozei (The investigation of the frequency and spread of the *Trichinella spiralis* parasite in domestic and wild animals in the R.P.R. Epizootological and trichinosis considerations). In: Zoonoze, Editura SSM, Bucharest, pp. 337–366.

Marian, I., Mihalca, A.D., Gherman, C.M., 2015. Prevalence of *Trichinella* spp. infection in large wild carnivore species from Romania between Jan 2014 and July 2015. Buasvm CN 72, 438–440. https://doi.org/10.15835/buasvmcn-vm:11631.

Neghină, R., Neghină, A.M., Marincu, I., Moldovan, R., Iacobiciu, I., 2009. Epidemiological and diagnostic findings during a 16-year-long trichinellosis surveillance in Timiş County, Romania. Vet. Parasitol. 159, 328–331. https://doi.org/10.1016/j.vetpar.2008.10.045.

Neghină, R., Neghină, A.M., Marincu, I., Moldovan, R., Iacobiciu, I., 2010a. Epidemiology and epizootology of trichinellosis in Romania 1868–2007. Vect. Borne Zoonotic Dis. 10, 323–328. https://doi.org/10.1089/vbz.2009.0084.

Neghină, R., Neghină, A.M., Marincu, I., Moldovan, R., Iacobiciu, I., 2010b. Trichinellosis and poverty in a Romanian industrial area: an epidemiological study and brief review of literature. Foodborne Pathog. Dis. 7, 757–761. https://doi.org/10.1089/fpd.2009.0496.

Neghină, R., Moldovan, R., Marincu, I., Calma, C.L., Neghină, A.M., 2012. The roots of evil: the amazing history of trichinellosis and *Trichinella* parasites. Parasitol. Res. 110, 503–508. https://doi.org/10.1007/s00436-011-2672-1.

Nesterov, V., Colofan, I., Nițulescu, A., Costiov, F., Dumitrescu, C., Milla, C., Popescu, S., 1991. Implicaţii ale activităţii umane în epizootologia trichinelozei silvatice (implications of human activity in the epizootiology of silvical trichinosis). Rev. Rom. Paraz. I 71–72.

Nicorescu, I.M.D., Ioniţă, M., Ciupescu, L., Buzatu, C.V., Tănăsuică, R., Mitrea, I.L., 2015. New insights into the molecular epidemiology of *Trichinella* infection in domestic pigs, wild boars, and bears in Romania. Vet. Parasitol. 212, 257–261. https://doi.org/10.1016/j.vetpar.2015.07.021.

Oltean, M., Kalmár, Z., Kiss, B.J., Marinov, M., Vasile, A., Sándor, A.D., Rosenthal, B.M., 2014. European mustelids occupying pristine wetlands in the Danube delta are infected with *Trichinella* likely derived from domesticated swine. J. Wildl. Dis. 50, 972–975. https://doi.org/10.7589/2013-12-335.

Olteanu, G., 2001. Trichinellosis in Romania: a short review over the past twenty years. Parasite 8, S98–S99. https://doi.org/10.1051/parasite/200108s2098.

Oprescu, I., Dărăbuş, Gh., Morariu, S., Mederle, N., Ilie, M., Imre, K., Imre, M., Mako, A., Nincov, I., 2007. The prevalence of trichinellosis in Covasna county between 1997–2007. In: Lucrări ştiinţifice Medicină Veterinară (Scientific works in Veterinary Medicine) XLII, pp. 64–69.

Pavel, R., Ursoniu, S., Păduraru, A.A., Lighezan, R., Lupu, M.A., Olariu, T.R., 2022. Seroprevalence and risk factors of *Trichinella spiralis* infection in blood donors from Western Romania. Medicina 58, 128.

Pozio, E., 2007. World distribution of *Trichinella* spp. infections in animals and humans. Vet. Parasitol. 149, 3–21. https://doi.org/10.1016/j.vetpar.2007.07.002.

Pozio, E., Zarlenga, D.S., 2005. Recent advances on the taxonomy, systematics and epidemiology of *Trichinella*. Int. J. Parasitol. 35, 1191–1204. https://doi.org/10.1016/j.ijpara.2005.07.012.

Pozio, E., Hoberg, E., La Rosa, G., Zarlenga, D.S., 2009. Molecular taxonomy, phylogeny and biogeography of nematodes belonging to the *Trichinella* genus. Infect. Genet. Evol. 9, 606–616. https://doi.org/10.1016/j.meegid.2009.03.003.

Șuteu, I., Cozma, V., 2012. Parazitologie clinică veterinară (Clinical Veterinarian Parasitology), 2nd Vol. Risoprint ed. Cluj-Napoca.
Tănase, M.A., Villard, L., Pitar, D., Apostol, B., Petrila, M., Chivulescu, S., Badea, O., 2019. Synthetic aperture radar sensitivity to forest changes: a simulations-based study for the Romanian forests. Sci. Total Environ. 689, 1104–1114. https://doi.org/10.1016/j.scitotenv.2019.06.494.
Trușcă, V., Alecu, M., 2005. Romania's Third National Communication on Climate Change under the United Nations Framework Convention on Climate Change. Available online: https://unfccc.int/sites/default/files/resource/romnc3Romania.pdf.

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Article

European Hares, *Lepus europaeus*, Represent a Reservoir Host for *Thelazia callipaeda* in Romania

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Abstract: Thelaziosis caused by *Thelazia callipaeda* is an emerging disease in Europe. Only two reports of naturally infected lagomorphs have been published so far. The aim of this study was to evaluate the status of the Romanian populations of European brown hares, *Lepus europaeus* as reservoir hosts for *T. callipaeda*. Between November 2019 and November 2021, the eyes of 326 *L. europaeus* carcasses were examined for the presence of ocular parasites. Nematodes were stored in plastic vials with physiological saline, followed by morphological and molecular identification. QGis 3.20 and EpiInfo™ 7 were used for mapping and statistical analysis. Four (1.23%) hares harbored *T. callipaeda* infection, with a total of 84 nematodes collected (mean intensity 21 nematodes/host), with 45 males, 39 females (two sexually immature, seven with only eggs, and 30 with eggs and larvae). One specimen from each host was successfully sequenced resulting in a 100% similarity with several other sequences of *T. callipaeda* haplotype 1. Statistical analysis revealed no significant results. The current study represents a first report of *T. callipaeda* in the European brown hare in Romania, and the second in Europe, also reiterating the role of lagomorphs as reservoir hosts for this zoonotic ocular nematode.

Keywords: *Thelazia callipaeda*; *Lepus europaeus*; reservoir host; Thelaziosis

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1. Introduction

Thelaziosis caused by *Thelazia callipaeda* (Spirurida, Thelaziidae) is a rapidly emerging zoonosis reported across most of Europe and Asia [1]. Domestic and wild carnivores are considered the primary vertebrate hosts of *T. callipaeda* [2]. Still, occasionally, adult nematodes were reported from other mammals such as other carnivores, lagomorphs, wild boars, and humans [3–7].

Infections in both domestic and wild carnivores are commonly reported across the distribution range of this nematode [1]. Human ocular infections follow an emerging trend in most countries where *T. callipaeda* had been reported in the main reservoir hosts [6–8]. These findings not only underline the zoonotic potential of this nematode but also highlight the clinical implications of the disease. Symptoms range from mild to severe conjunctivitis [9], further complicated by bacterial or fungal infections, which may lead to corneal ulcers [7].

In Romania, the disease was first diagnosed in 2014 [10], in a domestic dog from the western part of the country. Subsequent surveillance documented the spread across most of Romania's territory, in a wide variety of hosts: domestic dogs [11–13], domestic cats [13], jackals, wolves, wildcats [14], foxes [15], and mustelids [16]. However, despite its wide distribution in animals, no human cases have been documented in Romania, so

far. Although lagomorphs (hares, rabbits) are known as suitable hosts for *T. callipaeda* [3,4], there are no studies or reports of these hosts in Romania. Hence, we aimed to investigate the presence of *T. callipaeda* in hares, *Lepus europaeus* collected in various regions of Romania, and to evaluate their reservoir role.

2. Results

Four of the 326 European brown hares examined were positive for ocular nematodes (1.23%) (Table 1, Figures 1 and 2). A total of 84 nematodes were collected. All nematodes were morphologically identified as *T. callipaeda*. The intensity varied between 1 and 70 nematodes/hare, with a mean intensity of 21 (Table 2). Seven (18.9%) of the 37 mature females presented non-blastomerized eggs and blastomerized eggs, whereas 30 (81.1%) also presented larvated eggs as well as larvae inside the uterus.

Table 1. Sampled European brown hares according to the sex, altitude, and ecoregion.

Variable		Sampled	Positive	Prevalence (%)	95% CI
Sex	Males	132	2	1.52	0.42–5.36
	Females	136	2	1.47	0.4–5.2
	Undetermined	58	0	0	0–6.21
Altitude interval (meters)	0–50	15	1	6.66	1.19–29.82
	51–100	147	0	0	0–2.55
	101–200	50	0	0	0–7.13
	201–300	63	3	4.76	1.63–13.09
	301–500	41	0	0	0–8.57
	≥501	10	0	0	0–27.75
Ecoregion	Pannonian	161	0	0	0–2.33
	Continental	146	3	2.05	0.7–5.87
	Alpine	2	0	0	0–65.76
	Steppic	17	1	5.88	1.05–26.98
	Pontic	0	0	0	0
Sample season	Winter	227	4	1.76	0.69–4.44
	Spring	5	0	0	0–43.45
	Summer	1	0	0	0–79.35
	Autumn	85	0	0	0–4.32
	Unknown	8	0	0	0–32.44
Total		326	4	1.23	0.48–3.11

All four specimens selected for molecular analysis were successfully sequenced, showing a 100% similarity with several sequences of *T. callipaeda* haplotype 1 (GenBank: MK546436- MK546439, MF578281; MG913802; AP017700; OM470911).

Statistical analysis, using Pearson's chi-squared test, correlating sex ($p = 0.6563$), ecoregion ($p = 0.1171$), altitude intervals ($p = 0.1721$) to infection status revealed no significant results, the p values exceeding the 0.05 benchmark.

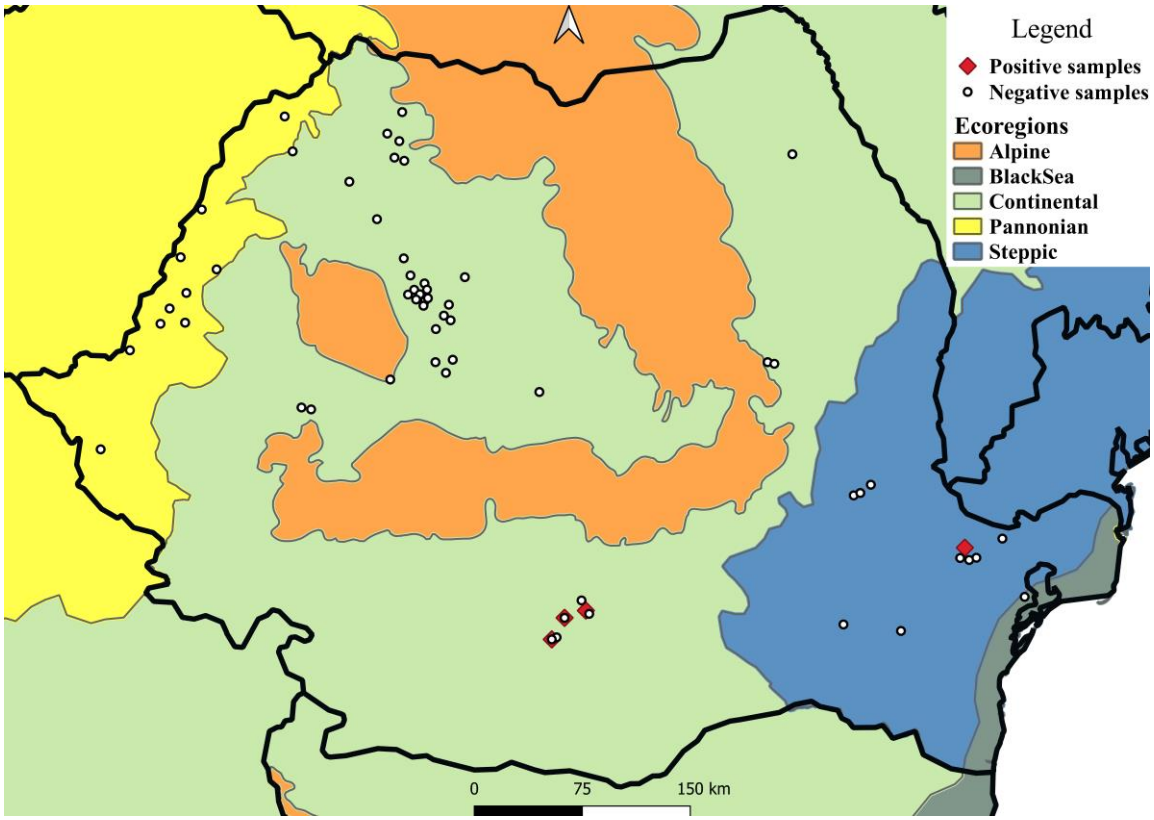

Figure 1. Sampling areas and distribution of *T. callipaeda* in European brown hares from Romania, by ecoregion.

Figure 2. *L. europaeus* with a specimen of *T. callipaeda* present in its left eye.

Table 2. *Thelazia callipaeda* infection intensity and population structure in European brown hares from Romania.

Positive Animals	*T. callipaeda* Collected	Infestation Type	Left Eye Intensity	Right Eye Intensity	*T. callipaeda* Population Structure		
					Male	Female	L5 Female
Hare 1	1	Unilateral	1	0	0	0	1
Hare 2	4	Unilateral	4	0	2	2	0
Hare 3	9	Bilateral	1	8	2	6	1
Hare 4	70	Bilateral	45	25	41	29	0
Median	6.5		2.5	4	2	4	0.5

3. Discussion

The current study serves as the first report of *T. callipaeda* in European brown hares in Romania and the second in Europe [3]. Moreover, the presence of larvae in the adult females of *T. callipaeda* is a strong indicator that hares are definitive (final) and also reservoir hosts, being able to transmit the infection in natural conditions. However, due to the low prevalence, we cannot suggest that hares are significant reservoir hosts in Romania, mainly as the area is known as hypernedmic for *T. callipaeda* in red foxes [15]. For instance, in Italy, the prevalence in hares was significantly higher (3/13, 23.1%) [3]. The higher prevalence in foxes could be linked to their crepuscular activity, which fits the one of *Phortica variegata* [17], whereas European brown hares are predominantly crepuscular and nocturnal, with a peak in the late afternoon, during the mating season, in spring [18,19]. Additionally, two wild European rabbits, *Oryctolagus cuniculus* from Portugal, also harbored *T. callipaeda* infection [4], highlighting the susceptibility of European lagomorphs to infection with this nematode.

Lagomorphs were only reported as natural hosts for *T. callipaeda* in Europe and Russia [3,4,20]. An experimental study, in Russia, concluded that the estimated life span of sexually mature forms of *T. callipaeda* in laboratory rabbits, *O. cuniculus* is of around six months [21], which, under natural conditions, could overlap with the activity period of the vectors. Moreover, the shallow burrowing behavior of brown hares during their inactive periods might leave them exposed to the activity of *P. variegata* [22].

The potential susceptibility of European brown hares as feeding hosts for *Phortica variegata* was suggested by Otranto et al. [3]. Interestingly, landscape diversity for the European hare has seen a shift towards woodlands, brushes, unimproved grasslands, and field margins [23], potentially making them more susceptible both to predators [24] and diseases. This follows the trends of the decline of farmland biodiversity, which can be attributed to the intensification of the agricultural industry in the late 20th century in Europe, according to several studies on agri-environment schemes [25–27].

Because of the scarcity of hare carcass availability outside of the hunting season, which takes place between November and January, the current study is mainly limited to mature and immature adult stages of *T. callipaeda*. Subsequently, any larval stages present during the peak infestation season March–June [28] will have either matured or died, affecting both the overall prevalence rate as well as the intensity within the host.

Therefore, there is an increasing need to determine the complexity of the sylvatic cycle and the diversity of reservoir hosts in relation to their ecology to better understand and implement preventive measures for limiting this zoonotic disease. This latter aspect has become a key point over the past ten years, as more human cases have emerged worldwide [6–8].

4. Materials and Methods

Between November 2019 and November 2021, 326 carcasses of European hares, *Lepus europaeus*, were examined as part of a broader survey of their parasites (unpublished data). Of these, six were collected outside of the November–February period, whereas another

eight had an unknown collection date (Table 1). The study area comprised 17 counties covering four ecoregions (Figure 1). The carcasses originated from legally hunted individuals or roadkills. The following information was recorded for each animal: date and location of collection, and sex.

As part of the necropsy procedure, the eyes of each carcass were examined under a stereo zoom microscope, with the lateral and medial canthus dissected to uncover the entire globe. Upon detection, nematodes were placed in a vial with physiological saline (0.9%), followed by a morphological examination.

Morphological identification of the ocular nematodes was performed using the keys provided by [20,29], during which each nematode's sex and developmental stages were recorded. Intact specimens underwent detailed morphometric analysis following preservation in 4% formalin solution. All measurements were done using an Olympus microscope (Olympus BX61) and dedicated software.

One randomly selected nematode from each brown hare was stored in 70% ethanol and used for molecular characterization. DNA extraction was performed individually from each nematode using the ISOLATE II Genomic DNA Kit (Bioline Meridian Bioscience, Luckenwalde, Germany), according to the manufacturer's instructions, and stored at −20 °C until further use. The samples were processed by PCR amplification of a 670-bp gene region the cytochrome c oxidase subunit 1 (cox 1), using a C1000™ Thermal Cycler (Bio-Rad, London, UK) and the NTF/NTR primer pair, as previously described [30]. Sequencing was performed by Macrogen Europe (Amsterdam, The Netherlands), while the assembled chromatograms and consensus sequences were translated and edited using the Geneious 4.8.5 software (Biomatter Ltd., Auckland, New Zealand). Lastly, using the Basic Local Alignment Search Tool (BLAST), the consensus sequences were compared with the available data in the GenBank® database.

Mapping was performed using the free open source QGis Geographic Information System (version 3.20 Odense, QGis Development Team, 2021), including ecoregions as a layer. Statistical analysis was performed using the EpiInfo™ 7 software (CDC, Atlanta, GA, USA, 2021), recording and calculating values for the frequency, prevalence, as well as 95% confidence interval of infestation, according to several parameters (Table 1).

5. Conclusions

The current study represents the first report of the presence of *T. callipaeda* in European brown hares in Romania, while also emphasizing the role as a reservoir host of lagomorphs for the aforementioned nematode.

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References

1. do Vale, D.; Lopes, A.P.; Fontes, M.d.C.; Silvestre, M.; Cardoso, L.; Coelho, A.C. Systematic review on infection and disease caused by *Thelazia callipaeda* in Europe: 2001–2020. *Parasite* **2020**, *11*, 98.
2. Anderson, R.C. *Nematode Parasites of Vertebrates: Their Development and Transmission*, 2nd ed.; CABI Publishing: Guilford, UK, 2000; p. 672.
3. Otranto, D.; Dantas-Torres, F.; Mallia, E.; DiGeronimo, P.M.; Brianti, E.; Testini, G.; Traversa, D.; Lia, R.P. *Thelazia callipaeda* (Spirurida, Thelaziidae) in wild animals: Report of new host and ecological implications. *Vet. Parasitol.* **2009**, *166*, 262–267. [CrossRef] [PubMed]
4. Gama, A.; Pires, I.; Canado, M.; Coutinho, T.; Lopes, A.P.; Latrofa, M.S.; Cardoso, L.; Dantas-Torres, F.; Ontranto, D. First report of *Thelazia callipaeda* infection in wild European rabbits (*Oryctolagus cuniculus*) in Portugal. *Parasites Vectors* **2016**, *9*, 236. [CrossRef]
5. Jin, Y.; Liu, Z.; Wei, J.; Wen, Y.; He, N.; Tang, L.; Lin, D.; Lin, J. A first report of *Thelazia callipaeda* infection in *Phortica okadai* and wildlife in national nature reserves in China. *Parasites Vectors* **2021**, *14*, 13. [CrossRef] [PubMed]
6. Morgado, A.C.T.; do Vale, B.; Ribeiro, P.; Coutinho, T.; Santos-Silva, S.; de Sousa Moreira, A.; Rodrigues, F.T.; Coelho, A.C.; Lopes, A.P.; Mesquita, J.R.; et al. First report of human *Thelazia callipaeda* infection in Portugal. *Acta Trop.* **2022**, *231*, 106436. [CrossRef]
7. Wei, X.; Liu, B.; Li, Y.; Wang, K.; Gao, L.; Yang, Y. A human corneal ulcer caused by *Thelazia callipaeda* in Southwest China: Case report. *Parasitol. Res.* **2020**, *119*, 3531–3534. [CrossRef]
8. Dolff, S.; Kehrmann, J.; Eisermann, P.; Dalbah, S.; Tappe, D.; Rating, P. Case Report: Thelazia callipaeda Eye Infection: The First Human Case in Germany. *Am. J. Trop. Med. Hyg.* **2020**, *102*, 350–351. [CrossRef]
9. Kregel-Weber, M.K.; Delling, C.; Dyachenko, V.; Luttgenau, H. Okulare Thelaziose bei einem Hund in Deutschland–Ein autochthoner Fall? *Tierärztliche Prax. Ausg. K Kleintiere/Heimtiere* **2020**, *49*, 55–59. [CrossRef]
10. Mihalca, A.D.; D'Amico, G.; Scurtu, I.; Chirilă, R.; Matei, I.A.; Ionică, A.M. Further spreading of canine oriental eyeworm in Europe: First report of Thelazia callipaeda in Romania. *Parasites Vectors* **2015**, *8*, 48. [CrossRef]
11. Ioniță, M.; Mitrea, I.L.; Ionică, A.M.; Morariu, S.; Mihalca, A.D. New cases of *Thelazia callipaeda* haplotype 1 in dogs suggest a wider disribution in Romania. *Vector-Borne Zoonotic Dis.* **2016**, *16*, 172–175. [CrossRef]
12. Tudor, P.; Bădicu, A.; Mateescu, R.; Tudor, N.; Mateescu, C.; Ionaşcu, I. First report of canine ocular thelaziosis in the Muntenia Region, Romania. *Parasitol. Res.* **2016**, *115*, 1741–1744. [CrossRef] [PubMed]
13. Dumitrache, M.O.; Györke, A.; Mircean, M.; Benea, M.; Mircean, V. Ocular thelaziosis due *Thelazia callipaeda* (Spirurida: Thelaziidae) in Romania: First report in domestic cat and new geographical records of canine cases. *Parasitol. Res.* **2018**, *117*, 4037–4042. [CrossRef] [PubMed]
14. Mihalca, A.D.; Ionică, A.M.; D'Amico, G.; Daskalaki, A.A.; Deak, G.; Matei, I.A.; Șimonca, V.; Iordache, D.; Modrý, D.; Gherman, C.M. Thelazia callipaeda in wild carnivores from Romania: New host and geographical records. *Parasites Vectors* **2016**, *9*, 350. [CrossRef]
15. Ionică, A.M.; Deak, G.; Matei, I.A.; D'Amico, G.; Cotuțiu, V.D.; Gherman, C.M.; Mihalca, A.D. *Thelazia callipaeda*, an Endemic Parasite of Red Foxes (*Vulpes vulpes*) in Western Romania. *J. Wildl. Dis.* **2018**, *54*, 829–833. [CrossRef]
16. Ionică, A.M.; Deak, G.; D'Amico, G.; Stan, G.F.; Chișamera, G.B.; Constantinescu, I.C.; Adam, C.; Lefkaditis, M.; Gherman, C.M.; Mihalca, A.D. Thelazia callipaeda in mustelids from Romania with the European badger, Meles meles, as a new host for this parasite. *Parasites Vectors* **2019**, *12*, 370. [CrossRef]
17. Otranto, D.; Cantacessi, C.; Dantas-Torres, F.; Brianti, E.; Pfeffer, M.; Genchi, C.; Guberti, V.; Capelli, G.; Deplazes, P. The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II: Helminths and arthropods. *Vet. Parasitol.* **2015**, *213*, 24–37. [CrossRef] [PubMed]
18. Tottewitz, F. Erste Ergebnisse zur Lebensraumnutzung un Aktitätsperiodik des Feldhasen (*Lepus europaeus*) in großflächig landwirtschaftlich genutzen Gebieten. *Beiträge Zur Jagd Wildforschung* **1993**, *18*, 135–139.
19. Pèpin, D.; Cargnelluti, B. Individual variations of daily activity patterns in radiotracked European hares during winter. *Acta Theriol.* **1994**, *39*, 399–409. [CrossRef]
20. Skrjabin, K.I.; Soboley, A.A.; Ivanshkin, V.M. Spirurata of animals and man and the diseases caused by them Part 4: Thelazioidea. In *Essential of Nematodology*; Skrjabin, K.I., Ed.; Academy of Sciences of the USSR: Moscow, Russia, 1967; Volume XVI, pp. 24–32.
21. Kozlov, D.P. The life cycle of the nematode *Thelazia callipaeda* parasite in the eye of man and carnivores. *Dokl. Akad. Nauk SSSR* **1961**, *142*, 732–733. (In Russian)
22. Trocchi, V.; Riga, F. I Lagomorfi in Italia. Linee guida per la conservazione e la Gestione. *Minist. Politiche Agric. For. Ist. Naz. Fauna Selvatica Doc. Tec.* **2005**, *25*, 128.
23. Petrovan, S.O.; Ward, A.; Wheeler, P.M. Habitat selection guiding agri-environment schemes for a farmland specialist, the brown hare. *Anim. Conserv.* **2012**, *16*, 344–352. [CrossRef]
24. Schmidt, N.M.; Asferg, T.; Forchhammer, M.C. Long-term patterns in European brown hare population dynamics in Denmark: Effects of agriculture, predation and climate. *BMC Ecol.* **2004**, *4*, 15. [CrossRef] [PubMed]

25. Stoate, C.; Boatman, N.; Borralho, R.; Carvalhob, C.R.; Snoo, G.; Eden, P. Ecological impacts of arable intensification in Europe. *J. Environ. Manag.* **2001**, *63*, 337–365. [CrossRef] [PubMed]
26. Robinson, R.A.; Sutherland, W.J. Post-war changes in arable farming and biodiversity in Great Britain. *J. Appl. Ecol.* **2002**, *39*, 157–176. [CrossRef]
27. Hackländer, K.; Klansek, E.; Steineck, T.; Ruf, T. Reproduction and juvenile mortality in European hare (*Lepus europaeus*) populations in intensive agricultural landscapes. *Mamm. Biol.* **2003**, *68*, 29–30.
28. Otranto, D.; Lia, R.P.; Buono, V.; Traversa, D.; Giangaspero, A. Biology of *Thelazia callipaeda* (Spirurida, Thelaziidae) eyeworms in naturally infected definitive hosts. *Parasitology* **2004**, *129*, 627–633. [CrossRef] [PubMed]
29. Otranto, D.; Lia, R.P.; Traversa, D.; Giannetto, S. *Thelazia callipaeda* (Spirurida, Thelaziidae) of carnivores and humans: Morphological study by light and scanning electron microscopy. *Parassitologia* **2003**, *45*, 125–133.
30. Casiraghi, M.; Anderson, T.; Bandi, C.; Bazzocchi, C.; Genchi, C. A phylogenetic analysis of filarial nematodes: Comparison with the phylogeny of *Wolbachia* endosymbionts. *Parasitology* **2001**, *122*, 93–103. [CrossRef]

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SHORT REPORT Open Access

Occurrence of *Giardia duodenalis* assemblages in farmed long-tailed chinchillas *Chinchilla lanigera* (Rodentia) from Romania

Călin Mircea Gherman, Zsuzsa Kalmár*, Adriana Györke and Viorica Mircean

Abstract

Background: *Giardia duodenalis* is a parasitic protist that infects a large number of species, being localized in the small intestine. Two of the eight recognized assemblages have zoonotic potential, but studies regarding their distribution in less important pet or farm species are scarce. Of these species, the long-tailed chinchilla is a host for *Giardia* spp., although data on the spread of infection and assemblages involved are confined. The present work aimed to determine the prevalence of *Giardia* infection and assemblage identification in farmed chinchillas in Romania. A total of 341 fecal samples were collected from 5 farms and microscopically examined using flotation test based on saturated sodium chloride solution. DNA from all positive samples was extracted and identified by PCR targeting the *gdh* gene.

Results: The overall prevalence of *Giardia* infection was 55.7% (190/341); there was no statistically significant difference ($P = 0.25$) in prevalence between young animals (58.8%) and adults (52.6%). Assemblages B (151/190), D (33/190) and E (6/190) were identified. Among assemblage B, sub-assemblages BIII (6/151) and BIV (145/151) were determined.

Conclusions: This study demonstrates that *Giardia* spp. infection is highly prevalent in farmed chinchillas from Romania, and the sub-assemblages identified are potentially zoonotic.

Keywords: Farmed long-tailed chinchilla, *Chinchilla lanigera*, *Giardia duodenalis*, Prevalence, Assemblages, Romania

Background

The genus *Giardia* contains six species of aerotolerant anaerobic enteric protozoan parasites isolated from mammals, birds and amphibians [1–4]. Of all these species, three infect mammals, *Giardia muris* and *G. microti* in rodents and *G. duodenalis* commonly in a broad range of mammalian hosts [5]. Within *G. duodenalis*, eight species assemblages, or genotypes, are currently recognized, named from A to G. The hosts of assemblages A and B of *G. duodenalis* are the humans and other primates, livestock, domestic carnivores and wild mammals; C and D infect canids, E is common in hoofed livestock, F is typical for cats, G infects rodents and H was isolated from marine mammals [6]. The most important are zoonotic assemblages A and B, within each of them being isolated by protein polymorphisms or allozyme electrophoresis four sub-assemblages (AI, AII, AIII, AIV and BI, BII, BIII, BIV, respectively) [7, 8].

In Romania, limited data exist regarding the prevalence of *Giardia* infection in animals. Recently, the presence of *G. duodenalis* was reported in domestic carnivores; the overall prevalence was 8.5% in dogs and 27.9% in cats [9, 10]. Furthermore, assemblages A (AII), B, C (10/60; 16.7%), D (42/60; 70.0%), and E (7/60; 11.7%) have been identified in domestic and wild animals (dogs, cats, foxes, deer, wolves, raccoon dogs and muskrats) [11–13]. Consequently, the study of *Giardia* spp. infection in Romania is a field of high importance.

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The long-tailed chinchillas (*C. lanigera*) are mountainous and crepuscular animals native to South America. Extensively hunted for their fur during the 19th century the species is now almost extinct in the wild, several colonies being identified only in Chile [14]. Due to their complex social behavior and attractive aspect, chinchillas became increasingly popular as pets across the world. At the same time, because of the softest, longest and finest furs among wild animals, the species became of interest for animal breeders. Farming of chinchilla dates back to 1923, when M. F. Chapman began to raise chinchillas in captivity, being the inception of what has become an industry [15]. Intensive farming exposed chinchillas to different pathogens, which are probably less common in the wild animals. Of these, water-borne parasitic diseases, particularly giardiasis, may cause clinical and sanitary problems and lead to production and economic losses [16]. Currently, there are about 75 chinchilla farms in Romania, with a production of 12,500 animals exported per year. It manifests also an increasing trend of chinchillas' farming, whose debut in Romania dates back about 10 years ago (http://agfcicr.ro/). Due to the increasing number of farmed chinchillas in Romania, and the lack of information on the occurrence and zoonotic potential of *G. duodenalis* in these animals, the present study aimed to investigate the prevalence of the infection and preliminary genotyping of the isolates in Romanian chinchilla farms.

Methods

Animals and collection sites

Five farms with an overall stock of 5500 animals were involved in the study. Of these 2200 were breeding animals and the rest were kits and young of different ages. The following abbreviations were used for the farms studied: BM, RG, SB, SM and LU. All farms use the intensive growth closed system, but farms BM and RG also buy animals from small farmers who grow chinchillas in polyspecific farms exposed to contact with other species. A total of 341 fecal samples were collected, representing 6.2% of the stock. Of these, 171 samples were from chinchilla mothers and 170 from young animals (Table 1).

Sample processing

Each fecal sample was individually examined by flotation technique using saturated sodium chloride solution (specific gravity 1.28) [17], followed by microscopic examination (light microscopy, magnification: 10×, 20×, 40×) for the identification of *Giardia* cysts. Briefly, 0.5 g of feces/sample was homogenized with 10 ml of distilled water, filtered and centrifuged at 3000× *g* for 10 min. The supernatant was discarded, and the sediment containing *Giardia* cysts was transferred to an Eppendorf tube and used for DNA extraction.

DNA extraction and PCR analysis

DNA extraction was performed from *Giardia*-positive samples, confirmed by microscopic examination, using Isolate Fecal DNA kit (Bioline, London, UK). To increase the specificity of DNA amplification, a semi-nested PCR reaction was performed targeting the glutamate dehydrogenase (*gdh*) gene in a T100 Thermal Cycler (Bio-Rad, Hercules, USA) [18, 19]. The PCR reaction mix contained 2× Red PCR Master mix (Rovalab, Teltow, Germany), 12 pmol of primers, 1 μl of genomic DNA; the reaction profile consisted of 1 cycle of initial denaturation at 95 °C for 5 min, followed by 40 cycles of 30 s each at 94 °C, annealing at 50 °C for 30 s for the primary reaction and 60 °C for secondary reaction, extension at 72 °C for 1 min and final extension at 72 °C for 5 min. Agarose gel (1.5%) electrophoresis stained with SYBR Safe DNA gel stain (Invitrogen, Carlsbad, USA) was performed for the visualization of PCR products.

RFLP

For discrimination of all assemblages of *G. duodenalis*, RFLP analysis was performed using *Rsa* I and *Nla*IV (Biolabs, New England, US) restriction enzymes [18]. The amplified fragments were digested in a total volume of 50 μl, as recommended by the manufacturer's instructions, 5 min at 37 °C for *Rsa*I, 1 h for *Nla*IV, and the reactions were stopped by 20 min of incubation at 60 °C. The digested products were visualized by electrophoresis on 3% agarose gel.

Table 1 Prevalence of *G. duodenalis* in fecal samples collected from long-tailed chinchillas in farms in Romania

Farm code	Total		Chinchilla mothers		Kits/young		Comparison	
	F	Prevalence (%) (95% CI)	F	Prevalence (%) (95% CI)	F	Prevalence (%) (95% CI)	Chi-square	*P*-value
BM	49/80	61.3 (49.7–71.9)	29/52	55.8 (41.3–69.5)	20/28	71.4 (51.3–86.8)	1.880	0.170
RG	56/80	70.0 (58.7–79.7)	24/28	85.7 (67.3–96.0)	32/52	61.5 (47.0–74.7)	5.065	0.024
SB	40/60	66.7 (53.3–78.3)	18/30	60.0 (40.7–77.3)	22/30	73.3 (54.1–87.7)	1.200	0.273
SM	19/60	31.7 (20.3–45.0)	9/30	30.0 (14.7–49.4)	10/30	33.3 (17.3–52.8)	0.077	0.781
LU	26/61	42.6 (30.0–55.9)	10/31	32.3 (16.7–51.4)	16/30	53.3 (34.3–71.7)	2.769	0.096
Total	190/341	55.7 (50.3–61.1)	90/171	52.6 (44.9–60.3)	100/170	58.8 (51.0–66.3)	1.325	0.2497

Abbreviations: *F* Frequency, *CI* confidence interval

DNA sequencing

The PCR products were purified by using QIAquick PCR purification kit (Qiagen, Hilden, Germany) and sequenced at Macrogen Europe (Amsterdam). Nucleotide sequence data from this study were submitted to the GenBank database under the accession numbers MG432793–MG432795.

Statistical analysis

The frequency of *Giardia*-positive samples, their prevalence and 95% confidence interval were calculated. The difference in prevalence between age groups and among farms was statistically analyzed by a Chi-square test. Statistical significance was set at a *P*-value of ≤ 0.05. All statistical analyses were performed using EpiInfo software version 3.5.1. (Centers for Disease Control and Prevention: http://wwwn.cdc.gov/epiinfo/).

Results

The occurrence of *Giardia* spp.

Giardia cysts were identified in 190 of 341 (55.7%, 95% CI: 50.3–61.0) fecal samples by microscopic examination. All 190 microscopically identified *Giardia*-positive samples were positive by PCR. General prevalence recorded the highest value in farm RG (56/80, 70%, 95% CI: 58.7–79.7%) and the lowest in farm SM (19/60, 31.6, 95% CI: 20.3–45.0%) ($\chi^2 = 28.83$, $df = 4$, $P < 0.001$). The infection was somewhat more frequent in young animals (100/170, 58.8%, 95% CI: 51.0–66.3%) compared to mother chinchillas (90/171, 52.6%, 95% CI: 44.9–60.3%) but the difference was not statistically significant ($\chi^2 = 1.09$, $df = 1$, $P = 0.25$) (Table 1).

Giardia spp. assemblages

In total, three *Giardia* assemblages (B, D and E) were found in the chinchilla farms studied. Assemblage B was the most prevalent (151/190, 79.5%), followed by D (33/190, 17.4%) and E (6/190, 3.1%). The identified sub-assemblages were BIV (145/190, 76.3%) and BIII (6/190; 3.1%) (Table 2).

Sequence analysis of fecal samples confirmed the infection with *G. duodenalis* sub-assemblages BIII, BIV, D and E (Table 2). Assemblages B (MG432795) and D (MG432793) were common in all farms in the study, in both age categories, and Assemblage E (MG432794) was identified only in farms BM and RG. No mixed assemblage infections were detected in animals in this study.

Discussion

The study of intestinal parasites in the long-tailed chinchilla is an important field of interest due to a permanent contact of this pet or farmed animal with humans. Among parasitic diseases identified in this species, giardiasis seems to be the most significant, due to the zoonotic character and increased values of prevalence reported worldwide (Table 3).

Table 2 Assemblages of *G. duodenalis* identified by PCR-RFLP and sequencing targeting the *gdh* gene in fecal samples of long-tailed chinchillas from farms in Romania

Farm	Age	Assemblage (RFLP)		Assemblage (sequencing) (no. of samples)			
		*Nla*IV	*Rsa*I	BIII	BIV	D	E
BM	CM	BIII/BIV/E/D	BIII; BIV	3	20	4	2
	Y	BIII/BIV/D	BIV		16	4	
RG	CM	BIII/BIV/D/E	BIV		19	3	2
	Y	BIII/BIV/D/E	BIV	1	22	7	2
SB	CM	BIII/BIV/D	BIV		15	3	
	Y	BIII/BIV/D	BIII; BIV	2	17	3	
SM	CM	BIII/BIV/D	BIV		4	5	
	Y	BIII/BIV	BIV		10		
LU	CM	BIII/BIV/D	BIV		8	2	
	Y	BIII/BIV/D	BIV		14	2	
Total				6	145	33	6

Abbreviations: *CM* chinchilla mothers, *Y* young

Table 3 Reported prevalence of *Giardia* spp. infection in the long-tailed chinchilla

Country	Husbandry system (pet/farmed/wild)	Prevalence (%)	Frequency	Detection method	Reference
Argentina	Farmed	34.42	84/244	Wet mounts, IFA	[46]
Belgium	Pet	66.3	53/80	SCF	[24]
Brazil	Farmed	8.0	20/250	ZCF	[47]
Brazil	Farmed	38.0	38/100	ZCF	[48]
Brazil	Pet	10.0	6/60	ZCF	[49]
Brazil	Farmed	31.37	80/255	ZCF	[28]
Chile	Wild	Negative	na		–
China	Pet	37.5	36/96	SF	[50]
China	Pet	27.1	38/140	PCR	[51]
Europe	Pet	61.4	326/531	ELISA	[31]
Italy	Farmed	39.4	41/104	DFA	[31]
Portugal	Pet	35.2–92.3	na	ZCF, SF	[52]
Russia	Pet	50.0	25/50	CFM	[53]
Russia	Pet	Positive	na	ANF	[29]
Peru	Wild	Negative	na		–
Romania	Farmed	55.7	190/341	NaClF	Present study

Abbreviations: *ANF* ammonium nitrate flotation, *CFM* combined flotation method, *DFA* direct fluorescent assay, *ELISA* enzyme-linked immunosorbent assay, *IFA* immunofluorescence assay, *na* not applicable, *NaClF* sodium chloride flotation, *SCF* sucrose gradient centrifugation-flotation technique, *SF* sugar flotation (Sheather's sugar solution), *ZCF* zinc-sulfate centrifugation-flotation

The prevalence revealed in the present study (55.7%) is slightly increased compared to that reported in farmed chinchilla from other regions (8.0–38.0% in Brazil, 34.4% in Argentina and 39.4% in Italy) but is comparable to those reported in pet animals (10.0–92.3%).

The discrepancies of prevalence recorded in existing studies can be explained by the different diagnostic value of copromicroscopic methods used, determined by the technique, less than the density of supersaturated solutions [20]. Moreover, coproscopic techniques have a lower diagnostic value, the prevalence determined by other modern serological or molecular methods (ELISA, IFA, PCR) being 2.6-fold higher in dogs and 3.7-fold higher in cats [21]. As such, we consider that the prevalence of infection revealed in this study, although high, can be appreciated as undervalued.

Prevalence of *Giardia* infection is generally influenced by many factors, such as the sensitivity of the diagnostic method used, the peculiarities of the biological cycle of the parasite (the discontinuities of cysts removal), the host, the age of host, the growth system, and the hygiene conditions (water, food, bedding) [22]. A variety of factors favor the emergence and transmission of infection in chinchilla populations. These risk factors may differ among pet and farmed chinchilla. Regarding pet chinchilla, participation in shows and contact with other pet animals, such as dogs, cats or other rodents, are significant [23, 24]. In farmed chinchillas, the age of animals, stress, poor husbandry system associated with low quality of water source, overcrowding and close contact with feces seems to act as predisposing factors. Juvenile chinchillas are more sensitive to acquire the infection [25]. Intensive rearing in plastic or metal cages, with fecal accumulation underneath and vulnerability of the drinking-water-processing system, favor the contact between animals and cysts of *Giardia* spp. [26, 27]. Captivity associated with specific stress emphasizes the sensitivity of chinchilla to *G. duodenalis* infection, an aspect demonstrated by the absence of *Giardia* spp. infection in wild animals [28, 29].

Chinchillas harbor various assemblages (A, B, C, D and E) of *G. duodenalis*, representing a potential zoonotic risk (Table 4). Assemblage B is the most common, being identified in almost all reported studies, except for an axenic isolate of *G. duodenalis* from Germany [30], in which assemblage A was identified. In our research, RFLP analysis of *G. duodenalis*-positive samples revealed a high occurrence of assemblage B isolates grouped into sub-assemblages BIII and BIV, representing the main assemblages involved in chinchilla's infection. In the present study, no mixed assemblage infections were detected, similar to previous studies [16, 31]. However, our data do differ from those reported in Belgium and Germany, which showed the presence of mixed assemblage A, B, C and E infections in chinchillas [24, 32].

Table 4 Assemblages of *G. duodenalis* identified in the long-tailed chinchillas

Country	Type of animal (pet/farmed)/ assemblage		Reference
	Pet	Farmed	
Austria	B	–	[32]
Belgium	A, AI, AII, B, BIV, C, E	–	[24]
Brazil	B	–	[16]
Brazil	BIV	–	[54]
China	AI, AII, BIV, BIV-1, BIV-2	–	[51]
Croatia	B	–	[55]
Czech Republic	B	–	[56]
Germany	A	–	[30]
Germany	A, B, D	–	–
Italy	–	B, C	[31]
Romania	–	BIII, BIV, D, E	Present study

The presence of C and D assemblages typical for canids, and E from hoofed livestock, in chinchillas is quite interesting. In this work, the existence of assemblages E in farms BG and RG can be explained by acquiring animals from farms in which ruminants were also kept, the direct or indirect contact between the two species being possible.

Generally, multiple factors can explain the diversity of assemblages identified across the world. Interspecies transmission is of particular importance for the zoonotic risk of infection, domestic animals being the source of human infection. Reverse or cross-species transmission of different assemblages (BIV, E) has also been demonstrated in areas where humans, primates and livestock overlap in their use of habitat [33]. Interspecific transmission is possible between species belonging to different taxa, from rodents to carnivores and from ruminants to humans [34]. It is also proven that *G. duodenalis* from the North American beaver (*Castor canadensis*) may infect Mongolian gerbils (*Meriones unguiculatus*); in this case, the transmission was carried out between two rodent species [35]. Transmission of *Giardia* spp. between different species of rodents is also confirmed in other older studies [36]. Nevertheless, Goltz [37] demonstrated that *G. chinchillae* from *C. lanigera* were not infective to laboratory mice, rats and guinea pigs. However, the interspecies transmission may explain the presence of assemblages D and E in our study, sustained by the existence of guard dogs and small ruminants in the examined farms.

Transport vectors can also play a significant role in the transmission of giardiasis [38]. It is confirmed that assemblage E of *G. duodenalis* is carried by flies, increasing the possibility of repeated infection or cross-transmission between sensitive species, by mechanical transmission [39].

As a result, despite of the strong host specificity and narrow host range of assemblage E, which is mostly identified in cloven-hoofed mammals, the involvement of the transport hosts can ensure the transmission of this assemblage to captive chinchillas [8].

Water source is also important in the circulation of *G. duodenalis* cysts, giardiasis being recognized as one of the major waterborne diseases [40]. Although the long-tailed chinchilla is a species adapted to aridity, with low water needs, it prefers the open dish drinker [41]. The best water supply in chinchilla farming is represented by bottled water, free of pathogens and chlorine [42]. Tap and well water are also accepted sources, but they present the risk of contamination with *Giardia* cysts. Surprisingly, in Romania, bottled water seems to have an increased risk of infection compared with wells or tap water [43]. Feces of different animal species can pollute water sources, shedding cysts into the water supply [44]. These cysts can pass through water treatment, even for pristine or filtered drinking water. Furthermore, *Giardia* spp. cysts have a demonstrated effective resistance to chlorination [45]. Tap water was the source in studied farms, without an additional water filtration; chlorination and filtration performed by water plant suppliers being the unique treatments. Combining predisposing factors as interspecific transmission, the possible involvement of vectors and deficiencies in water supply, the increased prevalence of *G. duodenalis* infection in farmed chinchilla from Romania may be explained.

Conclusions

This study revealed the increased prevalence of infection with *G. duodenalis* in farmed chinchilla from Romania and the presence of BIII, BIV, D and E assemblages. Further studies are needed to clarify the zoonotic risk for the owners and workers in chinchilla husbandry.

Abbreviations

BM: Baia Mare; LU: Luncani; RG: Reghin; SB: Suceava-Bejenaru; SM: Suceava-Morozan

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Availability of data and materials

Nucleotide sequence data from this study were submitted to the GenBank database under the accession numbers MG432793–MG432895.

Authors' contributions

GCM collected the samples, GA and MV made the flotation and microscopic examinations, KZ performed the DNA extraction, PCR, RFLP and sequencing. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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References

1. Morrison HG, McArthur AG, Gillin FD, Aley SB, Adam RD, Olsen GJ, et al. Genomic minimalism in the early diverging intestinal parasite *Giardia lamblia*. Science. 2007;317:1921–6.
2. Cavalier-Smith T. The excavate protozoan phyla Metamonada Grassé emend. (Anaeromonadea, Parabasalia, Carpediemonas, Eopharyngia) and Loukozoa emend. (Jakobea, Malawimonas): their evolutionary affinities and new higher taxa. Int J Syst Evol Microbiol. 2003;53:1741–58.
3. Lindmark DG. Energy metabolism of the anaerobic protozoon *Giardia lamblia*. Mol Biochem Parasitol. 1980;1:1–12.
4. Monis PT, Caccio SM, Thompson RC. Variation in *Giardia*: towards a taxonomic revision of the genus. Trend Parasitol. 2009;25:93–100.
5. Plutzer J, Ongerth J, Karanis P. *Giardia* taxonomy, phylogeny and epidemiology: facts and open questions. Int J Hyg Enviro Health. 2010; 213:321–33.
6. Ryan U, Cacciò SM. Zoonotic potential of *Giardia*. Int J Parasitol. 2013;43: 943–56.
7. Monis PT, Andrews RH, Mayrhofer G, Ey PL. Genetic diversity within the morphological species *Giardia intestinalis* and its relationship to host origin. Infect Gen Evol. 2003;3:29–38.
8. Feng Y, Xiao L. Zoonotic potential and molecular epidemiology of *Giardia* species and giardiasis. Clin Microbiol Rev. 2011;24:110–40.
9. Mircean V, Györke A, Jarca A, Cozma V. Prevalence of *Giardia* species in stool samples by ELISA in household cats from Romania and risk factors. J Fel Med Sur. 2011;13:479–82.
10. Mircean V, Györke A, Cozma V. Prevalence and risk factors of *Giardia duodenalis* in dogs from Romania. Vet Parasitol. 2012;184:325–9.
11. Onac D, Oltean M, Mircean V, Jarca A, Cozma V. Occurrence of *Giardia duodenalis* zoonotic assemblages in red foxes from Romania. Sci Parasitol. 2015;16:177–80.
12. Sommer MF, Beck R, Ionita M, Stefanovska J, Vasić A, Zdravković N, et al. Multilocus sequence typing of canine *Giardia duodenalis* from south eastern European countries. Parasitol Res. 2015;114:2165–74.
13. Györke A, Kalmar Z, Dumitrache OM, Gherman MC, Mircean V. *Giardia duodenalis* genotypes in domestic and wild animals from Romania identified by PCR-RFLP targeting the gdh gene. Vet Parasitol. 2016;217:71–5.
14. Spotorno A, Patton JL. Superfamily Chinchilloidea. In: Patton JL, Pardiñas UFJ, D'Elia G, editors. Mammals of South America. Rodents. Chicago: University of Chicago Press; 2015, pp. 762–786.
15. Anthony HM. Oldest chinchilla rancher visits chinchilla capital. Madera Daily Tribune. 1967;76:12.
16. Soares RM, de Souza SL, Silveira LH, Funada MR, Richtzenhain LJ, Gennari SM. Genotyping of potentially zoonotic *Giardia duodenalis* from exotic and wild animals kept in captivity in Brazil. Vet Parasitol. 2011;180:344–8.
17. Willis HH. A simple levitation method for the detection of hookworm ova. Med J Aust. 1921;2:375–6.
18. Read CM, Monis PT, Thompson RC. Discrimination of all genotypes of *Giardia duodenalis* at the glutamate dehydrogenase locus using PCR-RFLP. Infect Genet Evol. 2004;4:125–30.
19. Gillhuber J, Pallant L, Ash A, Thompson RC, Pfister K, Scheuerle MC. Molecular identification of zoonotic and livestock-specific *Giardia*-species in faecal samples of calves in southern Germany. Parasit Vectors. 2013;6:346.
20. Dryden MW, Payne PA, Ridley R, Smith V. Comparison of common fecal flotation techniques for the recovery of parasite eggs and oocysts. Vet Ther. 2005;6:15–28.
21. Bouzid M, Halai K, Jeffreys D, Hunter PR. The prevalence of *Giardia* infection in dogs and cats, a systematic review and meta-analysis of prevalence studies from stool samples. Vet Parasitol. 2015;207:181–202.

22. Oates SC, Miller MA, Hardin D, Conrad PA, Melli A, Jessup DA, et al. Prevalence, environmental loading, and molecular characterization of *Cryptosporidium* and *Giardia* isolates from domestic and wild animals along the Central California coast. Appl Environ Microbiol. 2012;78:8762–72.
23. Warburton AR, Jones PH, Bruce J. Zoonotic transmission of giardiasis: a case control study. Commun Dis Rep CDR Rev. 1994;4:R32–6.
24. Levecke B, Meulemans L, Dalemans T, Casaert S, Claerebout E, Geurden T. Mixed *Giardia duodenalis* assemblage a, B, C and E infections in pet chinchillas (*Chinchilla lanigera*) in Flanders (Belgium). Vet Parasitol. 2011; 177:166–70.
25. Fehr M. Chinchilla. In: Fehr M, Sassenburg L, Zwart P, editors. Krankheiten der Heimtiere. 6th ed. Hannover: Schluetersche; 2005. p. 183–213.
26. Neringer R, Andersson Y, Eitrem R. A water-borne outbreak of giardiasis in Sweden. Scand J Infect Dis. 1987;19:85–90.
27. Sudré AP. Carvalho Machado do Couto M, Bergamo do Bomfim TC. Occurrence of *Giardia intestinalis* in dairy goats and evaluation of risk factors for infection: research note. R Bras Ci Vet. 2012;19:149–53.
28. Fialho CG, Oliveira RG, Teixeira MC, Marques SMT, Oliveira RG, Oliveira RG, et al. Comparison of protozoan infection between chinchillas (*Chinchilla lanigera*) from a commercial breeding facility in southern Brazil and chinchillas from a natural reserve in Chile. Parasitol Latinoam. 2008;63:85–7.
29. Sivkova TN. Parasites of wild and domestic chinchilla. In: Proceedings of the Scientific Conference of the All-Russian Society of Helminthologists RAS "Theory and practice of struggle against parasitic diseases"; 2016, pp. 433–4.
30. Karanis P, Ey PL. Characterization of axenic isolates of *Giardia intestinalis* established from humans and animals in Germany. Parasitol Res. 1998; 84:442–9.
31. Veronesi F, Piergili Fioretti D, Morganti G, Bietta A, Moretta I, Moretti A, Traversa D. Occurrence of *Giardia duodenalis* infection in chinchillas (*Chincilla lanigera*) from Italian breeding facilities. Res Vet Sci. 2012;93:807–10.
32. Pantchev N, Broglia A, Paoletti B, Globokar Vrhovec M, Bertram A, et al. Occurrence and molecular typing of *Giardia* isolates in pet rabbits, chinchillas, guinea pigs and ferrets collected in Europe during 2006-2012. Vet Record. 2014;175:18.
33. Johnston AR, Gillespie TR, Rwego IB, McLachlan TL, Kent AD, Goldberg TL. Molecular epidemiology of cross-species *Giardia duodenalis* transmission in western Uganda. PLoS Negl Trop Dis. 2010;4:e683.
34. Davies RB, Hibler CP. Animal reservoirs and cross-species transmission of *Giardia*. In: Jakubowski W, Hoff JC, editors. Proceedings of a Symposium on Waterborne Transmission of Giardiasis, EPA-600/9-79-001. Cincinnati; 1978. p. 104–27.
35. Faubert GM, Belosevic M, Walker TS, MacLean JD, Meerovitch E. Comparative studies on the pattern of infection with *Giardia* spp. in Mongolian gerbils. J Parasitol. 1983;69:802–5.
36. Grant DR, Woo PTK. Comparative studies of *Giardia* spp. in small mammals in southern Ontario. II. Host specificity and infectivity of stored cysts. Can J Zoo. 1978;56:1360–6.
37. Goltz JP. Giardiasis in chinchillas, MSc thesis. Guelph, Ontario, Canada: University of Guelph; 1980.
38. Doiz O, Clavel A, Morales S, Varea M, Castillo FJ, Rubio C, et al. House fly (*Musca domestica*) as a transport vector of *Giardia lamblia*. Folia Parasitol (Praha). 2000;47:330–1.
39. Zhao Z, Dong H, Wang R, Zhao W, Chen G, Li S, et al. Genotyping and subtyping *Cryptosporidium parvum* and *Giardia duodenalis* carried by flies on dairy farms in Henan, China. Parasit Vectors. 2014;7:190.
40. Karanis P, Kourenti C, Smith H. Waterborne transmission of protozoan parasites: a worldwide review of outbreaks and lessons learnt. J Water Health. 2007;5:1–38.
41. Hagen K, Clauss M, Hatt JM. Drinking preferences in chinchillas (*Chinchilla laniger*), degus (*Octodon degu*) and guinea pigs (*Cavia porcellus*). J Anim Physiol Anim Nut. 2014;98:942–7.
42. Tapscott B. Standard guidelines for the operation of chinchilla ranches. 1998. http://www.omafra.gov.on.ca/english/livestock/alternat/facts/chinguid.htm. Accessed 15 Sep 2017.
43. Jarcă AD. Epidemiological, clinical, laboratory and therapeutic researches in humans and animals giardiasis. PhD Thesis, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania; 2011.
44. Lippy E. Water supply problems associated with a waterborne outbreak of giardiasis. In: Jakubowski W, Hoff JC. Proceedings of a Symposium on Waterborne Transmission of Giardiasis, EPA-600/9-79-001. Cincinnati; 1978 p. 164–74.
45. Steiner TS, Thielman NM, Guerrant RL. Protozoal agents: what are the dangers for the public water supply? Annu Rev Med. 1997;48:329–40.
46. Martino PE, Bautista EL, Gimeno EJ, Stanchi NO, Radman NE. Fourteen-year status report of fatal illnesses in captive chinchilla (*Chinchilla lanigera*). J Appl Anim Res. 2016;45:310–4.
47. Fagundes Gurgel AC, Dos Santos SA, Pacheco De Araújo FA. Protozoan parasites in captive chinchillas (*Chinchilla lanigera*) raised in the state of Rio Grande do Sul, Brazil. Parasitol Latinoam. 2005;60:186–8.
48. Petroneto BS, Calegari BF, Sansão P, Bonadiman DC, Moulin GN, Silva MA, et al. Intestinal protozoan in chinchillas (*Chinchilla lanigera*) created in captivity, the region serrana of state of Espirito Santo, Brasil. Acta Vet Bras. 2015;9:65–70.
49. Alves JG, Silva AS, Monteiro SG. First report of *Cryptosporidium* sp. in *Chinchilla lanigera* in Brazil. Rev FZVA. 2008;15:186–90.
50. Lv C, Wang H, Qi M, Zhang L. Survey of intestinal parasites in pet *Chinchilla lanigera*. Chin Anim Husb Vet Med. 2009;36:176–7.
51. Qi M, Yu F, Li S, Wang H, Luo N, Huang J, et al. Multilocus genotyping of potentially zoonotic *Giardia duodenalis* in pet chinchillas (*Chinchilla lanigera*) in China. Vet Parasitol. 2015;208:113–7.
52. Teixeira RSD. Survey of gastrointestinal parasites in two groups of *Chinchilla lanigera* in northern Portugal. Dissertação de Mestrado. Universidade de Lisboa, Faculdade de Medicina Veterinária, Lisboa, Portugal; 2013.
53. Kurnosova OP. Specific structure and features of distribution of intestinal protozoa at pets in Moscow. Ros Parazitol Zh. 2013;1:9–15.
54. Souza SLP. Caracterização genética de isolados de *Giardia* spp. provenientes de amostras fecais de origem humana e animal. Tese de Doutorado, Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, Brazil; 2007.
55. Cacciò SM, Beck R, Almeida A, Bajer A, Pozio E. Identification of *Giardia* species and *Giardia duodenalis* assemblages by sequence analysis of the 5.8S rDNA gene and internal transcribed spacers. Parasitology. 2010;137:919–25.
56. Šedinová J, Flegr J, Ey PL, Kulda J. Use of random amplified polymorphic DNA (RAPD) analysis for the identification of *Giardia intestinalis* subtypes and phylogenetic tree construction. J Eukaryot Microbiol. 2003;50:198–203.

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RESEARCH Open Access

Validity of genus *Perostrongylus* Schlegel, 1934 with new data on *Perostrongylus falciformis* (Schlegel, 1933) in European badgers, *Meles meles* (Linnaeus, 1758): distribution, life-cycle and pathology

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Abstract

Background: A century of debates on the taxonomy of members of the Metastrongyloidea Molin, 1861 led to many reclassifications. Considering the inconstant genus assignation and lack of genetic data, the main aim of this study was to support the validity of the genus *Perostrongylus* Schlegel, 1934, previously considered a synonym of *Aelurostrongylus* Cameron, 1927, based on new molecular phylogenetic data and to understand its evolutionary relationships with other metastrongyloid nematodes.

Results: Specimens of lungworm collected from European badgers in Germany, Romania and Bosnia and Herzegovina were morphologically and molecularly (rDNA, *cox*1) characterized. From a phylogenetic standpoint, *Perostrongylus* is grouped with high support together with the genera *Filaroides* van Beneden, 1858 and *Parafilaroides* Dougherty, 1946 and includes probably two species: *Perostrongylus falciformis* (Schlegel, 1933), a parasite of *Meles meles* in Europe and *P. pridhami* (Anderson, 1962), a parasite of *Neovison vison* in North America. *Perostrongylus* and *Aelurostrongylus* are assigned to different clades. *Aelurostrongylus* becomes a monotypic genus, with the only species *Aelurostrongylus abstrusus* (Railliet, 1898). In addition, we provide morphological and morphometric data for the first-stage (L1), second-stage (L2), and third-stage (L3) larvae of *P. falciformis* and describe their development in experimentally infected *Cornu aspersum* snails. The pathological and histopathological lesions in lungs of infected European badgers are also described. This is the first record of *P. falciformis* in Romania.

Conclusions: Molecular phylogenetic and morphological data support the validity of the genus *Perostrongylus*, most probably with two species, *P. falciformis* in European badgers and *P. pridhami* in minks in North America. The two genera clearly belong to two different clades: *Perostrongylus* is grouped together with the genera *Filaroides* and *Parafilaroides* (both in the family Filaroididae Schulz, 1951), whereas *Aelurostrongylus* belongs to a clade with no sister groups.

Keywords: *Perostrongylus*, *Aelurostrongylus*, Metastrongyloidea, European badgers, mustelids

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Background

A century of debates on the taxonomy of members of the superfamily Metastrongyloidea Lane, 1917 led to many reclassifications of these nematodes within a variety of families, subfamilies, genera and species [1, 2]. The genus *Aelurostrongylus* Cameron, 1927 was erected to accommodate *Aelurostrongylus abstrusus* (Railliet, 1898) [3] which was originally described by Mueller in 1890 [4] as *Strongylus pusillus* and renamed to *Strongylus abstrusus* by Railliet [5], because *S. pusillus* was preoccupied [6]. Later, the genus *Protostrongylus* Kamensky, 1905 was erected [7] and *Strongylus abstrusus* transferred to this new genus (as *Protostrongylus pusillus*), containing two other species, i.e. *Protostrongylus rufescens* (Leuckart, 1865), the type-species, and *Protostrongylus commutatus* (Diesing, 1851) [as *Protostrongylus terminalis* (Passerini, 1884)]. Interestingly, Kamensky used the early specific name as originally named by Mueller in 1890.

The definition of *Aelurostrongylus* as a new genus [3] was based mainly on the absence of cuticular bursal supports and the absence of "supporting fingers" of the spicular sheaths. Cameron [3] included only the type-species *A. abstrusus* in the newly erected genus.

Perostrongylus falciformis (Schlegel, 1933) was described from European badgers, *Meles meles* in Germany as *Strongylus falciformis* Schlegel, 1933 [8] due to the sickle-shaped spicules and one year later, transferred to the genus *Filaroides* van Beneden, 1858 as *Filaroides falciformis* [9]. In the same year, Schlegel [10] erected a new genus, *Perostrongylus* Schlegel, 1934 in order to include this nematode. The main characteristics to support the new genus were the reduced, truncated copulatory bursa of the males, and the presence of larvated eggs in the uterus of the females [10]. A few years later, Wetzel [11] suggested that *P. falciformis* should be transferred to the genus *Aelurostrongylus*, considering the genus *Perostrongylus* as a synonym, based on the morphology of the bursa and gubernaculum in males. In two subsequent reviews on this group of nematodes [12, 13], the genus *Perostrongylus* is listed as a synonym of *Aelurostrongylus*, based on Wetzel's suggestions, without further comments. In his taxonomic review, Dougherty [12] listed four species in the genus *Aelurostrongylus*: *Aelurostrongylus abstrusus* (Railliet, 1898); *Aelurostrongylus brauni* (von Linstow, 1897); *Aelurostrongylus. falciformis* (Schlegel, 1933); and *Aelurostrongylus fengi* (Hsü, 1935). The latter has been initially described as the type-species of *Pulmostrongylus* Hsü, 1935 [14], but Dougherty [12] considered this genus as a synonym of *Aelurostrongylus* while Anderson [15] and Lesage [16] as a subgenus of *Protostrongylus*. However, Seneviratna [13] as well as the authors of later keys and reviews [17, 18] maintained the validity of *Pulmostrongylus*. The generic allocation of *A. brauni* was also questioned [19]. Asakawa et al. [20] redescribed this species and assigned it to the newly established genus *Viverrostrongylus* Asakawa, 1986.

A new species belonging to genus *Aelurostrongylus*, *A. pridhami* was described by Anderson [21] in *Neovison vison* from Canada. Previously, the species was erroneously identified as *A. falciformis* [22]. The same author [19] highlighted that some species of the genus *Aelurostrongylus* (*A. abstrusus* and *A. fengi*) are oviparous, while others (*A. falciformis* and *A. pridhami*) are ovoviviparous. Moreover, *A. falciformis* and *A. pridhami* differ from *A. abstrusus* with regard to the morphology of the bursa and spicules. Based on these differences, Anderson [19] suggested that the genus *Perostrongylus* should be reinstated, but later placed it as a subgenus of *Aelurostrongylus* [15, 17]. Subsequent publications continued to use the genus name *Aelurostrongylus* (synonymy of the genus [23]; *A. falciformis* [24–29], *A. pridhami* [27, 30–33]), while others accepted and used *Perostrongylus* (*P. pridhami* [34–42], *P. falciformis* [37, 43]). It is evident, that most European studies maintained the validity of *Aelurostrongylus* while the American studies predominantly used *Perostrongylus*. However, based on the data from Anderson [19], the two species are congeneric, sharing the same features.

The life-cycles of both species of *Perostrongylus* have been described in detail. Wetzel [11] studied the development of *P. falciformis* in several snail and slug species, and described the L1-L3 larval stages, but illustrated only L1 and L3. He also established the prepatent period in experimentally infected European badgers. The development of *P. pridhami* was described in slugs as well as in various terrestrial and aquatic snails, with detailed description and illustrations for L1-L3 larval stages [21]. The development of *P. pridhami* in American minks has also been described in detail [21, 37]. Anderson [21] demonstrated the infectivity to minks of L3 of *P. pridhami* from paratenic hosts (rodents, birds, amphibians and fish).

Similarly to most metastrongyloid nematodes, *P. falciformis* has an indirect life-cycle. Females are ovoviviparous and deposit in the alveoli thin-shelled eggs with larvae which subsequently hatch. Larvae and mature adult parasites are found in the alveoli, alveolar ducts and terminal bronchioles. L1 are coughed-up, swallowed and shed through the faeces. Prepatency in experimental infections was shown to be 18–19 days. Larvae enter different species of land snails and moult twice to infective L3. European badgers become infected by eating snails or paratenic hosts. The development of L3 to adults in European badgers is not known [11]. Symptoms of infected European badgers vary from less severe to death, depending on lungworm infection rate and secondary bacterial infections. The associated lung lesions and different stages of verminous pneumonia in European badgers with minor to massive lungworm infections were

lobular bronchopneumonia, diffuse bronchitis and widespread emphysema [8, 10]. In minor infections, European badgers in good nutritional status often completely recovered after coughing out the worms, leading to the regression of inflammatory process and encapsulation and calcification of degraded worms and eggs in the form of small, cheesy calcareous nodules in the subpleura and parenchyma of lung lobes. The lungworm invasion affected predominantly young animals [8, 10, 11, 19].

Considering the rather inconstant genus assignation and lack of molecular data, the main aim of this study was to provide information to support the validity of the genus *Perostrongylus* based on new molecular phylogenetic data and to understand its evolutionary relationships with other lungworms of carnivores. In addition, morphological details of the larval stages of *P. falciformis*, as well as a detailed pathological and histopathological description of the lesions in European badgers are provided.

Methods

Sample collection

Thirty-two adult European badgers were collected from different localities in Europe (Table 1) and carcasses were examined by necropsy by removing the entire respiratory tract. The trachea, bronchi, and the bronchioles were longitudinally dissected and carefully examined under a stereomicroscope for the presence of parasites. Nematodes found encapsulated in small nodules on the surface of the lungs were collected, washed in saline solution and preserved in formaldehyde for morphological identification. Midbody fragments of nematodes were stored in 70% ethanol for DNA extraction and molecular identification.

Morphological analysis

Eight males and three females collected from two European badgers in Romania (CJ005077 from Hărman, Brașov County and CJ005086, from Charlottenburg, Timiș County) were examined as temporary mounts in lactophenol. Five morphometric features in males (body length, body width, oesophagus length, length of spicules, length of gubernaculum) and six morphometric features in females (body length, body width, oesophagus length, distance between the vulva and the caudal end, distance between the anus and the caudal end, distance between the vulva and anus) were evaluated. Furthermore, 50 L1 larvae collected from the lungs of the same European badgers were also measured (length and maximum width). Measurements were taken using an Olympus BX61 microscope, DP72 digital camera and the Cell^F imaging software (Olympus Corporation, Tokyo, Japan). One male was available for measurements in Germany. No adult specimens were available for measurement from the European badger collected in Bosnia and Herzegovina. Additionally, larvae collected from the experimentally infected snails were also examined.

Table 1 The examined samples and number of European badgers infected with *P. falciformis*

Country	No. of European badgers examined	Positive	Prevalence (%)
Romania	27	9	33.3
Bosnia and Herzegovina	1	1	nd
Germany	4	3	75

Abbreviation: *nd* not determined

Experimental life-cycle of *P. falciformis* in *Cornu aspersum*

First-stage larvae of *P. falciformis* were recovered from the lungs of a naturally infected European badger (CJ005086), hunted in Charlottenburg, Timiș County, Romania (45.975825°N, 21.518763°E) by the Baermann method [44]. The resulted solution was collected into two 50 ml Falcon tubes, centrifuged at 600× *g* for 3 min and the sediment examined under light microscopy. Larvae obtained were morphologically and molecularly identified as *P. falciformis* (based on sequence identity with morphologically confirmed adults). Single infective doses of 200 L1 each were collected and used for the infection of snails. *Cornu aspersum* snails not exposed to any nematodes of vertebrates were purchased from a commercial provider from Puglia, Italy. The snails were kept in a plastic box, covered with a fine mesh, in a temperature-controlled room (21 ± 2 °C) and fed lettuce every second day. Water was provided *ad libitum*. Moreover, the boxes were humidified twice a day. To exclude the presence of any previous parasitic infections, a subset of 10 snails were artificially digested and microscopically examined, one day before the infection. The experimental infection took place in the Unit of Parasitology of the Department of Veterinary Medicine of the University of Bari, Italy. *Cornu aspersum* snails (n = 30) were deprived of food 24 h before the infection and then placed individually into infection chambers, composed of six circular cell culture wells (Corning; CellBIND; Sigma-Aldrich, St. Louis, Missouri, USA). Each well contained a potato slice (0.3–0.4 mm thick, obtained with a circular puncher) with the infective dose on the surface. The infection chambers were covered with a wet gauze cloth and secured with rubber bands. The snails were maintained in the infection chamber for 24 h and then released in the rearing box.

Larval development of *P. falciformis* was assessed by artificial digestion [45] of five randomly selected snails at 3 (T1), 6 (T2), 10 (T3), 15 (T4), 20 (T5) and 30 (T6) days post-infection (dpi). At each dpi, the suspension obtained from the gastropod digestion was microscopically examined and, when present, larvae were isolated and preserved in 70% ethanol. Larvae were then cleared

and examined as temporary mounts in glycerol and digital images and measurements were taken using Leica LAS® AF 4.1 software.

Molecular analysis

The specimens used for molecular analysis are shown in Table 2. DNA was isolated from one male, one female fragment and three pools of L1 collected separately from three infected European badgers in Germany, using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. The ribosomal DNA (rDNA) region including partial *18S* rRNA gene, internal transcribed spacer 1 (ITS1), *5.8S* rRNA gene, internal transcribed spacer 2 (ITS2) and partial *28S* rRNA gene and a partial sequence of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1) were all amplified using nematode-specific primers. For the rDNA region we used combinations of the forward primers N18SF1, NF1 and NC1 and the reverse primers D3B, NC2 and NC5BR [44–49]. For the *cox*1 sequence we used the primers MetCOI-F1 and JB4.5 [50–52]. PCR was performed with HOT FIREPol® Blend Master Mix (Solis BioDyne, Tartu, Estonia), 200 nM final concentration of forward and reverse primers each and 100 ng of nematode DNA in a 50 μl reaction volume. PCR cycling conditions were as follows: 15 min activation/initial denaturation at 95 °C, 35 cycles of 20 s denaturation at 95 °C, 30 s annealing at 54 °C and 2 min extension at 72 °C, followed by a 5 min 72 °C final elongation. Amplicons were analysed on 1.5% agarose gels, gel-purified, cloned into pDrive vector (Qiagen, Hilden, Germany) and sequenced by an external service provider (LGC Genomics, Berlin, Germany). Sequence chromatograms were checked manually and complete sequences, assembled from overlapping amplicons, were submitted to the GenBank database under the accession numbers KY365435-KY365437.

Genomic DNA was extracted from two adult nematodes from Romania, using a commercial kit (Isolate II Genomic DNA Kit, Bioline, UK), according to the manufacturer's instructions. For each nematode, PCR amplification of a ~700 bp fragment of the *cox*1 gene and of the internal transcribed spacer 2 (ITS2, ~500 bp) of the rRNA gene were performed, using primers and protocols available in literature [47, 51]. The amplicons were purified using a commercial kit (Isolate II PCR and Gel Kit, Bioline, UK) and sequenced using an external service (performed by Macrogen Europe, The Netherlands).

Genomic DNA from L1 collected from one European badger from Bosnia and Herzegovina was extracted using a commercial kit (DNeasy Blood & Tissue Kit, Qiagen, GmbH, Hilden, Germany), in accordance with the manufacturer's instructions, and a partial fragment of the ribosomal internal transcribed spacer 2 (ITS2) was amplified as previously described [47]. Amplicons were purified and sequenced in both directions using the same primers as for PCR, employing the Taq Dye Deoxy Terminator Cycle Sequencing Kit (v.2, Applied Biosystems) in an automated sequencer (ABI-PRISM 377). Sequences were aligned using the Geneious R9 software package (http://www.geneious.com) and compared (BLASTn) with those available in the GenBank database (http:/ blast.ncbi.nlm.nih.gov/Blast.cgi).

Additionally, the DNA was isolated from three specimens of each larval stage collected from experimentally infected snails, following the same protocol used for nematodes from Bosnia and Herzegovina.

Phylogenetic analysis

For molecular phylogenetic analyses datasets of sequences obtained from BLAST searches of the NCBI nucleotide (nt/nr) database using complete and partial *Perostrongylus* sequences were trimmed to homologous ends and realigned using the multiple sequence alignment program MAFFT 7 [53] with the L-INS-i method for the *28S* D2-D3 and *cox*1 sequence data sets and the structure-aided Q-INS-i method for the ITS2 sequence

Table 2 Samples used for molecular analysis

Sample type	Sample code	Locality of origin	Country	Target gene	Primers	GenBank ID
Adult nematode (f)	CJ005077	Hărman	Romania	ITS2	NC1/NC2[a]	MG733142
Adult nematode (f)	CJ005086	Charlottenburg	Romania			
Adult nematode (f)	CJ005077	Hărman	Romania	*cox*1	LCO/HCO[b]	MG736730
Adult nematode (f)	CJ005086	Charlottenburg	Romania			
Adult nematode (m) + L1 pools	DE-FD-Mm3	Fulda	Germany	rDNA	NC18SF1/D3B	KY365435
Adult nematode (m) + L1 pools	DE-FD-Mm3	Fulda	Germany	*cox*1	MetCOIf1/JB4.5	KY365437
L1	OP137/17	Semizovac	Bosnia and Herzegovina	ITS2	NC1/NC[a]	MG910460
L1-L3 from experimentally infected *Cornu aspersum*				ITS2	NC1/NC2[a]	MG733142

[a]As in [47]
[b]As in [51]

data set. Phylogenetic trees were constructed using Bayesian analysis (MrBayes 3.2) (10,000 tree generations, sampling each 10, discarding first 250 trees) and TreeDyn for tree drawing at the phylogeny.fr platform [54]. The *28S* D2-D3 data set included 25 taxa and sequences homologous to nucleotides (nt) 3053–3968 of the *P. falciformis* sequence (KY365435). The ITS2 data set included 26 taxa and sequences homologous to nt 2513–2983 (including 15 nt of the flanking *5.8S* rRNA gene and *28S* rRNA gene). The *cox*1 data set included 15 taxa and sequences homologous to nt 200–650 of KY365437.

Histological analysis

Pieces of lung tissues originating from two fresh (unfrozen) European badgers collected in Romania (CJ005077 from Hărman, Brașov County and CJ005086, from Charlottenburg, Timiș County) containing nodules with the nematodes were fixed in 10% phosphate-buffered formalin for 24 h, routinely processed, embedded in paraffin wax, cut into 4 µm sections, and stained with haematoxylin and eosin (H&E).

Results

Out of the 32 examined European badgers, *P. falciformis* was found in 13 animals from all countries (Table 1).

Morphological description of the adults

Adult worms show a pronounced sexual dimorphism, the females being larger than the males. Both sexes have a cylindrical body, uniformly coloured, elongated, thread-like, very thin and extremely coiled inside nodules, making the removal of intact specimens difficult. The cuticle at the anterior end is smooth and the mouth opening is placed terminally. The buccal cavity is small, rudimentary, and opened into a clavate oesophagus which is composed of a cylindrical part in the anterior two-thirds of its length and a posterior bulbous region (Fig. 1).

The posterior end of females is slightly curved with the vulvar and anal openings on the lower curvature (Fig. 2). Morphometric data are shown in Table 3. In the uterus, larvated eggs are clearly visible (Fig. 3), demonstrating the ovoviviparity (Fig. 4).

The morphometric data for the males are shown in Table 3. At the posterior end, the males have a small copulatory bursa, two dissimilar and highly curved spicules, and a well-developed gubernaculum (Fig. 5). The copulatory bursa (Fig. 5a, b) is undeveloped, bilobed, with two symmetrical, transparent, and indistinguishable lateral lobes. The lobes are supported by rays with different appearance and origins: ventral, lateral, externo-dorsal and median. The ventral ray is short and distally split into two branches, the ventro-ventral and ventro-lateral, both similar in size. The lateral ray is divided into three short branches with lobate appearance: externo-lateral and medio-lateral having a common trunk, slightly separated from the postero-lateral branch. The externo-dorsal ray is undivided, small and lobated. The median-dorsal ray is short, thick, and has two lateral micro-lobes and a median, sharp and short expansion (Fig. 5a). The spicules are chitinous, brown, slightly dissimilar, sickle-shaped, short, but stout. The anterior end of each spicule is knob-shaped or hemispherical and is followed by a bent caudal half, sharpened on an edge and thickened on the opposite side (Fig. 6a, b). The gubernaculum is placed between the spicules, being attached to them through protractor and retractor muscles. It is triangular, with prolonged and tapered anterior half, while the posterior end is bifurcated with a bi-lobed shape at the base (Fig. 6c).

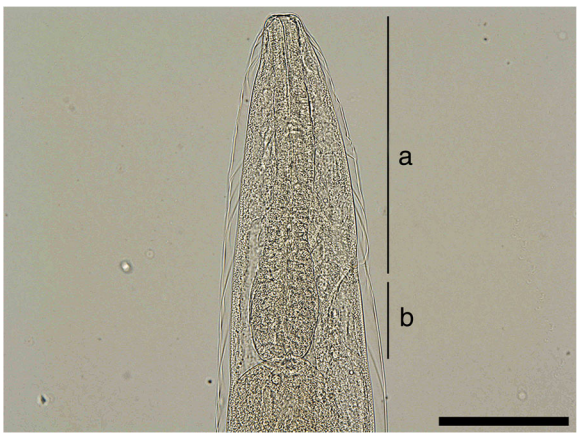

Fig. 1 Anterior end of *Perostrongylus falciformis*. **a** Cylindrical part of the oesophagus. **b** Bulbous region of the oesophagus. *Scale-bar*: 100 µm

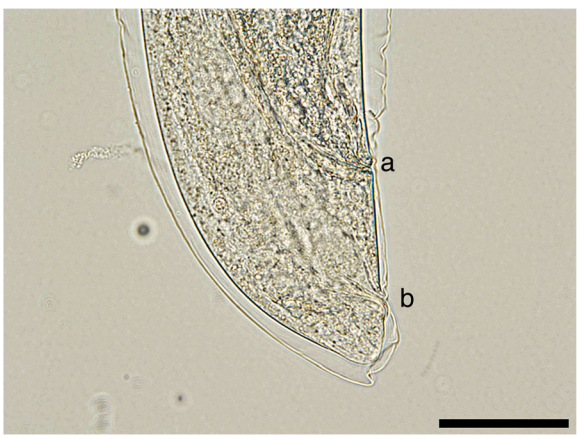

Fig. 2 Posterior end of female *P. falciformis*. **a** Vulva. **b** Anus. *Scale-bar*: 50 µm

Table 3 Comparative morphometric data for *Perostrongylus falciformis* obtained in the present study. Measurements are in micrometres unless indicated otherwise

Country	Romania	Germany[a]
Male	(*n* = 8)	(*n* = 1)
Body length (mm)	11.9–23.7	26.0
Body width	136–328	160
Cuticle thickness at mid-body	4–5	nd
Distance excretory pore to cephalic end	106–136	nd
Distance anus to caudal end	38–60	40
Oesophagus length (total)	207–395	209
Oesophagus length (cylindrical part)	137–145	131
Spicules length		
Shorter spicule	97–132	117[b]
Longer spicule	103–150	
Spicules maximum width	17–23	18
Gubernaculum length	39–54	48
Female	(*n* = 3)	
Body length (mm)	23–26	–
Body width	135–314	–
Cuticle thickness at mid-body	6–7	–
Distance excretory pore to cephalic end	132–222	–
Oesophagus length	267–273	–
Distance vulva to anus	94–105	–
Distance vulva to caudal end	176–184	–
Distance anus to caudal end	77–83	–
Larva	(*n* = 50)	–
Body length	310–408	–
Body maximum width	14–28	–

Abbreviation: *nd* not determined
[a]No females and larvae were measured from Germany
[b]The specimen was photographed from lateral view and only one spicule was visible

Development of *P. falciformis* in *Cornu aspersum*

Larval stages of *P. falciformis* were found in 27 out of 30 (90 %) experimentally infected snails. Numbers and developmental stages of larvae detected from experimentally infected snails are shown in Table 4. All control *C. aspersum* specimens digested prior to the infection (*n* = 10) were negative for helminths.

A total of 293 larvae were found at the gastropod digestions. First-stage larvae were found from T1 until the end of the study period, whereas the first L3 was detected as soon as 10 dpi and increasingly found until the end of the observational period (Table 4).

Morphology of the larval stages of *P. falciformis*

All measurements below are given in micrometres (Table 5). Metrical data are given as the range, with the mean in parentheses.

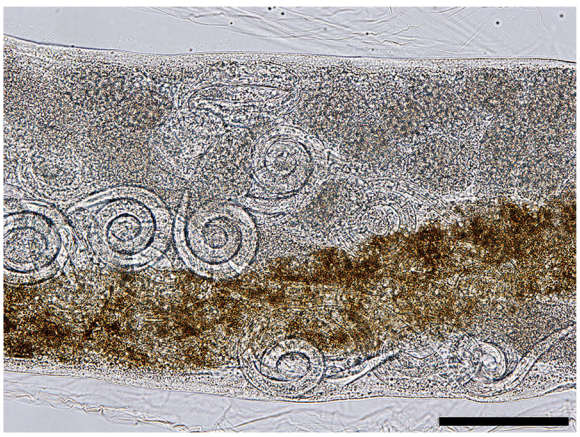

Fig. 3 Larvated eggs are clearly visible inside the uterus. *Scale-bar*: 100 μm

First-stage larvae collected from European badgers (Fig. 7a) measured 247–352 (317 ± 41) in length and 13–18 (14 ± 1) in width. The anterior extremity was featured by a narrowed, blunt end with a terminal buccal opening. The posterior extremity was 23–37 (32 ± 5) in length and characterized by a dorsal subterminal spine with a deep notch and a ventral bulge followed by an elongated sigmoid ending (Fig. 7a1).

Second-stage larvae (Fig. 7b) measured 403–443 (421 ± 13) in length and 30–33 (31 ± 1) in width. L2 were C-shaped and filled with granules. The button-like anterior extremity as well the tail (Fig. 7b1) resembled that of L1. The anterior and posterior extremities displayed an empty-like appearance due to the presence of the cuticle of the L2 and the sheet of the previous larval stage.

Third-stage larvae (Fig. 7c) had a ventrally curved body measuring 459–496 (484 ± 12) in length and 23–33 (27 ± 3) in width. Some L3 were still encased in the

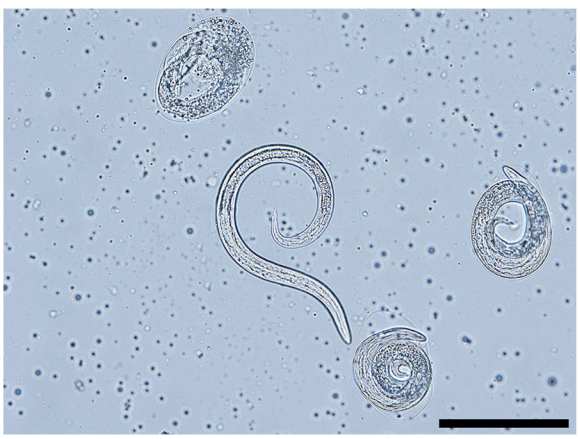

Fig. 4 Free L1 larva (center) and eggs with larvae of *P. falciformis*. *Scale-bar*: 100 μm

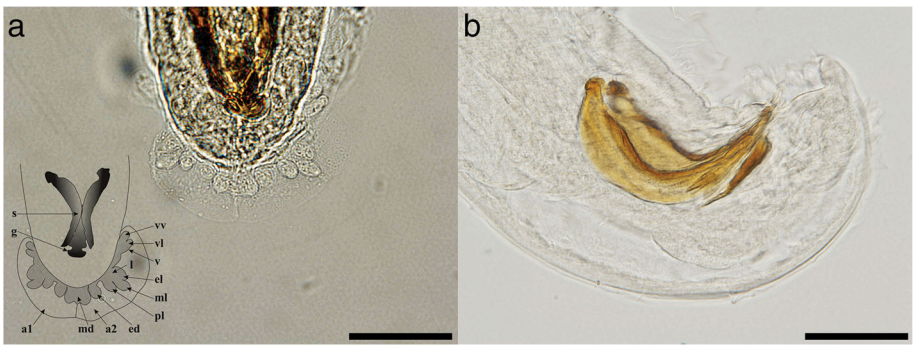

Fig. 5 Light microscopy and schematic representation of the posterior end of the males of *Perostrongylus falciformis*. **a** Copulatory bursa, dorsal view (inset: schematic representation: a1, a2, lateral lobes; v, ventral ray; vv, ventro-ventral branch of ventral ray; vl, ventro-lateral branch of ventral ray; l, lateral ray; el, externo-lateral part of lateral ray; ml, medio-lateral part of lateral ray; pl, posterio-lateral branch of lateral ray; ed, externo-dorsal ray; md, median-dorsal ray; s, spicules; g, gubernaculum) **b** Copulatory bursa, lateral view. *Scale-bars*: 50 μm

sheets of the previous stages. The anterior end was blunt, with a distinct buccal cavity followed by two stylet-like structures (Fig. 7c1). The muscular upper part of the oesophagus was cylindrical and followed by the glandular part which gradually enlarged in the bulbar oesophago-intestinal junction. The nerve-ring and the slightly posterior excretory pore were detected at 66–76 (72 ± 3) and 76–92 (84 ± 6) from the anterior extremity, respectively. The posterior extremity was 30–43 (39 ± 4) in length with a digitiform tip (Fig. 7c2).

Molecular data and phylogenetic position of *P. falciformis*

For molecular analysis of *P. falciformis* a region of 4021 bp of the ribosomal DNA including the near complete *18S*, ITS1, *5.8S*, ITS2 and partial *28S* and a partial region of 1075 bp of the mitochondrial cytochrome *c* oxidase subunit 1 gene (*cox*1) were sequenced from the German isolates. Furthermore, in order to compare geographical variants, the ITS2 region for *P. falciformis* isolates from Bosnia and Herzegovina and the ITS2 region and a partial *cox*1 gene for isolates from Romania were sequenced. The *cox*1 sequence from Romania was 99% identical to the German isolate, leading to three residue changes in the deduced amino acid sequence. The obtained ITS2 sequences of *P. falciformis* from the three countries were 100% identical, except one clone, suggesting an overall low geographical variation. The one exceptional rDNA clone (GenBank: KY365436) of the amplicon NF1-NC2 was from a female fragment from Germany, which had 15 SNPs (1802/1817 bp identities) compared to sequences from six other clones. This could be an additional haplotype or a rare intraspecific sequence variation in the rDNA repeats.

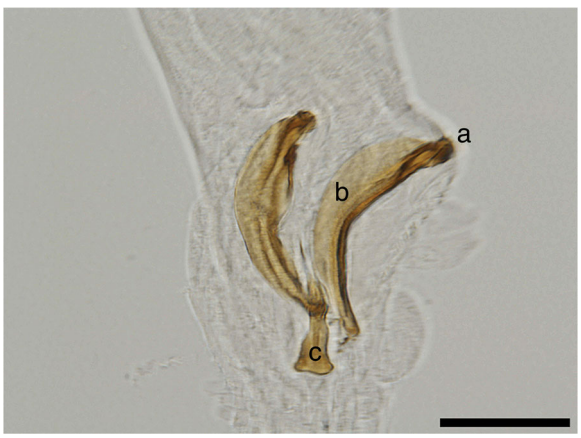

Fig. 6 Spicules and gubernaculum of *P. falciformis*. **a** Knob-shaped anterior end of the spicule. **b** The bent caudal half of the spicule. **c** Gubernaculum. *Scale-bar*: 50 μm

GenBank database searches with the *P. falciformis 18S* and *28S* rDNA sequences did not support the close phylogenetic relationship to *A. abstrusus* as would have been expected from the current taxonomic classification where *Perostrongylus* is considered as a subgenus of *Aelurostrongylus* [17]. Among the best matches for *P. falciformis*, according to alignment scores were species of the genera *Parafilaroides* and *Filaroides*. In contrast, the alignment score for *A. abstrusus* was lower and in the same range than to other metastrongyloid genera (not shown).

To further investigate the relationships among *Perostrongylus* and other metastrongyloid nematodes and to determine the molecular phylogenetic relation, analyses were performed with ITS2, partial *28S* (domains D2-D3) (Fig. 8) and partial *cox*1 sequences (Fig. 9) as biomarkers. These sequences were chosen due to their higher resolution at the species level and their length was adjusted to include a maximum of high scoring metastrongyloid nematodes from BLAST search on GenBank.

In the phylogenetic trees, *P. falciformis* and *A. abstrusus* were clearly separated, assigned to different clades,

Table 4 Total number (mean no. per snail ± SD) for the developmental stages of *Perostrongylus falciformis* larvae collected from five experimentally infected snails at 3 (T1), 6 (T2), 10 (T3), 15 (T4), 20 (T5) and 30 (T6) days post-infection

	First-stage larvae	Second-stage larvae	Third-stage larvae	Total
T1	19 (3.8 ± 2.7)	–	–	19 (6.3 ± 11.0)
T2	10 (2.0 ± 1.6)	–	–	10 (3.3 ± 5.8)
T3	13 (2.2 ± 1.8)	59 (11.8 ± 2.6)	1 (0.2 ± 0.4)	73 (24.3 ± 30.6)
T4	3 (0.6 ± 0.9)	78 (15.6 ± 7.2)	1 (0.2 ± 0.4)	82 (27.3 ± 43.9)
T5	4 (0.8 ± 1.1)	37 (7.4 ± 5.7)	4 (0.8 ± 1.1)	45 (15.0 ± 19.1)
T6	5 (1.0 ± 1.7)	36 (7.2 ± 3.8)	23 (4.6 ± 4.1)	64 (21.3 ± 15.6)

which was supported by all three genetic markers. Interestingly and consistent with discussed morphological similarities and forms of reproduction, the phylogeny inferred from the two rDNA sequence analyses, grouped *P. falciformis* together with species of the genera *Parafilaroides* and *Filaroides* (both within the family Filaroididae Schulz, 1951). The phylogenetic relationships of the Metastrongyloidea rDNA sequences correspond to previous studies [2]. There was strong support for groups of congeneric species and for the exclusion of the family Crenosomatidae from other metastrongyloid taxa. The relations obtained here for the partial *cox*1 sequence seem less consistent, because some morphologically proven congeneric species grouped with unrelated families, e.g. *A. abstrusus* with the Crenosomatidae and *A. costaricensis* with the Metastrongylidae.

Pathology caused by *P. falciformis* in European badgers

Grossly, multifocal, slightly elevated, well defined, small brown-black nodules were randomly distributed in the

Table 5 Measurements (in micrometres) of first- (L1), second- (L2) and third-stage (L3) larvae (n = 10 each) of *Perostrongylus falciformis*

Measurements	L1	L2	L3	Reference
Body length	247–352 (317 ± 41)	403–443 (421 ± 13)	459–496 (484 ± 12)	Present study
	270–370	350–420	340–440	[11]
	–	–	380–440	[63]
Maximum body width	13–18 (14 ± 1)	30–33 (31 ± 1)	23–33 (27 ± 3)	Present study
	10–17	26	30–32	[11]
	–	–	24-27	[63]
Tail length	23–37 (32 ± 5)	35–46 (41 ± 3)	30–43 (39 ± 4)	Present study
	33–40	–	30–46	[11]
Oesophagus length	99–163 (132 ± 25)	145–161 (153 ± 5)	162–196 (173 ± 10)	Present study
	130–150	161	130–183	[11]
Intestine length	130–150	204	176–260	[11]
Genital primordium, distance from posterior extremity	66–117 (88 ± 17)	–	109–166 (141 ± 15)	Present study
Nerve ring, distance from anterior extremity	–	–	66–76 (72 ± 3)	Present study
	85–90	–	–	[11]
Excretory pore, distance from anterior extremity	–	–	76–92 (84 ± 6)	Present study
	90–100	–	75–90	[11]
Ratio oesophagus length to body length	0.399–0.464	0.359–0.362	0.352–0.395	Present study
Ratio distance from posterior extremity to genital primordium to body length	0.267–0.331	–	0.237–0.334	Present study
Anus to posterior extremity	30	40	30	[11]

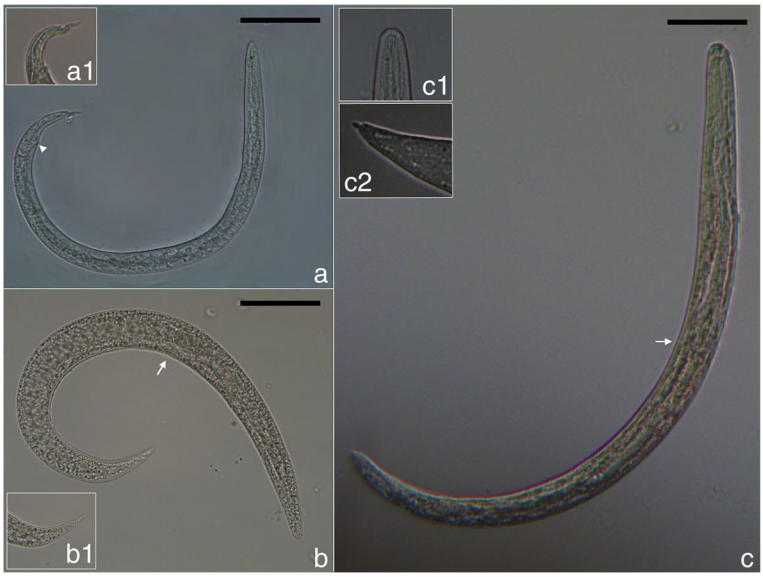

Fig. 7 Larval stages of *P. falciformis*. **a** First-stage larva and detail of the posterior extremity (a1) and well-visible anus (arrowhead). **b** Second-stage larva and detail of the posterior extremity (b1) and oesophago-intestinal junction (arrow). **c** Third-stage larva and details of the anterior (c1) and posterior (c2) extremities and oesophago-intestinal junction (arrow). *Scale-bars*: 50 μm

subpleural region of both lungs (Fig. 10a). Frequently, the parasite-containing nodules were associated with multifocal to coalescing areas of moderate alveolar emphysema (Fig. 10a). On the cut section, multiple, equally thin, blackish, partially coiled, *P. falciformis* adults were embedded in the lung parenchyma (Fig. 10b), without a significant preference for certain lung lobes. The bronchi were filled with a mucinous, foamy exudate (Fig. 10a).

Histologically, *P. falciformis* adults embedded in the lung parenchyma were present in high number in all examined histological sections. Few larval stages were also noted, especially in the bronchioles. *Perostrongylus falciformis* adults have a thin smooth cuticle, coelomyarian-polymyarian musculature and pseudocoelom with prominent intestine lined by cells that frequently contain brown-black granular pigment, and large uterus filled with developing eggs and larvae (Fig. 11c). Viable *P. falciformis* adults induce a mild inflammatory response consisting of macrophages (frequently laden with hemosiderin), occasional multinucleate giant cells, some lymphocytes and eosinophils (Fig. 11a-c). Few fibroblasts and thin collagen bundles were scattered between the above described cells. Smooth muscle hyperplasia of the terminal airways and alveolar emphysema were occasionally associated with the foci of interstitial pneumonia. Some viable adult nematodes are directly surrounded by a thin fibrous capsule. The inflammatory infiltrate extends from the parasite to the adjacent parenchyma, markedly expanding the alveolar walls. Alveolar emphysema was also focally associated with the foci of interstitial pneumonia. Bronchus-associated lymphoid tissue hyperplasia and alveoli filled by cellular infiltrate (as described above) and oedema were also observed.

Degenerated adult parasites (Fig. 11e) induced a marked inflammatory response consisting of poorly localized granulomas (sometimes centred on the parasite debris) and lympho-histiocytic and eosinophilic interstitial pneumonia. Occasionally, free *P. falciformis* larvae were present in the peribronchiolar and bronchiolar spaces (Fig. 11f). At these sites, the bronchiole walls were segmentally infiltrated by macrophages, epithelioid cells and multinucleate giant cells admixed with few eosinophils, fibroblasts and coiled larvae. Additionally, moderate to severe smooth muscle and bronchiolar epithelial hyperplasia with luminal obstruction by sloughed epithelial cells admixed with mucus, leukocytes (as described above) and parasite larvae were noted.

Discussion

This study provides the first molecular evidence for the validity of the genus *Perostrongylus*, which was previously synonymized with or considered a subgenus of *Aelurostrongylus*. With the exception of *A. abstrusus*, other species previously assigned to genus *Aelurostrongylus*, namely *A. brauni* and *A. fengi* were subsequently transferred to different genera. Due to the extremely poor description of *A. brauni* (a parasite originally described as *Strongylus brauni* from the Indian civet, *Viverra zibetha*), its classification as *species inquirenda* has already been suggested [19] and is now designated as *Viverrostrongylus brauni* [20]. *Aelurostrongylus fengi* was described from Crab-eating mongoose, *Herpestes*

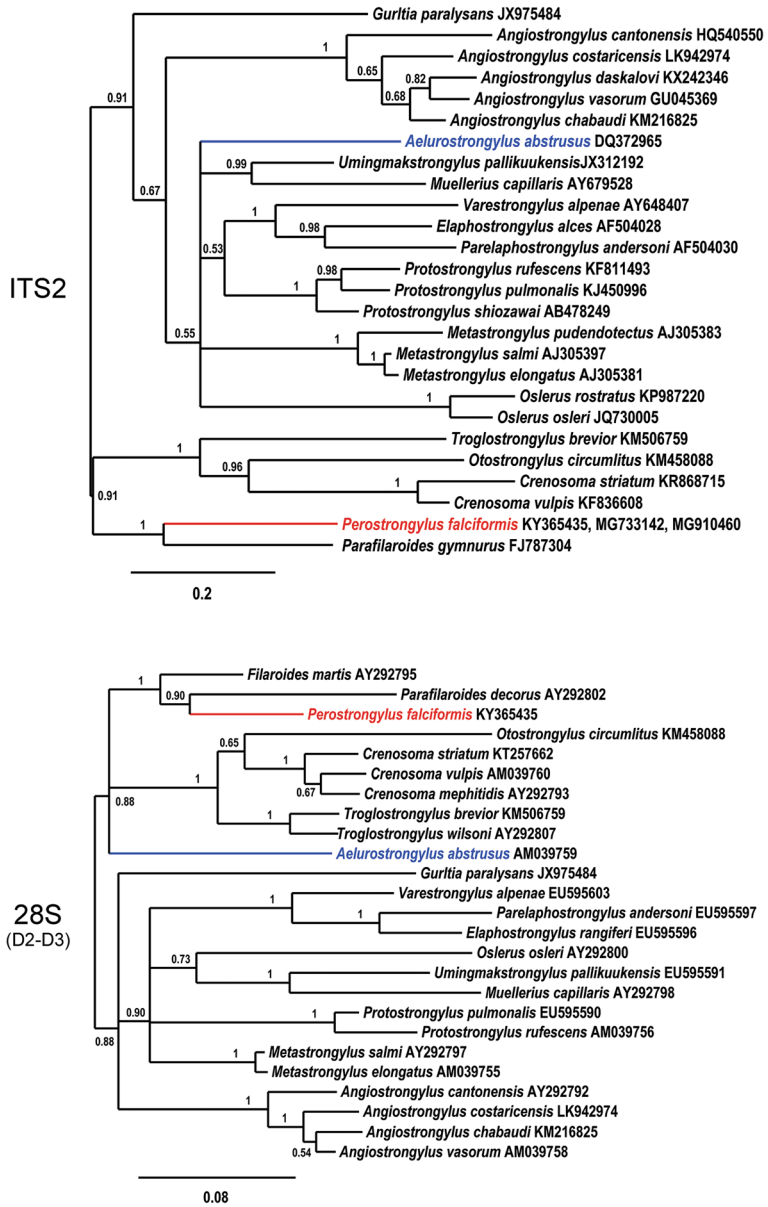

Fig. 8 Phylogenetic relationships of *P. falciformis* (red) with *A. abstrusus* (blue) and other metastrongyloid nematodes. Phylogenetic analysis based on ITS2 and partial *28S* rDNA (domains D2-D3) sequences of metastrongyloid genera. Trees are constructed using Bayesian inference. Posterior probability values are shown next to the nodes; branches with values < 0.5 are not shown. The scale-bar indicates the number of substitutions per site

urva, as *Pulmostrongylus fengi*. The taxonomic status of *Pulmostrongylus* (which includes already several species) is under debate, and currently it is considered a valid genus [18] or a subgenus of *Protostrongylus* [16]. Our molecular data provide evidence that *P. falciformis* and *A. abstrusus* are not congeneric. The conclusion drawn from this is the validity of the full genus status of *Perostrongylus* and the species name *P. falciformis* for the European badger lungworm.

Hence, the genus *Aelurostrongylus* becomes monotypic and includes only the type-species *A. abstrusus*. Considering the rigorous rules of taxonomy, an interesting fact arises regarding *A. abstrusus*. As the first description of the species used the specific name *pusillus* [4], the valid name for this species should be *Aelurostrongylus pusillus* (see above). However, due its veterinary importance and worldwide use since more than a century under the name *A. abstrusus*, we suggest the latter name to be used.

From a phylogenetic standpoint, *Perostrongylus* is a valid genus and most probably includes two species: *P. falciformis*, a parasite of *M. meles* and possibly *M.*

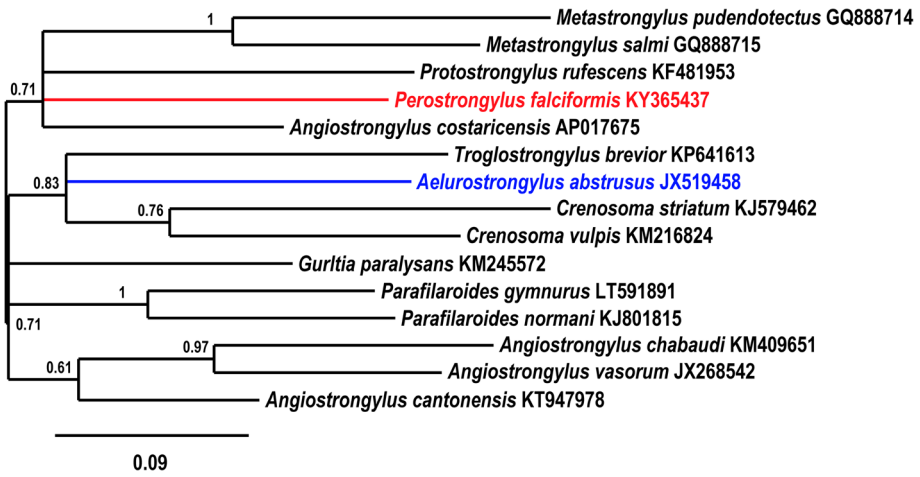

Fig. 9 Phylogenetic tree (Bayesian inference) using *cox*1 sequences for *P. falciformis* (red), *A. abstrusus* (blue) and species of other metastrongyloid genera. Posterior probability values are shown next to the nodes; branches with values < 0.5 are not shown. The scale-bar indicates the number of substitutions per site

erminea in Europe, and *P. pridhami* [21], a parasite of *N. vison* and *Mustela erminea* in North America. Our view is strongly supported by molecular phylogenetic data currently available only for *P. falciformis*. Further molecular studies are needed also for *P. pridhami* in North America, to conclude its phylogenetic position and relationships to *P. falciformis*.

Moreover, the phylogenetic analysis of the relationships of *P. falciformis* with species of other metastrongyloid genera clearly assigned *Perostrongylus* and *Aelurostrongylus* to different clades. *Perostrongylus falciformis* was grouped with high support together with the genera *Filaroides* and *Parafilaroides* (family Filaroididae), whereas *A. abstrusus* represented a single branch with no sister groups. Alignments of the internal transcribed spacer sequences (ITS1, ITS2) for different genera of the Metastrongyloidea are difficult because of the high variability in comparison to ribosomal RNA genes (*18S*, *28S*). We therefore performed the ITS2 multiple sequence alignment using a method of the program MAFFT which also considers secondary structures. However, the obtained correspondence between the analyses of ITS2 and the *28S* rDNA D2-D3 region corroborate the inferred phylogenetic position of *P. falciformis*

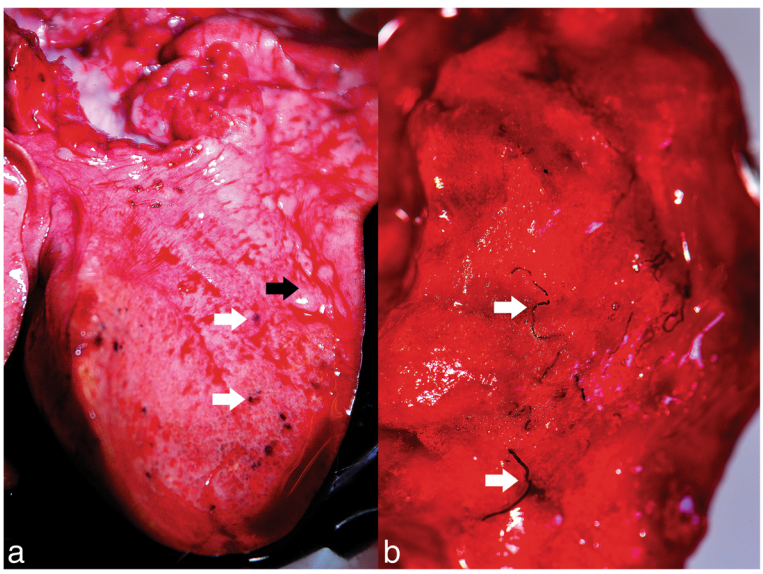

Fig. 10 Gross lesions produced by *P. falciformis*. **a** Adults in nodules in the subpleural space (white arrows) and the presence of alveolar emphysema (black arrow). **b** Adults in the pulmonary parenchyma (white arrows)

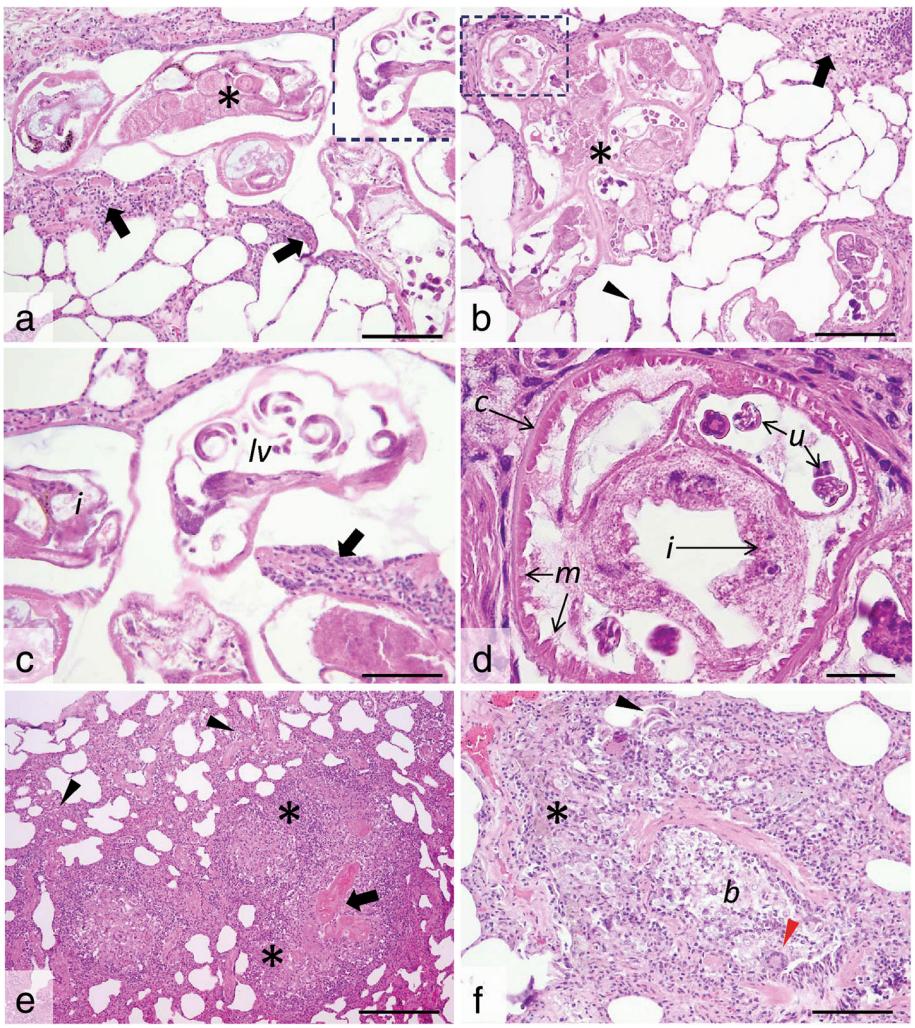

Fig. 11 Histological cross- and tangential sections of *P. falciformis* in the lung parenchyma. **a**, **b**. Viable *P. falciformis* adults (asterisks) coiled in the lung parenchyma and surrounded by a mild leukocyte reaction (many macrophages, occasional multinucleate giant cells, some lymphocytes and eosinophils) (black arrows) and smooth muscle hyperplasia. The alveolar walls are moderately expanded by the above described inflammatory cell population, with occasional alveolar wall rupture (arrowhead) and emphysema. **c**, **d** Detail of the marked areas in **a** and **b**. The parasites have a thin smooth cuticle (*c*) and pseudocoelom, coelomyarian-polymyarian musculature (*m*), intestine (*i*) with granular pigment and large uterus (*u*) filled with developing larvae (*lv*). The leukocyte reaction is also visible (black arrow in **c**). **e**, **f**. Free *P. falciformis* larvae (with thin walls and granular content) (black arrowhead in **f**) are present in the bronchiolar (*b*) and peribronchiolar spaces, associated with prominent inflammatory reaction consisting of many macrophage (some laden with hemosiderin) (asterisk in **f**), epithelioid cells and multinucleate giant cells (red arrowhead in **f**) and few eosinophils and fibroblast. Degenerated parasites (black arrow in **e**) induce a marked inflammatory response consisting of ill-defined granulomas (asterisks in **e**) and a locally-extensive interstitial lympho-histiocytic and eosinophilic pneumonia (arrowheads in **e**); H&E staining, ×20 (**a**, **b** and **f**), ×40 (**c**), ×100 (**d**) and ×10 (**e**). *Scale-bars*: **a**, **b**, **f**, 100 μm; **c**, 50 μm; **d**, 25 μm; **e**, 200 μm

close to the two genera of the Filaroididae within the superfamily Metastrongyloidea.

The close relationship of *Perostrongylus*, *Filaroides* and *Parafilaroides* in the molecular phylogeny is in good agreement with morphological characters and forms of reproduction. Males of species in all three genera have short, stout and arcuate spicules, a single-element gubernaculum and females are ovoviviparous. In contrast, males of *A. abstrusus* have slender (half the width of *P. falciformis*) and straight spicules, a gubernaculum of two joined equal elements and the females are oviparous [55]. The present paper also provides a very detailed morphological description of the adult stages of *P. falciformis* which was previously relatively brief [8, 10].

Both species of genus *Perostrongylus* are parasites of the Mustelidae. *Perostrongylus falciformis* has been reported so far only in European badgers from various countries in the western Palaearctic, including Germany [8, 11], Ukraine [56], Russia [57], Italy [58], UK [26], Norway [27], Poland [28], and Bosnia and Herzegovina

[29]. Here we report for the first time the presence of *P. falciformis* in Romania. In a study from UK on stoats, unidentified nematodes were shown in a histological section of a lung [59]. Later, Simpson et al. [60], hypothesized, based on the morphology of the female in these histological sections that the nematodes could be *P. falciformis*. However, without morphological or genetic data, this remains only a presumption and we do not list stoats as confirmed hosts for *P. falciformis*.

Perostrongylus pridhami has been reported mainly in the American mink, *N. vison* in North America: Ontario, Canada [21, 22, 37, 42], Newfoundland, Canada [30], and Montana, USA [40] and in stoats, *M. erminea* from Newfoundland [30]. Torres et al. [32] mentioned *P. pridhami* in European badgers in Spain. However, this is highly unlikely, as it probably represents a misidentification of *P. falciformis*.

Interestingly, one study on invasive American minks in Spain [33] reported a specimen identified as *Aelurostrongylus* spp. As the specimen was not identified to the species level, it is not known if this represents *P. pridhami*, a natural parasite of this host in North America, but in an invasive population, or *P. falciformis*, which could suggest an adaptation from European badgers to invasive American minks.

Lungworms of genus *Perostrongylus* seem to occur with variable prevalence in mustelids across their distribution range (Table 6). Additionally to the overview in this table, unidentified lungworms were reported in 18% (8/45) of European badgers in Germany, with pathological lesions consistent with those produced by *P. falciformis* [61]. In our opinion, a low prevalence or total absence of these parasites in studies with a reasonably large number of samples are likely the result of a rather superficial examination; probably *P. falciformis* is present in European badgers across their distribution range.

The data presented here indicate that *C. aspersum* is a suitable intermediate host of *P. falciformis*. However, in a series of laboratory infection studies, larval development from L1 to L3 was demonstrated to occur in two slug species [*Deroceras agreste* (Linnaeus, 1758) and *Arion hortensis* (Férussac, 1819)] and five species of terrestrial snails [*Trochulus hispidus* (Draparnaud, 1801), *Cepaea hortensis* (Müller, 1774), *C. nemoralis* (Linnaeus, 1758), *Euomphalia strigella* (Draparnaud, 1801) and *Succinea putris* (Linnaeus, 1758)] [11, 62].

After 24 hours, the L1 were found coiled between the muscle fibres of the snails' foot and the first moult occurred at 6–8 dpi at room temperature (or as long as 14 days at lower temperatures) [11, 62]. In the present study, the first L2 larvae were found at 10 dpi (however, the previous examination time was 6 dpi). Wetzel [11] mentions the second moult at 10–12 dpi. In our study, the first L3 were found at 10 dpi, but the largest numbers were present in snails at 30 dpi. Wetzel [11] also mentioned that these time frames are slightly variable according to the species of snail used, but no other details were provided. Wetzel [11, 62] described in detail L1-L3 larval stages and provided drawings for L1 and L3. These morphological details are largely consistent with our results (Table 5), with only minor differences. In addition, our present work provides the first detailed photomicrographs of L1-L3.

The life-cycle of *P. pridhami* described in North America [21] showed a role as potential intermediate hosts for several experimentally infected species of aquatic snails [*Physa integra* (Haldeman, 1841), *Gyraulus deflexus* (Sandberger, 1858), *G. crista* (Linnaeus, 1758), *Ampullaria cuprina* Reeve, 1856] as well as terrestrial snails [*Zonitoides arboreus* (Say, 1816), *Discus cronkhitei* (Newcomb, 1865), *Novisuccinea ovalis* (Say, 1817), *Anguispira alternate* (Say, 1816)] or slugs [*Deroceras gracile* (Müller, 1774)] [21]. As in *P. falciformis* [62], larvae of *P. pridhami* penetrate the foot of snails [21]. Stockdale [37] succeeded to infect terrestrial snails by

Table 6 Reported prevalence for *Perostrongylus* spp.

Species	Host	Country	Prevalence (%) (infected/examined)	Reference
P. falciformis	*Meles meles*	Germany	50.0 (6/12)	[10]
P. falciformis	*Meles meles*	Poland	22.2 (2/9)	[28]
P. falciformis	*Meles meles*	Norway	33.3 (3/9)	[27]
P. falciformis	*Meles meles*	UK	0.8 (1/118)	[26]
P. falciformis	*Meles meles*	Italy	52.6 (10/19)	[58]
P. falciformis	*Meles meles*	Spain	3.5 (3/85)	[32]
P. falciformis	*Mustela erminea*	UK	13.5 (5/37)	[59]
P. pridhami	*Neovison vison*	Canada	8.6 (13/152)	[42]
P. pridhami	*Neovison vison*	Canada	2.1 (1/48)	[30]
P. pridhami	*Mustela erminea*	Canada	12.5 (8/40)	[30]
Perostrongylus sp.	*Neovison vison*	Spain	2.0 (1/50)	[33]

injection. The dynamics of larval development in snails is not known for *P. pridhami*. The prepatent period for this species was 23–28 days in experimentally infected minks [21], slightly longer than for *P. falciformis* in European badgers [11, 62]. As demonstrated for other lungworms of carnivores, *P. pridhami* can be transmitted to minks after ingestion of paratenic hosts (mice, birds, amphibians and fish) [21] but no such information is available for *P. falciformis*.

Additional information is provided on the development of *P. pridhami* in minks experimentally infected *via* gastric tube with L3 from terrestrial snails [37]. The first moult in minks (L3 to L4) occurred 3 dpi and the final moult (L4 to L5) at 7 dpi [37]. The migration in the minks included penetration of the stomach wall, crossing the peritoneal cavity and the diaphragm, followed by penetration of the visceral pleura of the lungs, all these occurring within the first 24 hours [37]. The migration pattern and last two moults for *P. falciformis* in European badgers are not known. Anderson [21] also described the morphology of L1-L3 of *P. pridhami* which seem to be similar to that of the larvae of *P. falciformis* ([11, 62]; present study). L1-L3 of *P. pridhami* are all illustrated in detail [21].

As concluded by Hancox [25], although the European badger is affected by a wide range of parasites, average rates of infection will not have a significant effect on host population regulation. However, individuals may be affected by a high parasite load. Lesions and symptoms produced by *P. falciformis* in European badgers have been described previously [8, 10, 27, 29, 60]. Additionally, lesions consistent with a possible *P. falciformis* infection in *M. erminea* were found in the UK [59, 60]. In North America, lesions produced by *P. pridhami* in minks have also been described [21, 37, 38]. Generally, the pathology caused by the two species of *Perostrongylus* is similar and results in vomiting (though after experimental infection), coughing (usually when large numbers of parasites are present) and can be complicated by the presence of co-infections [i.e. with *Filaroides martis* (Werner, 1782) in minks]. However, the clinical signs are known only from experimental infections. The importance of co-infections with *Angiostrongylus daskalovi* Janchev & Genov, 1988 in European badgers [63] on the clinical outcome is unknown.

The lesions are produced either by infective larval migration and are located at various levels (stomach, peritoneum, pleura) or by larvae migrating through the lung parenchyma and adults. Generally, adults of *P. falciformis* are found either in subpleural granulomas or embedded in the lung parenchyma (present study). Similar subpleural nodules were found also in minks infected with *P. pridhami* [37, 38]. The histological lesions in our study are to date the most detailed and largely similar to the lesions observed in other studies on *P. falciformis* [27, 29, 59] or *P. pridhami* [38].

Conclusions

Molecular phylogenetic and morphological data support the validity of the genus *Perostrongylus*, with probably two species: *P. falciformis* in European badgers in Europe and *P. pridhami* in minks in North America. Moreover, *Aelurostrongylus* becomes a monotypic genus, with *A. abstrusus* as the type- and only species. Interestingly, the two genera clearly belong to two different evolutionary branches: *Perostrongylus* is grouped together with the genera *Filaroides* and *Parafilaroides* (both in family Filaroididae), whereas *Aelurostrongylus* belongs to a single branch, with no sister groups. The present study also demonstrated for the first time that *C. aspersum* snails are suitable intermediate hosts. Several aspects remain unknown. One question is which species of *Perostrongylus* is found in American minks invasive to Europe. The role of paratenic hosts in the life-cycle of *P. falciformis* is also a matter to be explored. Moreover, molecular data from *P. pridhami* will bring further proof for the phylogenetic position of *Perostrongylus* among metastrongyloids.

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Availability of data and materials

The data supporting the conclusions of this article are included within the article. Representative sequences were submitted to the GenBank database under the accession numbers KY365435-KY365437. Voucher specimens are available in the collection of the Museum of Parasitology, Department of Parasitology and Parasitic Diseases, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Romania under accession numbers CJ005077, CJ005086. The dataset and reference materials are available from the corresponding author upon request.

Authors' contributions

GD collected samples, performed necropsies and wrote the manuscript. ADM collected samples, wrote the manuscript and coordinated the study. JH: collected samples and performed the phylogenetic analysis. VC performed the experimental life-cycle; FAT carried out the histopathology analysis; MAC performed the larval morphological description and illustrations. FGB collected samples, participated in necropsies and revised the manuscript. CB collected samples, carried out the phylogenetic analysis, provided critical comments on the manuscript. AMI participated in the sample collection and performed the molecular analyses. AA essentially contributed to the sample collection and performed necropsies. DO critically revised the text, coordinated the experimental life-cycle. CMG performed the morphological identification, collected samples and coordinated the study. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study was performed in accordance with the national and European rules and regulations for research ethics. No live vertebrates were used in this study.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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References

1. Carreno RA, Hoberg EP. Evolutionary relationships among the Protostrongylidae (Nematoda: Metastrongyloidea) as inferred from morphological characters, with consideration of parasite-host coevolution. J Parasitol. 1999;85:638–48.
2. Carreno RA, Nadler SA. Phylogenetic analysis of the Metastrongyloidea (Nematoda: Strongylida) inferred from ribosomal RNA gene sequences. J Parasitol. 2003;89:965–73.
3. Cameron TWM. Studies on three new genera and some little-known species of the nematode family Protostrongylidae Leiper, 1926. J Helminthol. 1927;5:1–24.
4. Mueller A. Helminthologisch Mittheilungen. Dtsch Z Sportmed. 1927;17: 58–70.
5. Railliet A. Rectification de la nomenclature d'apres les traveaux recents. Rec Med Vet. 1898;75:171–4.
6. Fry W, Stewart JT Jr. Studies on *Aelurostrongylus abstrusus* (Railliet 1898) Cameron 1927. J Parasitol. 1931;18:34–9.
7. Kamensky SN. The systematic position of the genera *Metastrongylus* Wost. and *Protostrongylus* g. n. among the other Strongylidae. Sbornik Trudov Khar'kovokogo Vet Inst. 1905;7:17–50.
8. Schlegel M. Die lungenwurmseuche beim dachs. Berl Munch Tierarztl Wochenschr. 1933;49:341–4.
9. Böhm LK, Gebauer O. Zum System der Familie Metastrongylidae Leiper, 1908. Zool Anz. 1934;105:287–94.
10. Schlegel M. Die Lungenwurmseuche beim dachs. II. Berl Munch Tierarztl Wochenschr. 1934;50:369–73.
11. Wetzel R. Zur Biologie and systematischen Stellung des Dachslungenwurmes. In: Livro Jubilar do Professor Lauro Travassos. Rio de Janeiro: Instituto Oswaldo Cruz; 1938. p. 531–6.
12. Dougherty EC. The genus *Aelurostrongylus* Cameron, 1927 (Nematoda: Metastrongylidae), and its relatives; with descriptions of *Parafilaroides*, gen. nov., and *Angiostrongylus gubernaculatus*, sp. nov. Proc Helminthol Soc Wash. 1946;13:16–25.
13. Seneviratna P. Studies on the family Filaroididae Schulz, 1951. J Helminthol. 1959;33:123–44.
14. Hsü HF. A study of some Strongyloidea and Spiruroidea from French Indo-China and of *Thelazia chungkingensis* Hsü, 1933 from China. Z Parasitenkd. 1935;7:579–600.
15. Anderson RC. Keys to genera of the superfamily Metastrongyloidea. In: Anderson RC, Chabaud AG, Willmott S, editors. CIH keys to the nematode parasites of vertebrates No. 5. Farnham Royal Bucks, UK: Commonwealth Agricultural Bureau; 1978. p. 1–40.
16. Lesage C. Etude de la protostrongylose dans la population de lièvres européens (*Lepus europaeus*) dans le sud est de la France: approche épidémiologique et écologique. PhD Thesis. Reims: University of Reims Champagne-Ardenne; 2014.
17. Anderson RC, Chabaud AG, Willmott S. Keys to the Nematode Parasites of Vertebrates: Archival volume. Wallingford: CABI; 2009.
18. Gibbons LM. Keys to the Nematode Parasites of Vertebrates. Supplementary volume. Wallingford: CABI; 2010.
19. Anderson RC. Further studies on the taxonomy of metastrongyles (Nematoda: Metastrongyloidea) of Mustelidae in Ontario. Can J Zool. 1963;41:801–9.
20. Asakawa M, Ohbayashi M, Ow-Yang CK. Studies on the parasite fauna of Malaysia I. A redescription of *Strongylus brauni* Linstow, 1897, and the establishment of a new genus, *Viverrostrongylus*. Jpn J Vet Res. 1986;34:195–201.
21. Anderson RC. The systematics and transmission of new and previously described metastrongyles (Nematoda: Metastrongylidae) from *Mustela vison*. Can J Zool. 1962;40:893–920.
22. Anderson RC, Fyvie A. Observations on *Aelurostrongylus falciformis* (Schlegel) of *Mustela vison* in Ontario. J Parasitol. 1961;47(Suppl. 1):43.
23. Prestwood AK. *Didelphostrongylus hayesi* gen. et sp. n. (Metastrongyloidea: Filaroididae) from the opossum, *Didelphis marsupialis*. J Parasitol. 1976;62:272–5.
24. Stubbe M. Zur biologie der raubtiere eines abgeschlossenen waldgebietes. Sitzungsber Ges Naturf Freunde Berlin. 1965;11:73–102.
25. Hancox M. Parasites and infectious diseases of the Eurasian badger (*Meles meles* L.): a review. Mammal Rev. 1980;10:151–62.
26. Jones GW, Neal C, Harris EA. The helminth parasites of the badger (*Meles meles*) in Cornwall. Mammal Rev. 1980;10:163–4.
27. Davidson RK, Handeland K, Gjerde B. The first report of *Aelurostrongylus falciformis* in Norwegian badgers (*Meles meles*). Acta Vet Scand. 2006;48:6–10.
28. Demiaszkiewicz AW, Filip KJ, Pyziel AM. The first report of *Aelurostrongylus falciformis* (Schlegel, 1933) (Nematoda, Metastrongyloidea) in badger (*Meles meles*) in Poland. Ann Parasitol. 2017;63:117–20.
29. Stevanović O, Trbojević I, Nikolić S, Santrač V. The first reported case of advanced aelurostrongylosis in Eurasian badger (*Meles meles*, L. 1758) in Bosnia and Herzegovina: histopathological and parasitological findings. Parasitol Res. 2018. https://doi.org/10.1007/s00436-018-5984-6.
30. Jennings DH, Threlfall W, Dodds DG. Metazoan parasites and food of short-tailed weasels and mink in Newfoundland, Canada. Can J Zool. 1982;60:180–3.
31. Kennedy MJ. Synopsis of the Parasites of Vertebrates of Canada - Helminths and Protozoa of Terrestrial Mammals. Edmonton: Department of Agriculture, Animal Health Division; 1986.
32. Torres J, Miquel J, Motjé M. Helminth parasites of the Eurasian badger (*Meles meles* L.) in Spain: a biogeographic approach. Parasitol Res. 2001;87:259–63.
33. Martínez-Rondán FJ, de Ybáñez MR, Tizzani P, López-Beceiro AM, Fidalgo LE, Martínez-Carrasco C. The American mink (*Neovison vison*) is a competent host for native European parasites. Vet Parasitol. 2017;247:93–9.
34. Dorney RS, Lauerman LH Jr. A helminthological survey of wild mink in Wisconsin. Bull Wildl Dis Assoc. 1969;5:35–6.
35. Kontrimavichus VL. Helminths of Mustelids and Trends in Their Evolution. Moscow, Russia: Nauka; 1969.
36. Dailey MD. The transmission of *Parafilaroides decorus* (Nematoda: Metastrongyloidea) in the California sea lion (*Zalophus californianus*). Proc Helminthol Soc Wash. 1970;37:215–22.
37. Stockdale PHG. The development, route of migration, and pathogenesis of *Perostrongylus pridhami* in mink. J Parasitol. 1970;56:559–66.
38. Stockdale PHG. Pulmonary lesions in mink with a mixed infection of *Filaroides martis* and *Perostrongylus pridhami*. Can J Zool. 1970;48:757–9.
39. Ko RC, Anderson RC. Tissue migration, growth, and morphogenesis of *Filaroides martis* (Nematoda: Metastrongyloidea) in mink (*Mustela vison*). Can J Zool. 1972;50:1637–49.
40. Barber DL, Lockard LL. Some helminths from mink in southwestern Montana, with a checklist of their internal parasites. Great Basin Nat. 1973;33:9.
41. Gibbons LM, Krishnasamy M. *Malayometastrongylus diardinematus* n. g., n. sp. (Metastrongyloidea: Angiostrongylidae) from *Rattus rattus diardii* in Malaysia and a redescription of *Thaistrongylus harinasutai* Ohbayashi, Kamiya & Bhaibulaya, 1979. Syst Parasitol. 1986;8:107–15.
42. Martin PA, McDaniel TV, Hughes KD, Hunter B. Mercury and other heavy metals in free-ranging mink of the lower Great Lakes basin, Canada, 1998–2006. Ecotoxicology. 2011;20:1701–12.
43. Varodi EI, Malega AM, Kuzmin YI, Kornyushin VV. Helminths of wild predatory mammals of Ukraine nematodes. Vestn Zool. 2017;51:187–202.
44. Willcox HP, Coura JR. A new design of the Baermann, Moraes, Coutinho's technique for the isolation of nematode larva. Mem Inst Oswaldo Cruz. 1989;84:563–5.
45. Colella V, Giannelli A, Brianti E, Ramos RA, Cantacessi C, Dantas-Torres F, Otranto D. Feline lungworms unlock a novel mode of parasite transmission. Sci Rep. 2015;5:13105.
46. Nunn G. Nematode molecular evolution. An investigation of evolutionary patterns among nematodes based upon DNA sequences. PhD Thesis. Nottingham: University of Nottingham; 1992.

47. Gasser RB, Chilton NB, Hoste H, Beveridge I. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. Nucleic Acids Res. 1993;21:2525–6.
48. Chilton NB, Huby-Chilton F, Gasser RB, Beveridge I. The evolutionary origins of nematodes within the order Strongylida are related to predilection sites within hosts. Mol Phylogenet Evol. 2006;40:118–28.
49. Porazinska DL, Giblin-Davis RM, Faller L, Farmerie W, Kanzaki N, Morris K, et al. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. Mol Ecol Res. 2009;9:1439–50.
50. Bowles J, Blair D, McManus DP. Genetic variants within the genus *Echinococcus* identified by mitochondrial DNA sequencing. Mol Biochem Parasitol. 1992;54:165–73.
51. Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3:294–9.
52. Muñoz P, Hirzmann J, Rodriguez E, Moroni M, Taubert A, Gibbons L, Hermosilla C, Gómez M. Redescription and first molecular characterization of the little known feline neurotropic nematode *Gurltia paralysans* (Nematoda: Metastrongyloidea). Vet Parasitol Reg Stud Rep. 2017;10:119–25.
53. Katoh K, Standley DM. MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol. 2013; 30:772–80.
54. Dereeper A, Guignon V, Blanc G, Audic S, Buffet S, Chevenet F, et al. Phylogeny.fr: robust phylogenetic analysis for the non-specialist. Nucleic Acids Res. 2008;36:465–9.
55. Gerichter CB. Studies on the nematodes parasitic in the lungs of Felidae in Palestine. Parasitology. 1949;39:251–62.
56. Kadenatsii, AN. Helminthofauna of mammals of Crimea and experience of recovery of domestic animals from basic helminthiases. Helminthol. Laboratory of Acad. Sciences of the USSR. Chair of parasitology of Omsk Vet. in-ta. 1957; p. 137.
57. Kontrimavichus VL, Delyamure SL, Boev SN. Metastrongyloids of domestic and wild animals. Fundamentals of Nematology 1976;26:1–237. (Translated by Oxonian Press Pvt. Ltd., New Delhi, India, 1985).
58. Magi M, Banchi C, Barchetti A, Guberti V. The parasites of the badger (*Meles meles*) in the north of Mugello (Florence, Italy). Parassitologia. 1999;41:533–6.
59. McDonald RA, Day MJ, Birtles RJ. Histological evidence of disease in wild stoats (*Mustela erminea*) in England. Vet Rec. 2001;149:671–5.
60. Simpson VR, Tomlinson AJ, Stevenson K, McLuckie JA, Benavides J, Dagleish MP. A post-mortem study of respiratory disease in small mustelids in south-west England. BMC Vet Res. 2016;12:72.
61. Rudolph R. Ceroidbildung und deren ätiologie in dachslungen. Berl Munch Tierarztl Wochenschr. 1968;81:13–5.
62. Wetzel R. Zur entwicklung des dachslungenwurmes *Filaroides falciformis* (Schlegel, 1933). Sitzungsber Ges Naturf Freunde Berlin. 1937;1:1–3.
63. Gherman CM, Deak G, Matei IA, Ionică AM, D'Amico G, Taulescu M, et al. A rare cardiopulmonary parasite of the European badger, *Meles meles*: first description of the larvae, ultrastructure, pathological changes and molecular identification of *Angiostrongylus daskalovi* Janchev & Genov, 1988. Parasit Vectors. 2016;9:423.

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REVIEW Open Access

A synoptic overview of golden jackal parasites reveals high diversity of species

Călin Mircea Gherman and Andrei Daniel Mihalca*

Abstract

The golden jackal (*Canis aureus*) is a species under significant and fast geographic expansion. Various parasites are known from golden jackals across their geographic range, and certain groups can be spread during their expansion, increasing the risk of cross-infection with other carnivores or even humans. The current list of the golden jackal parasites includes 194 species and was compiled on the basis of an extensive literature search published from historical times until April 2017, and is shown herein in synoptic tables followed by critical comments of the various findings. This large variety of parasites is related to the extensive geographic range, territorial mobility and a very unselective diet. The vast majority of these parasites are shared with domestic dogs or cats. The zoonotic potential is the most important aspect of species reported in the golden jackal, some of them, such as *Echinococcus* spp., hookworms, *Toxocara* spp., or *Trichinella* spp., having a great public health impact. Our review brings overwhelming evidence on the importance of *Canis aureus* as a wild reservoir of human and animal parasites.

Keywords: Golden jackal, *Canis aureus*, Parasites

Background

The golden jackal, *Canis aureus* (Carnivora: Canidae) is a medium-sized canid species [1] also known as the common or Asiatic jackal [2], Eurasian golden jackal [3] or the reed wolf [4]. Traditionally, *Canis aureus* has been regarded as a polytypic species (Table 1), with 14 subspecies distributed across a vast geographical territory in Europe, Asia and Africa [5, 6]. Recently, phylogenetic studies have demonstrated that at least two of the African subspecies need a formal recognition as distinct species. Koepfli et al. [3] suggested that *C. aureus anthus* forms a distinct monophyletic lineage to *C. aureus* and should be recognized as a separate species. Similarly, the phylogenetic comparison of the Egyptian jackal (*C. aureus lupaster*) with other wolf-like canids showed a close relationship with the gray wolf species complex rather than with other subspecies of golden jackals [7]. Nevertheless, because most of the studies dealing with parasites of golden jackals do not mention the subspecies, for the purpose of this review we have considered the entire group, without excluding the two former subspecies.

The distribution of golden jackals is limited to the Old World [8]. Molecular evidence supports an African origin for all wolf-like canids including the golden jackal [8]. It is considered that the colonization of Europe by the golden jackal took place during the late Holocene and early Neolithic, through the Balkan Peninsula [9]. During the last century, the species has recorded at least two geographic expansion events. A notable expansion started in the 1950s, with a second one following during the 1980s. This is particularly evident in Europe. Stable reproductive populations have been recorded in about 20 European countries, while in other nine, vagrant specimens were observed [10]. The factors that facilitate the territorial expansion of golden jackals are unclear, but land use [11], climate change [12, 13], and the lack of intra-genus competition have been suggested [12–14].

Golden jackals have an opportunistic nutritional behaviour with an extremely varied diet [15]. They prey or scavenge on small mammals, birds and their eggs, amphibians, reptiles, even invertebrates, and they take carrion when available. Occasionally, jackals also feed on vegetables or fruits. Additionally, their relatively broad home range, varying from 1.1 to 20.0 km^2 [16, 17], increases the chance of contact with various parasites but also with other hosts.

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Table 1 Subspecies and geographical distribution of the golden jackal, *Canis aureus*

Subspecies	Common name	Range	Synonyms
Canis a. aureus Linnaeus, 1758	Common jackal	Middle Asia; Afghanistan; Iran; Iraq; Arabian Peninsula; Baluchistan; northwestern India	*C. balcanicus*; *C. caucasica*; *C. dalmatinus*; *C. hadramauticus*; *C. hungaricus*; *C. kola*; *C. lanka*; *C. maroccanus*; *C. typicus*; *C. vulgaris*
Canis a. algirensis Wagner, 1841	Algerian wolf	Algeria; Morocco; Tunisia	*C. barbarus*; *C. grayi*; *C. tripolitanus*;
Canis a. anthus Cuvier, 1820	Senegalese wolf; grey jackal; slender jackal	Senegal	*C. senegalensis*
Canis a. bea Heller, 1914	Serengeti wolf; Serengeti jackal	Kenya; northern Tanzania	
Canis a. cruesemanni Matschie, 1900	Siamese jackal; South East Asian jackal	Thailand; Myanmar; East India	
Canis a. ecsedensis Kretzoi, 1947	Pannonian jackal	Pannonian Basin	*C. minor*; *C. balcanicus*; *C. hungaricus*
Canis a. indicus Hodgson, 1833	Indian jackal; Himalayan jackal	Pakistan; India; Nepal; Bhutan; Burma	
Canis a. lupaster Hemprich & Ehrenberg, 1833	African wolf; Egyptian wolf; Egyptian jackal	Egypt; Algeria; Mali; Ethiopian Highlands; Senegal	*C. lupaster*; *C. lupus lupaster*; *C. sacer*
Canis a. moreoticus Geoffroy Saint-Hilaire, 1835	European jackal; Caucasian jackal; Reed wolf	Southeastern Europe; Asia Minor; Caucasus	*C. graecus*
Canis a. naria Wroughton, 1916	Sri Lankan jackal	Southern India; Sri Lanka	*C. lanka*
Canis a. palaestina Khalaf, 2008		Palestine; Israel	
Canis a. riparius Hemprich & Ehrenberg, 1832	Somali wolf	Somalia; Ethiopia; Eritrea	*C. hagenbecki*; *C. mengesi*; *C. somalicus*
Canis a. soudanicus Thomas, 1903	Variegated wolf; Nubian wolf	Sudan; Somalia	*C. doederleini*; *C. nubianus*; *C. thooides*; *C. variegatus*
Canis a. syriacus Hemprich & Ehrenberg, 1833	Syrian jackal	Israel; Lebanon; Jordan	

All these biological and behavioural features create premises for their infection with a broad range of pathogens, including parasites. Golden jackals are known to host a large spectrum of viral, bacterial and parasitic pathogens [18–20]. The literature survey indicates that the studies published on golden jackal parasites are usually limited to a country or, more commonly to a region, and there is no synoptic overview on this potentially important topic. The aim of the present work was to review all the published data on the parasite fauna of golden jackals in a comprehensive and updated list. The goal is consistent with the demographic and territorial expansion tendency of this species and increased contact with domestic animals and humans.

Literature survey methodology

The list of the golden jackal parasites was compiled on the basis of an extensive literature search published from historical times until April 2017. Abstracts in conference proceedings and theses were also considered. The search queries were performed in the several databases: Pub Med [21], Science Direct [22], Web of Science [23], Helminthological Abstracts [24], Biological Abstracts [25], BioOne [26], Host-Parasite Database of the Natural History Museum (London) [27] and the web search engine Google Scholar [28]. Additionally, two Russian databases, namely the Russian Scientific Electronic Library [29] and the Scientific Library Earth Papers [30] were also used as sources of information.

The parasites are listed in tables, organized according to their taxonomic rank, and species within families are alphabetically listed. Taxonomy follows Adl et al. [31] for protists; Gibson et al. [32], Jones et al. [33], and Bray et al. [34] for trematodes; Kahlil et al. [35] and Nakao et al. [36] for cestodes; De Ley & Blaxter [37] for nematodes; Amin [38] for acanthocephalans; and the database "Catalogue of Life: 2016 Annual Checklist" by Roskov et al. [39] for arthropods. The names of the species were updated according to the current taxonomy, but synonyms used by different authors are also indicated. Each species is indexed together with the country of the report, the method of examination and reference. The records within a species are listed according to the alphabetical order of the country name. If two or more reports for the same country are registered, the ranking was made chronologically, according to the year publication. The prevalence, frequency and intensity of infection are also given, when available. The prevalence was provided or calculated only when the sample size was at least 10. In the case of experimental infection studies, the country has not been specified. Articles that report infections in captive jackals and doubtful records are mentioned and/or discussed accordingly.

Protists

Eight families with 21 species were reported in golden jackals in 23 countries. Additionally, several protists were identified only to the generic level or were doubtfully considered as parasites of golden jackals (Table 2) [40–85].

Leishmania

The sand fly-borne kinetoplastids of the genus *Leishmania* were reported in golden jackals from 13 countries (Table 2), showing a large geographical distribution in Asia, Africa and Europe. At least three species of *Leishmania* have been identified by molecular methods in naturally infected golden jackals (*L. donovani*, *L. infantum* and *L. tropica*). Additionally, golden jackals were experimentally shown to be receptive for the infection with *L. major* [85], but this species has never been found in naturally infected specimens. The multiple records of *Leishmania* spp. in golden jackals suggest a reservoir role for this carnivore, for both visceral and cutaneous leishmaniasis in humans, as well as for canine leishmaniasis. Infected jackals have been found also at the margin of the endemic area for canine leishmaniasis (i.e. Romania), where this finding has been temporally correlated with the re-emergence of the disease in domestic dogs [86]. Although there is no clear link between the emergence of leishmaniasis in dogs and the spreading of jackals, this is an issue to be further investigated, mainly as the jackal continues to spread into areas at the margin of canine leishmaniasis endemicity. This was previously demonstrated when infected dogs were newly introduced to non-endemic areas in Europe [87].

Tick-borne protists (Babesiidae, Theileriidae and Hepatozoidae)

Experimental evidence showed that golden jackals are receptive to the infection with *Babesia canis* [40] and *B. gibsoni* [43]. However, there are surprisingly few records of natural infections with piroplasms in golden jackals (Table 2) despite the large variety and number of studies on ticks (see below). In Europe, the only *Babesia* species molecularly confirmed in golden jackals is *B. canis*, recently reported in Romania [42]. The other report of *B. canis* in jackals is from Nigeria [41], but the species identification was based on blood smears and in captive animals. We consider this record doubtful, as the typical vector for *B. canis*, *Dermacentor reticulatus*, does not occur in Nigeria. Probably the species in this case belongs to the same complex group of large canine *Babesia* known in this area, *B. rossi* or *B. vogeli* [88]. *Babesia gibsoni*, which is widely distributed in Asia, has been reported only once in golden jackals, in India. Although *Babesia rossi* is common in domestic and wild carnivores in Africa [89], so far there are no records of this species in golden jackals. The scarcity of reports of *Babesia* spp. in this wild canid is probably related to the low number of studies and the lack of

Table 2 Protist parasites of the golden jackal, *Canis aureus*

Family	Species	Origin	Prevalence (%)	Frequency	Method	Reference
Phylum Apicomplexa						
Class Aconoidasida						
Babesiidae	*Babesia canis* (syn. *Piroplasma canis*)	na (as *P. canis*)	na	na	EI	[40]
		Nigeria[a]	na	1/6	BS	[41]
		Romania	9.2	5/54	MI	[42]
	Babesia gibsoni	na	na	na	EI	[43]
		India	na	na	BS	[44]
Theileriidae	"Theileria annae"	Romania	3.7	2/54	MI	[42]
Class Conoidasida						
Eimeriidae	*Eimeria* sp.[b]	Bulgaria	5.8	3/56	CO	[45]
	Eimeria aurei[b]	India	na	na	CO	[46]
	Isospora sp.	Bulgaria	5.8	3/56	CO	[45]
		India[a]	case report		CO	[47]
		Iran	7.1	4/56	CO	[48]
		Serbia	6.6	4/60	CO	[49]
	Isospora dutoiti	former USSR	na	na	CO	[50]
		Turkmenistan	na	na	CO	[51]
	Isospora kzilordiniensis	Kazakhstan	na	2/9	CO	[52]
	Isospora neorivolta	Russia	9.3	14/150	CO	[53]
	Isospora ohioensis	Russia	5.3	8/150	CO	[53]
	Isospora theileri	Azerbaijan	na	na	CO	[54]
		Turkmenistan	na	na	CO	[51]
Hepatozoidae	*Hepatozoon* sp.	Algeria	na	2/5	MI	[55]
		Mauritania	25.0	4/16	MI	[55]
	Hepatozoon canis	Austria	case report		MI	[56]
			case report		MI	[42]
		Croatia	30.4	14/46	MI	[57]
		Czech Republic	na	1/1	MI	[42]
		Hungary	57.9	33/57	MI	[57]
			60.0	na	MI	[58]
		Israel	2.1	1/46	BS	[19]
		Montenegro	na	2/2	MI	[57]
		Romania	72.2	39/54	MI	[42]
		Serbia	67.5	140/206	MI	[57]
Sarcocystidae	*Cystoisospora canis*	Hungary	15.0	3/20	CO	[59]
	Neospora caninum	Israel	1.7	2/114	IFAT	[60]
	Sarcocystis sp.	Bulgaria	1.9	1/56	CO	[45]
	Sarcocystis cruzi (syn. *S. bovicanis*)	Russia (as *S. bovicanis*)	20.0	30/150	CO	[53]
	Sarcocystis tenella (syn. *S. ovicanis*)	Russia (as *S. ovicanis*)	34.0	51/150	CO	[53]
	Sarcocystis tropicalis (syn. *Isospora tropicalis*)	India (as *I. tropicalis*)	case report		CO	[61]
		India	case report		CO	[62]
	Toxoplasma-type oocysts	Hungary	5.0	1/20	CO	[59]
	Toxoplasma gondii	Iran	33.3	na	LAST	[63]

Table 2 Protist parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Method	Reference
			77.5	31/40	ELISA	[64]
"Flagellates"[c]						
Hexamitidae	*Giardia* sp.	Iraq[a]	100	4/4	CO	[65]
	Giardia duodenalis (syn. *G. canis*)	Croatia	12.5	1/8	IF, MI	[66]
		Iran	7.1	4/56	CO	[48]
		Russia (as *G. canis*)	1.3	2/150	CO	[53]
Trichomonadidae	*Pentatrichomonas hominis* (syn. *P. canis auri*)	India (as *P. canis auri*)	case report		CO	[67]
Trypanosomatidae	*Leishmania* sp.	Iran	2.5	4/161	SIO	[68]
			12.5	6/48	IFA	
		Serbia	6.9	15/126	MI	[69]
		Spain[a]	case report		H	[70]
	Leishmania donovani	Bangladesh	5 cases	na	MI	[71]
		Georgia	na	1/4	SIO	[72]
		Kazakhstan	na	na	na	[73]
		Iran	5.0	1/20	SIO	[74]
		na	na	na	EI	[75]
	Leishmania infantum	Algeria	case report		IFI, MI	[76]
		Georgia	2.5	1/39	IA	[77]
		Iran	10.0	1/10	DAT, ELISA, IFAT	[78]
			11.6	7/60	DAT	[78]
			1.6	1/60	SIO	
		Iraq	59.6	90/151	SIO, Cult, IFAT, ELISA	[79]
		Israel	7.6	4/53	ELISA	[80]
			6.5	3/46	ELISA	[19]
			1.3	1/77	MI	[81]
		Kazakhstan	na	na	na	[82]
		Romania	2.7	1/36	MI	[42]
		Tajikistan	na	na	na	[83]
		Turkmenistan	2 specimens	na	na	[84]
	Leishmania major	na	na	na	EI	[85]
	Leishmania tropica	Israel	6.5	5/77	na	[81]

Abbreviations: BS blood smear May-Grünwald-Giemsa stained, *CO* coprological examination, *Cult* cultures from the viscera, blood and other tissues, *DAT* direct agglutination test, *EI* experimental infection, *ELISA* enzyme-linked immunosorbent assay, *H* histopathology, *IA* immunochromatographic assay, *IF* immunofluorescence assay, *IFAT* indirect fluorescent antibody test, *IFI* indirect fluorescent immunoassay, *LAST* latex agglutination slide test, *MI*- molecular identification, *SIO* smears from internal organs stained with standard Giemsa, *na* not applicable/unknown

[a]Animals kept in captivity

[b]Doubtful record

[c]Various opinions on the higher taxonomy of these groups are available, hence we keep the generic term "flagellates"

more sensitive/specific methods, as the typical vector ticks [*D. reticulatus* for *B. canis*, *Rhipicephalus sanguineus* (*sensu lato*) for *B. vogeli* and *Haemaphysalis leachi* for *B. rossi*] have been reported on various occasions on these hosts.

An interesting recent report indicates the presence of "Theileria annae" in golden jackals from Romania [42]. Currently, the taxonomic status of this species is debated and it is most commonly referred to as "*Babesia microti*-like". This group has been reported predominantly in red foxes, but also in several other wild carnivores in North America, Asia and Europe [89]. However, so far, the role of golden jackals in its ecology remains unknown.

The first report of *Hepatozoon canis* in golden jackals is relatively recent [19] and has been followed in the last years by several records, mainly in Europe and North Africa (Table 2). Surprisingly, despite the wide distribution

of *H. canis* in canids [90], this tick-borne apicomplexan has never been found in jackals from sub-Saharan Africa or Asia. Nevertheless, the large number of records and the presence of its main vector, *R. sanguineus* (*s.l.*) suggest a reservoir role of golden jackals for *H. canis* at least in Europe, Middle East and North Africa.

Intestinal homoxenous coccidia (Eimeriidae)

Various species of intestinal coccidia of the family Eimeriidae have been found in, or even described from jackals (Table 2). We consider all records of *Eimeria* as pseudoparasites, as previously suggested [91]. Three species of the genus *Isospora* have been described from golden jackals but currently their taxonomic status is listed as doubtful [91]: *Isospora dutoiti* is a misidentification with *Hammondia* spp. or *Neospora caninum*, while *I. theileri* and *I. kzilordiniensis* are probably invalid names (as they might be synonyms with other *Isospora* species from canids). Two other species, *I. neorivolta* and *I. ohioensis*, which are known to infect several species of canids [91], were reported in golden jackals. Interestingly, all these *Isospora* reports in golden jackals are from countries in the former USSR, and this probably reflects a greater interest of researchers from this area for this group of parasites rather than the real geographical distribution. Few reports of unnamed *Isospora* sp. in golden jackals are known from Asia and the Balkans (Table 2).

Heteroxenous coccidia (Sarcocystidae)

Various records list golden jackals host to Sarcocystidae. Sporocysts of *Sarcocystis* (*S. cruzi*, *S. tenella* and *S. tropicalis*) and oocysts of *Cystoisospora canis* have been reported in the faeces of golden jackals in Europe, Russia and India, suggesting their role as definitive hosts (Table 2). Although antibodies against *Neospora caninum* have been detected in *C. aureus* in Israel [60], the role of golden jackals as definitive hosts for this parasite has never been demonstrated and needs to be investigated. So far, various canid species were demonstrated to shed oocysts of *N. caninum*: dogs (*Canis familiaris*) [92], coyotes (*C. latrans*) [93], dingoes (*C. lupus dingo*) [94], and gray wolves (*C. lupus*) [95]. Interestingly, Takacs et al. [59] reported "*Toxoplasma*-like" oocysts in the faeces of jackals but, unfortunately, no morphometric data were provided and there was no attempt to characterize them molecularly. We can only assume that these were small oocysts which, in our opinion, could represent any of the small canine coccidia *N. caninum*, *Besnoitia* spp. or *Hammondia* spp., none of them confirmed so far in golden jackals.

Helminths

The highest number of studies on the parasitic fauna of golden jackals are related to helminths. Our literature survey found at least 178 publications in 38 countries reporting helminths in golden jackals, with 119 species belonging to three phyla: Platyhelminthes, Nematoda and Acanthocephala [96–119].

Trematodes

The diversity of trematodes in golden jackals is relatively high (27 species from nine families) (Table 3). Most of the studies originate in the countries of the former USSR, Asia and North Africa, with few scattered records in Europe. There are no trematodes recorded in golden jackals in sub-Saharan Africa. This situation reflects probably the impact of the Russian helminthological school and the lack of studies in other regions rather than the influence of ecological factors or feeding behaviour of jackals. Among the various records of trematodes in golden jackals, two groups could be identified: the canid- or Carnivora-specific trematodes and other trematodes (specific rather to other mammal groups or birds).

The most commonly reported and widely distributed trematode in golden jackals is *Alaria alata*, found in Caucasus, Russia and Central Asia to Middle East and the Balkans (Table 3). We consider the report of *Alaria americana* in Iran doubtful, as the species is known otherwise only in canids from North America [120].

Jackals have been commonly reported as hosts for fish-borne trematodes typically associated with carnivores. Such examples include species of the genera *Ascocotyle*, *Cryptocotyle*, *Heterophyes*, *Metagonimus* (Heterophyidae), *Echinochasmus*, *Euparyphium* (Echinostomatidae), *Pseudamphistomum*, *Opisthorchis* (Opisthorchiidae) mainly in Asia and northern Africa. The fish-borne *Nanophyetus salmincola* was identified in India, but its geographical distribution is limited to the Pacific Northwest of the USA [121]; with high probability, the report might represent a misidentification with *N. schikhobalowi*, an Asian troglotrematid [122]. The diversity of trematode species in golden jackals is completed by other groups which use various invertebrates (i.e. arthropods) (*Plagiorchis massino*, *Microphallus narii*) or non-fish small vertebrates (i.e. amphibians) (*Pharyngostomum cordatum*) as second intermediate hosts, reflecting the wide diet composition of this carnivore.

Interestingly, *Dicrocoelium dendriticum*, a hepatic fluke typically associated with herbivores, has been found on several occasions in the bile ducts of golden jackals [97, 98] in Russia. As the infection source for this parasite is represented by ant second intermediate hosts, the infection of jackals is probably accidental.

Several of these trematodes reported in golden jackals have zoonotic potential. Human alariosis caused by *Alaria* mesocercariae manifests in various clinical signs which range from cutaneous symptoms to respiratory disorders, a diffuse unilateral neuroretinitis even to an anaphylactic shock with fatal outcome [123]. However,

Table 3 A comprehensive list of trematode parasites of the golden jackal, *Canis aureus*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Phylum Platyhelminthes							
Class Trematoda							
Dicrocoeliidae	*Dicrocoelium dendriticum* (syn. *D. lanceatum*)	Russia	5.0	1/20	5.00 ± 4.45	necropsy	[96][a]
			26.1	na	na	necropsy	[97]
			na	na	na	necropsy	[98]
Diplostomidae	*Alaria alata*	Azerbaijan	22.4	20/89	2–30	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	9.0	na	na	necropsy	[101]
			1.9	1/56	na	necropsy	[45]
		Croatia	na	na	na	na	[102]
		Chechnya	100	16/16	22–268	necropsy	[103]
		Greece	20.0	1/5	na	necropsy	[104]
		Hungary	10.0	2/20	na	necropsy	[59]
		Iran	na	na	na	necropsy	[105]
		Russia	10.0	2/20	3.00 ± 2.68	necropsy	[96]
			34.8	na	na	necropsy	[97]
			na	na	na	necropsy	[98]
			13.3	8/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
			26.0	39/150	2–23	necropsy	[53]
		Serbia	0.9	4/447	19.00 ± 3.63	necropsy	[108]
			30.0	18/60	na	necropsy	[49]
		Uzbekistan	na	na	na	na	[109]
	Alaria americana (syn. *A. canis*)[b]	Iran	5.0	1/20	34	necropsy	[110]
			10.0	1/10	na	necropsy	[111]
	Pharyngostomum cordatum (syn. *P. fausti*)	Azerbaijan	na	na	na	necropsy	[100]
		Azerbaijan (as *P. fausti*)	8.3	1/12	2	necropsy	[99]
			na	na	na	necropsy	[100]
		Russia	6.6	4/60	na	necropsy	[106]
Echinostomatidae	*Echinochasmus corvus*	India	na	na	na	na	[112]
	Echinochasmus schwartzi	Iran	na	na	na	necropsy	[105]
	Euparyphium sp.	Iran	na	na	na	necropsy	[105]
	Euparyphium melis	Russia	16.6	10/60	na	necropsy	[106]

Table 3 A comprehensive list of trematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Heterophyidae	*Ascocotyle italica* (syn. *Parascocotyle italica*)	Russia	8.3	5/60	na	necropsy	[106]
	Ascocotyle sinoecum (syn. *Phagicola sinoecum*)	Iran	na	na	na	necropsy	[105]
	Cryptocotyle lingua[b]	Russia	2.7	4/150	3–18	necropsy	[53]
	Heterophyes sp.	Egypt	na	3/5	na	necropsy	[113]
	Heterophyes aequalis	Egypt	na	na	na	necropsy	[113]
	Heterophyes dispar	Egypt	na	na	na	necropsy	[113]
	Heterophyes heterophyes	Egypt	2 specimens	na	na	necropsy	[113]
	Metagonimus ciureanus (syn. *Dexiogonimus ciureanus*)	Georgia	na	na	na	necropsy	[114]
	Metagonimus yokogawai	Iran	14.2	4/28	na	necropsy	[115]
		Italy	case report		6	necropsy	[116]
		Serbia	1.6	1/60	na	necropsy	[49]
Microphallidae	*Microphallus narii* (syn. *Spelotrema narii*)	India	na	na	na	na	[117]
			na	na	na	na	[112]
Opisthorchiidae	*Metorchis xanthosomus*[b]	Russia	21.8	na	na	necropsy	[97]
	Opisthorchis sp.	Bangladesh	na	1/5	na	necropsy	[118]
	Pseudamphistomum truncatum	Serbia	0.2	1/447	2	necropsy	[108]
			3.3	2/60	na	necropsy	[49]
Plagiorchiidae	*Plagiorchis* sp.	Iran	na	na	na	necropsy	[105]
	Plagiorchis elegans[b]	Russia	3.3	2/60	na	necropsy	[106]
	Plagiorchis massino	Uzbekistan	na	na	na	na	[109]
Schistosomatidae	*Schistosoma spindale*[b]	India	case report		na	CO	[119]
Troglotrematidae	*Nanophyetus salmincola*[b]	India	case report		na	CO	[47]

Abbreviations: *na* not applicable/unknown, *CO* coprological examination
[a]Unknown site of infection
[b]Doubtful record

all human cases originate in North America (and are probably caused by *A. americanum*). The zoonotic potential of *A. alata* in Eurasia remains unknown. Adults *Heterophyes dispar* and *H. heterophyes* may produce diarrhoea, abdominal pain and discomfort in humans [124], while *Metagonimus yokogawai* is considered to be the most common intestinal trematode infection in the Far East, highly important due to the ability of their eggs to invade the blood stream thus causing serious complications [125]. Hence, golden jackals might have a significant role in the environmental contamination with such parasites and represent an indirect source for human contamination. Hepatic and biliary trematodes *D. dendriticum*, *Pseudamphistomum truncatum* and *Opisthorchis felineus* are also able to infect humans, causing abdominal pain, weight loss, chronic relapsing watery diarrhea and hepatobiliary system damages [126, 127].

However, for many other trematode species, golden jackals, as other carnivores, are probably accidental hosts, or most likely, present a pseudoparasitism following ingestion of birds or rodents, as they typically infect other vertebrate groups. For instance, *Cryptocotyle lingua* is mainly a parasite of different gull species in Europe, North America and Japan [128]; *Plagiorchis elegans* is a parasite of raptors, waterfowl, passerines and several mammals as the wood mouse, rat, gerbil and hamster [129]; *Metorchis xanthosomus* is specific for birds in Anseriformes, Gaviiformes, Podicipediformes and Gruiformes [130]; and *Schistosoma spindale* has been described in ruminants and rodents in southeastern Asia [131].

Cestodes

Cestode infections in golden jackals have been recorded across all their distribution range, with a relatively high species diversity (Table 4) [132–152].

Among all the cestode species, *Aelurotaenia cameroni* is the only one known exclusively in the golden jackal. However, as the species was only recently described [132], its absence from other carnivores cannot be excluded until further studies. It is not surprising that all other identified tapeworm species are characteristic to carnivores, confirming the low specific affinity of the adult parasites [153]. As such species infect usually a wider range of canid or non-canid carnivores, this demonstrates a close environmental connection between multiple carnivore species and the use of the same trophic source.

The most commonly reported tapeworms in golden jackals are *Dipylidium caninum*, *Mesocestoides* spp., *Echinococcus granulosus* and *Taenia* spp., found across a wide geographical range (Europe, Asia and Africa). The cosmopolitan character of all these cestodes is attributed to the abundance and diversity of intermediate hosts and the lack of specificity for the definitive hosts [153]. Hence, the jackal, together with other carnivores, represents an important source of environmental contamination. Several of these species are known to be zoonotic, some with a minor impact (i.e. *D. caninum*), but other being a major public health threat (i.e. *E. granulosus*).

Dipylidium caninum occurs across the globe, human cases being reported in European and Asian countries after accidental ingestion of the infected cat and dog fleas with cysticercoid larvae [154]. Although the jackal is not a domestic species, hence not a direct source of infection to humans, it may transmit fleas to hunting, shepherd or stray dogs and participates together with other wild canids in the natural cycle of this cestode.

Several species of the genus *Mesocestoides* have been found in golden jackals in various regions. *Mesocestoides lineatus* is spread in Africa, Asia and Europe; it was rarely found in humans, with about 20 cases being described to date across the world [155]. Although the main definitive hosts are carnivores, humans can also act as accidental final hosts following ingestion of raw or undercooked meat of birds, amphibians, reptiles or small mammals [156]. The zoonotic potential of the other species of *Mesocestoides* in unknown.

The most well-represented family of tapeworms found in jackals is the Taeniidae. The high diversity of the Taeniidae in golden jackals reflects furthermore the wide range of mammalian prey species on which they feed. Golden jackals are hosts to both zoonotic species of *Echinococcus*. *Echinococcus granulosus* and *E. multilocularis* have been reported in this wild canid on multiple occasions and across a wide geographical range. The unilocular or cystic hydatidosis produced by larvae of *E. granulosus* (*sensu lato*) is a ubiquitous infection with high prevalence in various parts of the world [157]. Human multilocular or alveolar echinococcosis caused by *E. multilocularis* has recorded a significant increase in the incidence in northern Eurasia since 1990 [157, 158]. In several regions, high prevalences with both species were reported in golden jackals (Table 4). Reports of *Echinococcus* spp. in areas where this canid has recently spread or increased in abundance (i.e. central and eastern Europe) raise the important question on its role as a potentially new natural reservoir and infection source for humans and livestock.

Among species of genus *Taenia* and *Multiceps*, the most commonly reported species in golden jackals are *T. hydatigena*, *T. pisiformis*, *T. ovis* and *M. multiceps*. Other species (*T. polyacantha*, *T. taeniaeformis*, *T. krabbei*, *T. krepkogorski*, *T. crassiceps* and *M. serialis*) have been also found but only occasionally, mainly within the limited geographical range of Caucasus and central Asia (Table 4). The zoonotic potential of these species is limited, and only few human cases have been reported so far: *T. taeniaeformis* [159–162], *T. crassiceps* [163], *T. hydatigena* [164], *T. ovis* [165, 166], *M. multiceps* and *M. serialis* [167]. *Taenia krabbei*, *T. krepkogorski* and *T. polyacantha* are considered non-zoonotic

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Phylum Platyhelminthes							
Class Cestoda							
Dilepididae	*Aelurotaenia cameroni*	India	na	na	na	na	[132]
Diphyllobothriidae	*Diphyllobothrium* sp.	India[a]	na	na	na	CO	[133]
	Diphyllobothrium latum	Bangladesh	20.0	6/30	na	necropsy	[134]
		India	na	na	na	na	[112]
	Spirometra sp.	India[a]	na	na	na	CO	[135]
		Iran	7.1	1/14	4	necropsy	[136]
	Spirometra erinaceieuropaei (syn. *S. erinacei*)	Azerbaijan	25.0	19/76	1–19	necropsy	[99]
			na	na	na	necropsy	[100]
		Azerbaijan (as *S. erinacei*)	3.5	4/114	2–21	necropsy	[137]
		Iran (as *S. erinacei*)	na	na	na	necropsy	[105]
	Spirometra houghtoni	Iran	na	na	na	necropsy	[105]
	Spirometra mansoni (syn. *Bothriocephalus mansoni*)	Italy	case report	na	necropsy	[138]	
Dipylidiidae	*Diplopylidium noelleri*	Iran	5.0	na	na	necropsy	[139]
		Tunisia	16.0	5/31	1–66	necropsy	[140]
	Dipylidium caninum (syns *Taenia elliptica*, *T. cucumerina*)	Azerbaijan	na	na	na	necropsy	[100]
		Bangladesh	26.6	8/30	na	necropsy	[134]
		Bulgaria	3.8	na	na	necropsy	[141]
			63.6	7/11	na	necropsy	[142]
		Chechnya	100	16/16	3–12	necropsy	[103]
		Hungary	5.0	1/20	4	necropsy	[59]
		India	na	na	na	na	[143]
			na	na	na	na	[112]
		India[a]	5.0	3/60	na	CO	[144]
		Iran	7.1	1/14	4	necropsy	[136]
			10.0	4/40	na	necropsy	[145]
			20.0	2/10	na	necropsy	[111]
			10.1	8/79	na	necropsy	[139]
			33.9	19/56	na	necropsy	[48]
		Israel	46.6	7/15	na	necropsy	[146]
			5.8	1/17	na	CO	[19]
		Italy	na	na	na	necropsy	[138]
		Kazakhstan	16.6	3/18	2–8	necropsy	[147]
		Russia	47.8	na	na	necropsy	[97]
			5.0	1/20	1.00 ± 0.25	necropsy	[96]
			10.0	6/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
			8.0	12/150	1–13	necropsy	[53]
		Serbia	1.6	7/447	4.8 ± 0.6	necropsy	[108]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	na	1/5	na	necropsy	[149]
			16.0	5/31	4–67	necropsy	[140]
		Turkey	na	na	na	necropsy	[150]
		Uzbekistan	na	na	na	necropsy	[151]

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			na	na	na	necropsy	[152]
	Joyeuxiella echinorhynchoides	Azerbaijan	30.2	23/76	1–30	necropsy	[99]
			na	na	na	necropsy	[100]
		Iran	27.8	5/18	na	necropsy	[334]
			7.5	3/40	na	necropsy	[145]
		Turkey	na	na	na	necropsy	[150]
	Joyeuxiella pasqualei	Iran	30.0	3/10	na	necropsy	[111]
Mesocestoididae	*Mesocestoides* sp. group A (oval to elongate cirrus-pouch and short cirrus)	Israel	8.1	7/86	na	necropsy, ME	[335]
	Mesocestoides sp. group B (broad-oval cirrus-pouch and long, more or less strongly coiled cirrus)	Israel	15.2	13/85	na	necropsy, ME	
	Mesocestoides sp.	Greece	na	3/5	na	necropsy	[104]
		Tunisia	na	2/5	na	necropsy	[149]
		Iran	na	1/1	na	necropsy	[336]
		Bulgaria	34.6	na	na	necropsy	[45]
	Mesocestoides corti	Azerbaijan	na	na	na	necropsy	[100]
		Tunisia	12.9	4/31	1–10	necropsy	[140]
	Mesocestoides lineatus (syn. *M. carnivoricolus*)	Azerbaijan	2.6	3/114	9–47	necropsy	[137]
			37.7	37/98	2–63	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	27.0	na	na	necropsy	[101]
			72.7	8/11	na	necropsy	[142]
		Hungary	20.0	4/20	na	necropsy	[59]
		India	na	na	na	na	[112]
		India (as *M. carnivoricolus*)	na	na	na	necropsy	[337]
		Ingushetia	na	1/2	na	necropsy	[338]
		Iran	15.0	3/20	na	necropsy	[110]
			70.0	7/10	na	necropsy	[111]
			36.7	29/79	na	necropsy	[139]
			30.3	17/56	na	necropsy	[48]
			61.1	11/18	na	necropsy	[334]
		Russia	26.1	na	na	necropsy	[97]
			5.0	1/20	1.00 ± 0.53	necropsy	[96]
			40.0	24/60	na	necropsy	[106]
			na	na	na	necropsy	[339]
			na	na	na	necropsy	[98]
			40.0	60/150	1–128	necropsy	[53]
		Serbia	5.8	26/447	69.7 ± 9.3	necropsy	[108]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	74.0	23/31	na	necropsy	[140]
		Turkey	na	na	na	necropsy	[340]
		Ukraine	na	1/1	5	necropsy	[341]
		Uzbekistan	na	na	na	necropsy	[151]
			na	na	na	necropsy	[152]

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
	Mesocestoides litteratus	Serbia	4.7	21/447	64.3 ± 15.1	necropsy	[108]
		Tunisia	23.0	7/31	6–130	necropsy	[140]
	Mesocestoides petrowi	Azerbaijan	na	na	na	necropsy	[100]
		Russia	na	na	na	necropsy	[342]
	Mesocestoides zacharovae	Azerbaijan	case report	na	necropsy	[343]	
Taeniidae	*Echinococcus granulosus*	Azerbaijan	16.3	16/98	2–400	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh	20.0	6/30	na	necropsy	[134]
		Bulgaria	23.0	na	na	necropsy	[101]
			na	3/3	na	PCR	[344]
			9.0	1/11	na	necropsy	[142]
			1.9	na	na	necropsy	[45]
		Ceylon	case report	7	necropsy	[345]	
		Chad	1.2	1/82	na	necropsy	[346]
		Chechnya	na	na	na	necropsy	[347]
			12.0	2/16	8–16	necropsy	[103]
		Chechnya, Ingushetia	na	2/7	74–217	necropsy	[348]
		Hungary	10.0	2/20	na	necropsy	[59]
		India	na	na	na	CO	[349]
		Iran	5.0	1/20	48	necropsy	[110]
			na	na	na	necropsy	[105]
			16.0	na	na	necropsy	[350]
			2.3	2/86	na	necropsy	[351]
			40.0	16/40	na	necropsy	[145]
			40.0	16/40	na	necropsy	[352]
			8.9	7/79	na	necropsy	[139]
			20.0	2/10	na	necropsy	[353]
			66.7	6/9	na	CO	[353]
			na	1/1	na	PCR	[354]
			3.5	2/56	na	necropsy	[48]
			na	na	na	PCR	[355]
		Italy	case report	1	necropsy	[356]	
		Kazakhstan	5.9	na	3–29	necropsy	[147]
		Kenya	27.2	6/22	< 200	necropsy	[357]
		Palestine	na	na	na	na	[358]
		Pakistan	9.0	9/100	na	necropsy	[359]
		Russia	12.5	2/16	na	necropsy	[360]
			82.6	na	na	necropsy	[97]
			69.8	na	na	necropsy	[361]
			5.0	1/20	4.00 ± 2.36	necropsy	[96]
			66.0	10/15	na	CO	[362]
			3.3	2/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
		Tajikistan	30.7	4/13	> 1,000	necropsy	[363]
		Tunisia	na	1/5	72	necropsy	[149]
			na	2/2	na	PCR	[364]

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			9.7	3/31	11–98	necropsy	[140]
	E. multilocularis (syn. *Alveococcus multilocularis*)	Azerbaijan	3.7	2/54	3–5	necropsy	[99]
		Hungary	9.0	1/11	412	necropsy	[365]
		Ingushetia	na	1/2	na	necropsy	[338]
		Iran	16.0	4/25	na	necropsy	[366]
			50.0	5/10	na	necropsy	[353]
			na	9/9	na	CO	[353]
		Russia (as *Alveococcus multilocularis*)	18.7	3/16	na	necropsy	[360]
		Serbia	14.3	4/28	4–57	necropsy	[367]
		Tajikistan	7.7	1/13	na	necropsy	[363]
		Uzbekistan	na	1/4	na	necropsy	[368]
	Multiceps multiceps (syn. *Taenia multiceps*	Azerbaijan	8.9	8/89	2–11	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh (as *T. multiceps*)	10.0	3/30	na	necropsy	[134]
		Bulgaria	9.0	na	na	necropsy	[101]
			9.0	1/11		necropsy	[142]
		Chechnya	na	na	na	necropsy	[347]
		Iran	7.5	3/40		necropsy	[145]
		India	na	na	na	necropsy	[369]
		Kazakhstan	11.1	2/18	4–16	necropsy	[147]
		Russia	39.1	na	na	necropsy	[97]
		Serbia	1.6	7/447	3.00 ± 0.53	necropsy	[108]
		Tajikistan	na	2/6	na	necropsy	[370]
		Ukraine	na	1/1	na	necropsy	[341]
	Taenia serialis	Kazakhstan	5.5	1/18	10	necropsy	[147]
		Kenya	na	2/2	42	PCR	[371]
		Serbia	1.1	5/447	2.7 ± 0.2	necropsy	[108]
	Taenia sp.	Bulgaria	23.0	na	na	necropsy	[141]
		Greece	na	1/5	na	necropsy	[104]
		India	na	na	na	na	[143]
		India[a]	11.6	7/60	na	CO	[144]
		Iran[a]	na	2/2	11 ± 2 epg	CO	[372]
		Kenya	60.0	3/5	na	necropsy	[373]
		Tunisia	19	6/31	1–29	necropsy	[140]
	Taenia crassiceps	Azerbaijan	na	na	na	necropsy	[100]
		Hungary	40.0	8/20	na	necropsy	[59]
		Russia	25.0	15/60	na	necropsy	[106]
	Taenia hydatigena (syn. *T. marginata*)	Azerbaijan	15.3	14/91	1–18	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	55.0	na	na	necropsy	[101]
			27.2	3/11	na	necropsy	[142]
		Chechnya	na	na	na	necropsy	[347]
			50.0	8/16	3–6	necropsy	[103]
		Hungary	15.0	3/20	na	necropsy	[59]
		India	na	na	na	necropsy	[369]

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
		Iran	7.1	1/14	2	necropsy	[136]
			40.0	16/40	na	necropsy	[145]
			10.0	1/10	na	necropsy	[111]
			7.6	6/79	na	necropsy	[139]
			7.1	4/56	na	necropsy	[48]
			5.6	1/18	na	necropsy	[334]
		Italy (as *T. marginata*)	na	na	na	na	[138]
		Kazakhstan	22.0	4/18	2–8	necropsy	[147]
		Russia	6.2	1/16	na	necropsy	[360]
			36.8	14/38	1–3	necropsy	[374]
			34.8	na	na	necropsy	[97]
			5.0	1/20	3.00 ± 2.18	necropsy	[96]
			1.6	1/60	na	necropsy	[106]
			na	na	na	necropsy	[98]
			na	na	na	necropsy	[107]
		Serbia	0.9	4/447	3.75 ± 1.80	necropsy	[108]
		Tajikistan	na	na	na	necropsy	[148]
		Uzbekistan	na	na	na	necropsy	[152]
	Taenia krabbei	Azerbaijan	0.8	1/114	3	necropsy	[137]
			na	na	na	necropsy	[100]
	Taenia krepkogorski (syn. *Hydatigera krepkogorski*)	Azerbaijan	na	na	na	necropsy	[100]
	Taenia ovis	Azerbaijan	5.1	2/39	1–2	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	case report		na	necropsy	[375]
		Iran	5.6	1/18		necropsy	[334]
		Russia	39.1	na	na	necropsy	[97]
		Tajikistan	na	na	na	necropsy	[148]
		USSR (former)	na	na	na	necropsy	[376]
	Taenia pisiformis (syn. *T. serrata*)	Azerbaijan	15.7	14/89	1–6	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	18.0	na	na	necropsy	[101]
			54.5	6/11		necropsy	[142]
		Chechnya	100	16/16	3–18	necropsy	[103]
		Greece	na	1/5	na	necropsy	[104]
		Hungary	20.0	4/20	na	necropsy	[59]
		India (as *T. serrata*)	na	na	na	na	[377]
			na	na	na	necropsy	[369]
		Iran	na	na	na	necropsy	[105]
		Kazakhstan	5.5	1/18	3	necropsy	[147]
		Russia	17.4	na	na	necropsy	[97]
			3.3	2/60		necropsy	[106]
			na	na	na	necropsy	[107]
			16.7	25/150	1–3	necropsy	[53]
		Serbia	1.8	8/447	10.1 ± 2.1	necropsy	[108]
		Tajikistan	na	na	na	necropsy	[148]

Table 4 A comprehensive list of cestode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
		Tunisia	3.2	1/31	1	necropsy	[140]
	Taenia polyacantha (syn. *Tetratirotaenia polyacantha*)	Azerbaijan (as *Tetratirotaenia polyacantha*)	na	na	na	necropsy	[100]
		Turkey	na	na	na	necropsy	[340]
	Taenia taeniaeformis (syn. *Hydatigera taeneiformis*)	Azerbaijan (as *H. taeneiformis*)	na	na	na	necropsy	[100]
		Tajikistan	na	na	na	necropsy	[148]
		Uzbekistan	na	na	na	necropsy	[152]

Abbreviations: *CO* coprological examination; *ME* microscopic/morphological examination; *PCR* polymerase chain reaction; *epg* eggs per gram faeces; *na* not applicable/not available
[a]Animals kept in captivity

tapeworms [158, 168]. Considering the common findings of a wide range of Taeniidae in golden jackals, the high spatial mobility of these hosts and the high resistance of taeniid eggs in the environment [169], the role of jackals as natural reservoirs and infection source for humans and domestic animals should be considered potentially important.

Species of *Spirometra* (Diphyllobothriidae) identified in golden jackals from Europe and Asia (*S. mansoni*, *S. houghtoni* and *S. erinaceieuropaei*) cause sparganosis in intermediate hosts. Humans may acquire the infection after drinking water contaminated with infected copepods or by ingestion of uncooked meat, and occasionally may lead to blindness, paralysis, and death [170, 171]. *Diphyllobothrium latum* is also reported in humans due to consumption of raw or undercooked fish, in cold water areas from the Holarctic Eurasia, overlaid to those regions where the species is recorded in jackal [172]. However, due to the limited number of reports, the role of golden jackals in the natural cycle of these diphyllobothriid cestodes remains unknown.

Tapeworm species with a limited geographic distribution are also reported in jackals. *Diplopylidium noelleri* and *Joyeuxiella* spp. are spread only in warm regions from Asia and Europe, probably due to the high abundance and diversity of reptiles, known as common intermediate hosts [173].

Acanthocephalans

Although the diet of golden jackals generally includes invertebrates, wild birds, reptiles and small mammals which are intermediate or paratenic hosts in the life-cycle of thorny-headed worms [174–176], compared to other groups of helminths, there are only few and geographically limited reports of acanthocephalans in golden jackals. The diversity of acanthocephalans identified in this canid includes at least six species (Table 5) [176–181].

Macracanthorhynchus catulinus has been reported on several occasions in jackals in former USSR and Bulgaria, while the congeneric species *M. hirudinaceus* was found only in Tunisia and Iran. It is unclear if the reports of *M. hirudinaceus* (a parasite typically found in pigs; [155]) represent cases of pseudoparasitism or misidentifications with *M. catulinus* (a parasite typically found in canids), as most papers referring to these findings do not provide details on the identification methods. There are few scattered records of other carnivore-specific acanthocephalan species in golden jackals (*Oncicola canis*, *Pachysentis canicola*, *Centrorhynchus itatsinis* and *Echinorhynchus pachyacanthus*) in central Asia and Italy (Table 5).

Nematodes

Nematodes constitute the most well-represented group of parasites in golden jackals, with 41 species identified (28 species in Chromadorea and 13 species in Enoplea) (Table 6) [182–256].

Ascarids

Ascarids, primarily considered heteroxenous nematodes, have lost their intermediate hosts and have adapted to direct transmission or through paratenic hosts [257]. Four species are reported in golden jackals, with *Toxocara canis* and *Toxascaris leonina* being ubiquitous. *Baylisascaris devosi* is a species typically found in mustelids inhabiting the northern hemisphere [258]. Its presence in golden jackals has been reported only once, in Azerbaijan [99]. This broad distribution and common presence of ascarids in this wild canid can be explained by the intervention of numerous paratenic hosts, possible preys for jackals, in the life-cycle of these nematode species (mostly rodents and invertebrates such as earthworms and insects) [259].

Strongyloides

The cosmopolitan and zoonotic *Strongyloides stercoralis* infects about 200 million people, more commonly in tropical and subtropical climates [260]. Despite the large number of records in domestic dogs from various countries, the species has been reported only once in golden jackals (Table 6). The lack of reports in other parts of the jackal's range could be explained by a low receptivity of this host or by failures of finding the parasites during necropsy due to their small size. A moderate prevalence of 5.6% is also recorded in dogs from northeastern Iran [261]

Table 5 Acanthocephalan parasites of the golden jackal, *Canis aureus*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Class Archiacanthocephala							
Oligacanthorhynchidae	*Macracanthorhynchus* sp.	Iran	10.0	1/10		necropsy	[111]
	Macracanthorhynchus catulinus	Azerbaijan	0.8	1/114	24	necropsy	[137]
			17.0	14/82	1–6	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	3.8	na	na	necropsy	[45]
		Kazakhstan	5.5	1/18	3	necropsy	[147]
		Russia	6.6	4/60	n/a	necropsy	[106]
			6.7	10/150	1–6	necropsy	[53]
		Tajikistan	na	na	na	necropsy	[148]
		Turkmenistan	na	na	na	necropsy	[177]
	Macracanthorhynchus hirudinaceus[a]	Tunisia	na	1/5	na	necropsy	[149]
			3.2	1/31	6	necropsy	[140]
		Iran	case report		na	necropsy	[178]
			62.5	25/40	na	necropsy	[179]
			30.0	3/10	na	necropsy	[111]
			5.0	4/79	na	necropsy	[139]
			3.5	2/56	na	necropsy	[48]
	Oncicola sp.	Iran	na	na	na	necropsy	[178]
	Oncicola canis	Iran	case report		na	necropsy	[178]
			12.6	10/79	na	necropsy	[139]
	Pachysentis canicola	Iran	case report		na	necropsy	[180]
Class Palaeacanthocephala							
Centrorhynchidae	*Centrorhynchus itatsinis*	Azerbaijan	1.4	1/71	na	necropsy	[181]
			na	na	na	necropsy	[100]
Incertae sedis	*Echinorhynchus pachyacanthus*	Italy	na	na	na	necropsy	[138]

Abbreviation: *na* not applicable/not available/no answer
[a]Doubtful record

which is higher than estimated prevalence in humans across the country that ranges between 0.1 and 0.3% [262]. Although carnivores can be a source of infection for humans via larvae that develop in the environment, the principal reservoirs of *S. stercoralis* are humans. The role of domestic and wild carnivores in the epidemiology of strongyloidiasis remains to be clarified [155].

Hookworms and other digestive tract strongylids

Several hookworm (Ancylostomatidae) species have been reported in golden jackals, with *Ancylostoma caninum* and *Uncinaria stenocephala* commonly reported across the entire geographical range of these hosts. Additionally, *A. guentini* was described from golden jackals in India, being so far the only known host for this parasite [191]. The remaining two records (*Placoconus lotoris*, known otherwise only from new world procyonids, and *Ancylostoma braziliense*, typically found in the Americas) we list as doubtful and as these are probably misidentifications of other hookworm species. The opportunistic behaviour of the golden jackals leads them to venture close to human habitats to feed [2]. The proximity with domestic dogs allows interspecific transmission of ancylostomid species, due to the high rate of soil and grass contamination [263]. In this regard, increased prevalence recorded in Russia, ranging between 5.0 and 52.2%, is correlated with similar values in dogs, between 2.06 and 62.3% [264]. *Ancylostoma caninum* and *U. stenocephala* possess a zoonotic potential causing dermal larva migrans in humans [260]. Although a direct relationship between the numerous reports in golden jackals and the presence of disease in humans cannot be established, this carnivore species probably contributes to the presence of Ancylostomatidae larvae in rural and peri-urban areas.

Molineus patens is a hookworm commonly reported in a wide range of carnivores in the Palaearctic and Nearctic [265], including two records from jackals in Russia. However, its zoonotic role has not been documented.

Table 6 Nematode parasites of the golden jackal, *Canis aureus*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Phylum Nematoda							
Class Chromadorea							
Ancylostomatidae	*Ancylostoma* sp.	India[a]	na	3/3	na	CO	[182]
			na	5/5	na	CO	[183]
			31.6	19/60	na	CO	[144]
			case report		700 epg	CO	[184]
			case report		na	CO	[47]
		Iran[a]	na	2/2	10.5 epg	CO	[372]
	Ancylostoma/Uncinaria sp.	Bulgaria	84.6	na	na	necropsy	[141]
		India[a]	na	na	na	CO	[135]
		India	na	3/6	na	CO	[185]
		Iran	na	na	na	necropsy	[105]
		Serbia	33.3	20/60	na	necropsy	[49]
		Tunisia	na	5/5	na	necropsy	[149]
	Ancylostoma braziliense[b]	India	na	na	na	na	[117]
	Ancylostoma caninum	Azerbaijan	17.3	17/98	1–20	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh	46.6	14/30	na	necropsy	[134]
		Bulgaria	54.5	6/11	na	necropsy	[142]
			11.5	na	na	necropsy	[45]
		Chechnya	50.0	8/16	3–18	necropsy	[103]
		Egypt	na	na	na	necropsy	[186]
		Greece	na	1/5	na	necropsy	[104]
		Hungary	40.0	8/20	na	necropsy	[59]
		India	na	na	na	na	[187]
			na	na	na	na	[143]
			na	na	na	na	[188]
		India[a]	na	na	na	CO	[189]
			100	12/12	100–400 epg	CO	[190]
		Iran	100	20/20	27.1	necropsy	[110]
			7.1	1/14	4	necropsy	[136]
			2.5	2/79	na	necropsy	[139]
			case report		na	necropsy	[155]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			3.5	2/56	na	necropsy	[48]
		Israel	33.3	5/15	na	necropsy	[146]
			76.0	13/17	50–800 epg	CO	[19]
		Russia	52.2	na	na	necropsy	[97]
			5.0	3/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
			12.0	18/150	3–265	necropsy	[53]
		Serbia	0.2	1/447	2	necropsy	[108]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	9.7	3/31	2–4	necropsy	[140]
		Uzbekistan	na	na	na	necropsy	[151]
	Ancylostoma guentini	India	na	na	na	necropsy	[191]
	Placoconus lotoris (syn. *Uncinaria lotoris*)[b]	Russia	16.6	2/12	4	necropsy	[98]
	Uncinaria stenocephala	Afghanistan	na	na	na	necropsy	[192]
		Azerbaijan	50.0	49/98	1–404	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	64.0	na	na	necropsy	[101]
			na	na	na	necropsy	[237]
			45.4	5/11	na	necropsy	[142]
			84.6	na	na	necropsy	[45]
		Chechnya	50.0	8/16	4–23	necropsy	[103]
		Greece	na	4/5	na	necropsy	[104]
		Hungary	40.0	8/20	na	necropsy	[59]
		Iran	85.0	17/20	11.1	necropsy	[110]
			6.3	5/79	na	necropsy	[139]
		Russia	34.8	na	na	necropsy	[97]
			na	na	na	necropsy	[339]
			30.0	18/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
			89.3	134/150	3–550	necropsy	[53]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	68.0	21/31	1–54	necropsy	[140]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
		Turkey	na	na	na	necropsy	[150, 194]
		Uzbekistan	na	na	na	necropsy	[151]
			na	na	na	necropsy	[152]
Ascarididae	*Baylisascaris devosi*	Azerbaijan	17.7	14/79	1–16	necropsy	[99]
	Toxascaris sp.	Kazakhstan	11.1	2/18	2–8	necropsy	[147]
	Toxascaris leonina	Afghanistan	na	na	na	necropsy	[192]
		Armenia[a]	case report		na	CO	[195]
		Azerbaijan	0.8	1/114	1	necropsy	[137]
			31.8	29/91	1–19	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	36.0	na	na	necropsy	[101]
			5.8	na	na	necropsy	[141]
		Chechnya	100	16/16	1–12	necropsy	[103]
		Egypt	na	na	na	necropsy	[186]
		Hungary	15.0	3/20	na	necropsy	[59]
		Iran	30.0	3/10	na	necropsy	[111]
		Iran[a]	na	2/2	166.5 epg	CO	[372]
		Kazakhstan	11.1	2/18	2–11	necropsy	[147]
		Russia	43.5	na	na	necropsy	[97]
			10.0	2/20	4.00 ± 3.14	necropsy	[96]
			15.0	9/60	na	necropsy	[106]
			na	na	na	necropsy	[98]
			na	na	na	necropsy	[107]
			4.7	7/150	2–23	necropsy	[53]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	6.5	2/31	1–7	necropsy	[140]
		Turkey	na	na	na	necropsy	[150]
		Uzbekistan	na	na	na	necropsy	[151]
			na	na	na	necropsy	[152]
	Toxocara sp.	India[a]	21.6	13/60	na	CO	[144]
			na	na	na	CO	[196]
			na	2/2	40–700 epg	CO	[197]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			na	3/6	na	CO	[185]
	Toxocara canis (syn. *Belascaris marginata*)	Azerbaijan	32.6	32/98	1–21	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh	40.0	12/30	na	necropsy	[134]
			na	2/5	na	CO	[118]
		Bulgaria	54.5	6/11	na	necropsy	[142]
			7.7	na	na	necropsy	[45]
		Chechnya	50.0	8/16	3–18	necropsy	[103]
		Greece	na	2/5	na	necropsy	[104]
		Hungary	20.0	4/20	na	necropsy	[59]
		India (as *B. marginata*)	na	na	na	na	[187]
			na	na	na	na	[143]
			na	na	na	na	[112]
		India[a]	na	na	na	CO	[189]
		Iran	10.0	2/20	na	necropsy	[110]
			7.1	1/14	10	necropsy	[136]
			10.0	1/10	na	necropsy	[111]
			5.0	4/79	na	necropsy	[139]
			12.5	7/56	na	necropsy	[48]
		Iran[a]	na	2/2	25 ± 5 epg	CO	[372]
		Russia	60.9	na	na	necropsy	[97]
			5.0	1/20	2.00 ± 2.13	necropsy	[96]
			6.6	4/60	na	necropsy	[106]
			na	na	na	necropsy	[98]
			na	na	na	necropsy	[107]
			23.3	35/150	1–22	necropsy	[53]
		Serbia	1.6	7/447	7.8 ± 2.1	necropsy	[108]
			23.3	14/60	na	necropsy	[49]
		Tajikistan	na	na	na	necropsy	[148]
		Tunisia	16.0	5/31	1–9	necropsy	[140]
		Turkey	na	na	na	necropsy	[150]
		Uzbekistan	na	na	na	necropsy	[151]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			na	na	na	necropsy	[152]
	Toxocara cati (syns *Ascaris mystax*, *T. mystax*)[b]	Italy	na	na	na	necropsy	[138]
		Russia	26.1	na	na	necropsy	[97]
			5.0	1/20	2.0 ± 1.9	necropsy	[96]
		Uzbekistan	na	na	na	necropsy	[152]
Crenosomatidae	*Crenosoma vulpis*	Azerbaijan	14.6	12/82	1–19	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh	na	1/5	na	necropsy	[118]
		Chechnya	100	16/16	10–23	necropsy	[103]
		Hungary	30.0	6/20	na	necropsy	[59]
		Russia	26.1	n/a	na	necropsy	[97]
			10.0	6/60	na	necropsy	[106]
			na	na	na	necropsy	[107]
			12.0	18/150	6–297	necropsy	[53]
	Crenosoma petrowi	Chechnya	37.5	6/16	16–38	necropsy	[103]
Diaphanocephalidae	*Kalicephalus schadi fotedari*[b]	India	na	na	na	necropsy	[198]
Dracunculidae	*Dracunculus medinensis*	India	na	na	na	na	[112]
		Kazakhstan	16.6	3/18	2–4	necropsy	[147]
		Tajikistan	na	na	na	necropsy	[148]
Gnathostomatidae	*Gnathostoma spinigerum*	India, Bangladesh, Burma	na	na	na	na	[112]
		Bangladesh	26.6	8/30	na	necropsy	[134]
Gongylonematidae	*Gongylonema* sp.[b]	Serbia	0.4	2/447	1	necropsy	[108]
Metastrongylidae	*Angiostrongylus vasorum*	na	na	1/1	na	EI	[199]
		Hungary	10.0	2/20	na	necropsy	[59]
Molineidae	*Molineus patens*	Russia	8.3	5/60	na	necropsy	[106]
			26.0	39/150	3–104	necropsy	[53]
Onchocercidae	*Acanthocheilonema reconditum* (syn. *Dipetalonema reconditum*)	Bulgaria	30.0	3/10	na	K	[200]
		Kenya (as *Dipetalonema reconditum*)	na	na	na	na	[201]
	Dirofilaria immitis	Azerbaijan	na	na	na	necropsy	[100]
		Bangladesh	na	4/5	na	necropsy	[118]
		Bulgaria	8.9	5/56	2–16	necropsy/K	[202]
			9.6	na	na	necropsy	[45]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			70.0	7/10	na	K	[200]
			37.5	122/325	1–19	necropsy	[203]
		Greece	case report		4	necropsy	[204]
		Hungary	7.4	2/27	1–10	necropsy	[205]
		India[a]	na	na	na	na	[206]
		Iran	28.5	4/14	13	necropsy	[136]
			na	na	na	necropsy	[207]
			11.4	9/79	na	necropsy	[139]
			1.7	1/56	na	necropsy	[48]
			8.9	4/45	1–10	necropsy, molecular	[208]
		Romania	18.52	10/54	1–7	necropsy	[209]
			9.26	5/54	na	molecular	[209]
		Russia	8.3	1/12	29	necropsy	[98]
			8.3	5/60	na	necropsy	[106]
			20.0	na	na	necropsy	[339]
			22.7	34/150	2–23	necropsy	[53]
		Serbia	7.3	32/437	na	necropsy	[210]
		Uzbekistan	na	na	na	necropsy	[152]
	Dirofilaria repens	Egypt	na	na	na	necropsy	[186]
		Iran	10.0	2/20	na	necropsy	[110]
		Romania	1.8	1/54	na	molecular	[209]
		Russia	3.3	2/60	na	necropsy	[106]
		Uzbekistan	na	na	na	necropsy	[152]
Oxyuridae	*Syphacia* sp.[b]	Kazakhstan	5.5	1/18	7	necropsy	[147]
Physalopteridae	*Physaloptera* sp.	Iran	na	na	na	necropsy	[105]
		Kazakhstan	5.5	1/18	4	necropsy	[147]
	Physaloptera sibirica	Azerbaijan	na	na	na	necropsy	[100]
		Kazakhstan	11.1	2/18	1–3	necropsy	[147]
Rictulariidae	*Rictularia* sp.	Egypt	na	na	na	necropsy	[186]
		Greece	na	1/5	na	necropsy	[104]
	Rictularia affinis (syn. *Pterygodermatites affinis*)	Azerbaijan	36.3	28/77	1–29	necropsy	[99]
			na	na	na	necropsy	[100]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
		Bulgaria	7.7	na	na	necropsy	[45]
		India (as *P. affinis*)	na	na	na	na	[117]
			na	na	na	na	[188]
			na	na	na	na	[112]
		Iran	50.0	5/10	na	necropsy	[111]
			5.0	4/79	na	necropsy	[139]
		Kazakhstan	11.1	2/18	1–3	necropsy	[147]
		Tunisia (as *P. affinis*)	6.5	2/31	1	necropsy	[140]
		Turkmenistan	na	na	na	necropsy	[177]
		Uzbekistan	na	na	na	necropsy	[151]
			na	na	na	necropsy	[152]
	Rictularia cahirensis	Egypt	na	na	na	necropsy	[186]
		Iran	10.0	2/20	na	necropsy	[110]
		Turkey	na	na	na	necropsy	[340]
		Uzbekistan	na	na	na	necropsy	[151]
Spirocercidae	*Spirocerca arctica*	Azerbaijan	16.6	2/12	5–14	necropsy	[99]
			na	na	na	necropsy	[100]
	Spirocerca lupi (syn. *Filaria sanguinolenta*)	Azerbaijan	23.4	23/98	1–29	necropsy	[99]
			na	na	na	necropsy	[100]
		India	case report		na	necropsy	[211]
			na	na	na	na	[112]
		India[a]	case report		na	necropsy	[212]
		Iran	2.5	2/79	na	necropsy	[139]
		Italy (as *F. sanguinolenta*)	na	na	na	necropsy	[138]
		Uzbekistan	na	na	na	necropsy	[151]
	Spirocerca sanguinolenta	India	na	na	na	necropsy	[213]
Strongyloididae	*Strongyloides* sp.	India[a]	na	na	na	CO	[214]
		Iran[a]	na	2/2	5.5 ± 0.7 epg	CO	[372]
	Strongyloides stercoralis	Iran	na	na	na	necropsy	[105]
Subuluridae	*Oxynema linstowi*	Tunisia	3.2	1/31	2	necropsy	[140]
Thelaziidae	*Thelazia callipaeda*	Romania	1.6	1/64	70	necropsy	[215]
Class Enoplea							

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
Capillariidae	*Capillaria* sp.	India[a]	na	na	na	CO	[214]
		Russia	2.7	4/150	1–45	necropsy	[53]
	Eucoleus aerophilus (syn. *Thominx aerophilus*)	Azerbaijan (as *T. aerophilus*)	na	na	na	necropsy	[100]
		Hungary	5.0	1/20	na	necropsy	[59]
		Russia	8.3	5/60	na	necropsy	[106]
			37.3	56/150	1–6	necropsy	[53]
	Capillaria boehmi	Russia	30.0	45/150	1–11	necropsy	[53]
	Capillaria plica	Azerbaijan	14.8	4/27	1–4	necropsy	[99]
			na	na	na	necropsy	[100]
		Bulgaria	16.4	na	na	necropsy	[45]
		Hungary	45.0	9/20	na	necropsy	[59]
		Russia	11.6	7/60	na	necropsy	[106]
			33.3	50/150	1–123	necropsy	[53]
	Capillaria putorii	Azerbaijan	na	na	na	necropsy	[100]
Dioctophymatidae	*Dioctophyme renale*	Azerbaijan	3.5	4/114	1	necropsy	[137]
		Iran	35.0	7/20	na	necropsy	[110, 216]
		Russia	3.3	2/60	na	necropsy	[106]
		Tajikistan	na	na	na	necropsy	[148]
		Uzbekistan	na	na	na	necropsy	[151]
			na	na	na	necropsy	[152]
	Dioctophyme skrjabini	Russia	17.4	na	na	necropsy	[97]
Trichinellidae	*Trichinella* sp.	Armenia	33.3	5/15	na	TRIC	[217]
		Azerbaijan	30.1	na	na	TRIC	[218]
			28.6	na	na	TRIC	[219]
		Bulgaria	50.0	na	na	TRIC	[220]
			33.3	15/45	na	TRIC	[221]
			21.2	7/33	na	TRIC	[222]
		Croatia	na	na	na	AD	[102]
		Georgia	80.0	na	na	TRIC	[218]
		Romania	53.7	29/54	na	TRIC	[223]
		Russia	14.3	na	na	TRIC	[224]
			case report		na	AD	[225]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
			25.0	75/302	na	TRIC	[226]
			case report		na	AD	[227]
		Tajikistan	na	na	na	TRIC	[228]
		Thailand	case report		na	TRIC	[229]
			case report		na	TRIC	[230]
		USSR (former)	36.5	na	na	TRIC	[231]
		Yugoslavia (former)	25.0	na	na	TRIC	[232]
	Trichinella britovi	Algeria	case report		na	PCR	[233]
		Azerbaijan	case report		na	AD	[234]
		Iran	11.1	2/18	na	AD, PCR	[235]
		Kazakhstan	na	na	na	TRIC	[234]
		Lithuania	na	3/4	0.9–1.4 lpg	AD, PCR	[236]
		Romania	case report		55 lpg	AD, PCR	[237]
		Serbia	4.7	2/42	na	AD, PCR	[238]
			15.4	2/13	na	AD, PCR	[239]
			27.8	25/90	1.1–57.6 lpg	AD, PCR	[240]
		Turkmenistan	case report		na	AD	[234]
		Uzbekistan	case report		na	AD	[234]
	Trichinella nativa	Kazakhstan	18.9	4/18	7 lpg	AD	[147]
		Lithuania	n/a	1/4	0.9–1.4 lpg	AD, PCR	[236]
		URSS (former)	61.5	na	na	TRIC	[241]
	Trichinella nelsoni	Iran	na	2/2	na	TRIC	[242]
		Kazakhstan	16.2	3/18	6 lpg	na	[147]
	Trichinella pseudospiralis	Russia	2.7	4/150	na	TRIC	[53]
	Trichinella spiralis	Afghanistan	case report		214 lpg	TRIC	[243]
		Azerbaijan	20.4	17/83	1–7 lpg	TRIC	[244]
			21.1	25/114	1–22 lpg	TRIC	[137]
			17.5	16/91	1–44 lpg	TRIC	[99]
		Bulgaria	na	na	na	TRIC	[245]
			45.0	na	na	TRIC	[101]
			40.0	na	na	AD	[45]
		Georgia	36.6	na	na	TRIC	[246]

Table 6 Nematode parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species (synonym)	Origin	Prevalence (%)	Frequency	Intensity	Method	Reference
		Hungary	9.0	1/11	na	AD, PCR	[365]
		Iran	60.3	38/63	na	TRIC	[247]
			55.5	10/18	na	TRIC	[248]
			na	3/3	na	TRIC	[249]
			55.7	59/106	na	TRIC	[250]
			84.0	na	na	TRIC	[251]
		Kazakhstan	5.5	1/18	3 lpg	TRIC	[147]
		Russia	43.5	na	na	TRIC	[97]
			21.6	13/60	na	TRIC	[106]
			55.3	83/150	na	TRIC, AD	[53]
		Senegal	33.0	na	na	TRIC	[252]
		Serbia	na	3/3	1.9–21.4 lpg	AD, PCR	[253]
			8.3	1/12	3 lpg	AD, PCR	[254]
			7.9	3/38	3 lpg	AD, PCR	[255]
			14.2	6/42	na	AD, PCR	[238]
			38.4	5/13	na	AD, PCR	[239]
			71.1	64/90	0.59–152.8 lpg	AD, PCR	[240]
		Tunisia	na	2/2	na	TRIC	[256]
Trichuroididae	*Trichuris* sp.	India[a]	15.0	9/60	na	CO	[144]
	Trichuris georgicus (syn. *Trichocephalus georgicus*)	Azerbaijan	14.4	12/83	1–12	necropsy	[99]
			na	na	na	necropsy	[100]
	Trichuris vulpis (syn. *Trichocephalus vulpis*)	Azerbaijan (as *Trichocephalus vulpis*)	11.2	11/98	1–11	necropsy	[99]
			na	na	na	necropsy	[100]
		Bangladesh	na	1/5	na	CO	[118]
		Bulgaria	30.7	na	1–156	necropsy	[141]
			36.3	4/11	na	necropsy	[142]
		Russia (as *Trichocephalus vulpis*)	21.6	13/60	na	necropsy	[106]
			35.3	53/150	1–70	necropsy	[53]
		Serbia	11.6	4/60	na	necropsy	[49]
		Hungary	10.0	2/20	na	necropsy	[59]

Abbreviations: AD artificial digestion, *CO* coprological examination, *EI* experimental infection, *epg* eggs per gram faeces, *lpg* larvae per gram tissue, *K* Knott test, *PCR* polymerase chain reaction, *TRIC* trichinelloscopy, *na* not applicable/not available

[a]Animals kept in captivity

[b]Doubtful record

Metastrongyloids

Compared to foxes, little is known about the respiratory and cardiovascular strongylids of golden jackals. *Crenosoma vulpis* has been reported on several occasions in Asia and Europe, with variable prevalence (Table 6). There is a single report of *Crenosoma petrowi* in golden jackals, a parasite otherwise known mainly from mustelids in North America [266], one report from bears (also in North America) and another one from stone martens in Italy [267]. Surprisingly, there is only one record of *Angiostrongylus vasorum* in golden jackals, suggesting either a lack of habitat overlap or a low detection sensitivity during necropsy.

Filarioids

Zoonotic filarioids *Acanthocheilonema reconditum*, *Dirofilaria immitis* and *D. repens* have been all reported in golden jackals in various countries (Table 6). They have been found both as adults during necropsies but also as microfilariae demonstrating the reservoir role of jackals. *Dirofilaria* spp. are responsible in humans of conjunctivitis, focal pulmonary infarction with granuloma formation and subcutaneous and submucosal lesions in the lung and conjunctiva [268–270]. A recent review listed 1782 human *Dirofilaria* spp. infections, out of which 372 were pulmonary (in Australia, North and South America) and 1410 were subcutaneous/ocular cases (mostly in Europe and Asia) [271]. *Acanthocheilonema reconditum* is considered non-pathogenic for canids [155], but a single human case is well documented as being caused by this species and at least other two, by other *Acanthocheilonema* species [270, 272, 273]. The recorded prevalence of *D. immitis* has significantly varied in different areas between 7.3% in Serbia and 80.0% in Bangladesh, and seems to be consistent with the prevalence registered in dogs, generally between 40 and 70% in endemic areas [155]. The prevalence of *D. repens* in golden jackals ranged between 3.3 and 10.0%, resembling that recorded in dogs, generally varying from 5 to 20% [155].

The oriental eye-worm *Thelazia callipaeda* has been identified in golden jackals only in Romania [215], but this is probably due to lack of proper examinations of the eyes during the necropsy in other studies rather than a resistance of this host. Dogs originating in the Far East were initially considered the main host of the nematode [257]. Over the last 15 years, *T. callipaeda* has shown an increase in the distribution area mainly in Europe, with many new host records [274]. Human thelaziosis followed the same geographical spreading, with recent cases of infection diagnosed [274]. In this epidemiological picture, the golden jackal occurs as a new reservoir host.

Capillariids

Respiratory capillariids of carnivores (*C. aerophila*, *C. boehmi* and *C. putorii*) are considered primarily homoxenous, but the earthworms often act as facultative intermediate hosts [1, 275]. Along with the heteroxenous species *Capillaria plica* found in the urinary bladder, all species are cosmopolitan [257]. They have been found in golden jackals in Russia and other former Soviet Union countries. Only *C. plica* and *C. aerophila* are known to be zoonotic [276, 277], but their public health impact is minor. As the primary source of infection with *Capillaria* is the soil contaminated by infective eggs [155], the jackal can play an epidemiological role and secondary source of infection for domestic carnivores and humans.

Trichinella

Trichinellids are an important group of meat-borne zoonotic parasites [278] for which the golden jackals represent an important reservoir. Five species, *T. britovi*, *T. nativa*, *T. nelsoni*, *T. pseudospiralis* and *T. spiralis*, were recorded in mountainous and lowland regions across the distribution range of the golden jackal in Europe and Asia. However, only three of these (*T. britovi*, *T. nativa* and *T. spiralis*) have been confirmed molecularly (Table 6). We consider all the records by artificial digestion or trichinelloscopy (see Table 6) where the species is named as hypothetic, as there is no reliable morphological means of differentiation between species, hence we recommend to consider these as *Trichinella* sp. Nevertheless, the zoonotic potential have been shown for most *Trichinella* species, hence, the golden jackal represents an important natural sylvatic reservoir for these nematodes [240].

Other nematodes

Various other groups of nematodes have been found in golden jackals (families Spirocercidae, Dracunculidae, Gnathostomatidae, Physalopteridae, Rictulariidae, Subuluridae), but the reports are occasional (Table 6). Other groups (*Kalicephalus*, *Syphacia* and *Gongylonema*) are with high probability pseudoparasites, originating in prey hosts.

Spirocerca lupi is a rare zoonotic nematode species also identified in golden jackals in Europe, central and southern Asia (Table 6). Although a single human infection has been reported [279], the jackal may represent a reservoir host that maintains the life-cycle of the parasite in a certain region. Two other species of the genus *Spirocerca* (*S. arctica* and *S. sanguinolenta*, both described from domestic dogs) have been also reported in jackals, but their taxonomic status and biology are unknown.

Dracunculus medinensis has been identified in the golden jackal from several central and southern Asian countries. Currently the disease in humans has been declared extinct in the vast majority of the countries, with only three (Chad, South Sudan and Ethiopia) reporting cases in 2016 [280]. Dogs are considered to be important reservoirs for human infection [155, 281, 282]. In 2016,

more than 1000 dogs in Chad, 14 dogs in Ethiopia, and 11 dogs in Mali were reported with guinea-worm [280]. In this context, understanding the role of wild canids (including golden jackals) remains a crucial aspect in the management of the ongoing eradication campaign.

Another zoonotic species identified in golden jackals from tropical Asian countries (India, Bangladesh, Myanmar) is *Gnathostoma spinigerum*. Gnathostomiasis, a major food-borne parasitic zoonosis and a significant public health problem, is considered an emerging imported disease in Europe and a common human infection in central and South America, and Asia [283]. Domestic and wild mammals are the final hosts and numerous intermediate and paratenic hosts are the source for the human infection. The golden jackals maintain the sylvatic focus of the parasites and interfere with the domestic cycle, at least in several Asian countries (Table 6).

Various other carnivore-specific spirurids have been found in golden jackals (*Physaloptera sibirica*, *Rictularia affinis*, *R. cahirensis* and *Oxynema linstowi*), but the role of this host species in their natural cycle remains unknown.

The cosmopolitan species *Dioctophyme renale* causes a severe kidney destruction in the carnivore definitive hosts. Although with limited zoonotic importance, so far around 20 human cases have been reported [284]. American minks seem to be the main reservoirs of the parasite [257], but an increased prevalence is also recorded in other wild and domestic carnivores. In golden jackals, *D. renale* has been reported in Asia, where the prevalence ranged between 3.3–35.0% (Table 6). Interestingly, this wild canid has shown a twice higher prevalence than stray dogs in the same geographical region [110], demonstrating the role of the jackal in the development of parasite's cycle in nature.

Trichuris vulpis has been found on various occasions in golden jackals in Europe and Asia (Table 6). The high prevalence of *T. vulpis* infection in golden jackals (10.0–36.3%) is in line with the value recorded in dogs originating from the same areas: 25% in India [285], 20% in Bulgaria [286] and 8.95% in Russia [264]. Although the prevalence of *T. vulpis* in domestic and wild canids is generally high, only around 60 human cases have been recorded [155].

Arthropods

A great variety of Arthropods have been found in golden jackals (Table 7) [287–328].

Ticks

Due to the large geographical range, the diversity of ticks parasitizing golden jackals is high. Ticks from 37 species belonging to six genera have been recorded in jackals throughout Europe, Asia and Africa. Nevertheless, the number of studies on tick-borne pathogens is surprisingly low. The common tick species found in golden jackals in Europe, i.e. *Dermacentor reticulatus*, *D. marginatus*, *Haemaphysalis concinna*, *H. punctata*, *Ixodes canisuga*, *I. hexagonus*, *I. ricinus* and *Rhipicephalus sanguineus* (*s.l.*), show that they share these ticks with other wild canids, like foxes [329] or with domestic dogs [330]. The two most commonly reported ticks in golden jackals from Europe are *D. reticulatus* and *I. ricinus*. These ticks are known to be important vectors for *Babesia canis* and important tick-borne bacteria, *Borrelia burgdorferi* (*s.l.*) and *Anaplasma phagocytophilum*. However, the reports of these pathogens in *C. aureus* are scarce (a single report of *Babesia canis* from Romania [42]). In Asia, the most common ticks on jackals are several species of genus *Haemaphysalis*, with a high diversity of species reported: *H. leachi*, *H. adleri*, *H. bispinosa*, *H. canestrinii*, *H. flava*, *H. indoflava*, *H. intermedia*, *H. kutchensis* and *H. parva*. However, studies on the pathogens they might transmit are absent. Several of these *Haemaphysalis* species are shared with domestic dogs or other wild carnivores, raising the question of the reservoir role of jackals for certain tick-borne pathogens. Except for *Haemaphysalis* ticks, another commonly reported tick on golden jackals from Asia is *Rhipicephalus haemaphysaloides*, a tick which prefers ungulates and known as vector of several viral and protozoan diseases [331]. Studies in golden jackals from arid regions (northern Africa and Middle East) demonstrated the predominant presence of ticks from the genus *Rhipicephalus*: *R. sanguineus* (*s.l.*), *R. turanicus* and *R. leporis*. Surprisingly, there are no reports of ticks on golden jackals in sub-Saharan Africa.

Mites

Compared to foxes, jackals seem to be less affected by mange-causing mites (Table 7). So far, there is a single report of *Sarcoptes scabiei* in golden jackals, in Israel [287] and a single report of *Otodectes cynotis*, in Iran [295]. It is unclear if the scarcity of data regarding *Sarcoptes* is because of the low prevalence or because of the lack of studies and/or reports. Except sarcoptid mites, there are few records of *Demodex* in golden jackals, but its clinical significance is not known (Table 7).

Fleas and lice

The diversity of fleas reported in golden jackals is relatively high, with at least seven species reported (Table 7), with the most common being *Pulex irritans*, *Ctenocephalides canis* and *C. felis*. Most of the reports of fleas in golden jackals originate in Russia and other former USSR countries and western and southern Asia. Surprisingly, there are no reports of fleas in golden jackals in Europe. The reports of lice in golden jackals are scarce with only three species occasionally reported (Table 7).

Table 7 Arthropod parasites of the golden jackal, *Canis aureus*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity (A, ♂, ♀, N, L)[b]	Reference
Class Arachnida						
Demodecidae	*Demodex* spp.	Israel	na	1/1	na	[287]
	Demodex canis	Russia	3.3	5/150	na	[53]
	Demodex folliculorum	Bangladesh	na	na	na	[118]
Ixodidae	*Amblyomma* sp.	Nepal	na	na	na	[288]
	Amblyomma varanense (syn. *Aponomma gervaisi lucasi*)	India	na	na	na	[289]
	Amblyomma variegatum	Haute-Volta	na	na	7 N	[290]
		Senegal	na	na	54 L	[291]
	Dermacentor marginatus	Russia	15.3	23/150	1–26	[53]
		Serbia	45.0	9/20	na	[292]
	Dermacentor reticulatus	Austria	na	1/1	17 ♂; 2 ♀	[56]
		Hungary	na	4/4	10 A	[293]
		Italy	na	1/1	na	[116]
		Romania	12.6	10/79	46 ♂; 25 ♀	[294]
		Russia	62.0	93/150	1–26	[53]
	Haemaphysalis sp.	Iran	1.7	1/56	na	[48, 295, 296]
		Nepal	na	na	na	[288]
	Haemaphysalis adleri	Iraq	na	na	na	[297]
		Israel	na	2/2	4 ♂; 1 ♀	[298]
			na	3/3	na	[299]
	Haemaphysalis bispinosa	India	na	na	na	[300]
		Nepal	na	na	na	[288]
	Haemaphysalis canestrinii	India	na	1/1	9 ♂; 3 ♀,	[301]
			na	na	na	[300]
		Pakistan	na	3/3	3 ♂; 1 ♀	[301]
	Haemaphysalis concinna	Austria	na	1/1	1 N	[56]
		Hungary	na	4/4	7 N; 4 L	[293]
		Romania	1.2	1/79	1 N	[294]
	Haemaphysalis flava	India	na	na	A	[289]
	Haemaphysalis indoflava	India	na	na	na	[302]
	Haemaphysalis intermedia	India	na	2/2	8 ♂; 17♀; 28 N; 13 L	[303]
			na	na	na	[300]
	Haemaphysalis kutchensis	India	na	na	10 ♂; 1 ♀	[348]
	Haemaphysalis leachii (syns *H. leachii leachii*, *H. leachii indica*)	Egypt (as *H. leachii leachii*)	na	na	na	[305]
		India	na	1/1	9 ♂; 3 ♀	[306]
		India (as *H. leachii indica*)	na	na	na	[289]
			na	2/2	7 ♂; 2♀; 4 N; 1 L	[307]
		Nepal (as *H. leachii indica*)	na	3/3	6 N	[307]
	Haemaphysalis longicornis (syn. *H. neumanni*)	Ceylon	na	na	na	[308]
	Haemaphysalis paraleachi	Sudan	100	10/10	42 ♂; 4 ♀	[309]

Table 7 Arthropod parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity (A, ♂, ♀, N, L)[b]	Reference
	Haemaphysalis parva (syn. *H. otophila*)	India	na	na	na	[310]
		Israel	na	3/3	na	[299]
		Israel (as *H. otophila*)	na	6/6	37	[311]
	Haemaphysalis punctata	Romania	na	4/4	na	[312]
			2.5	2/79	1 ♂; 2 N	[294]
	Hyalomma sp.	Russia	0.7	1/150	1	[53]
		Tajikistan	na	na	na	[148]
	Hyalomma aegyptium	USSR (former)	na	na	na	[313]
	Hyalomma anatolicum	Tajikistan	na	na	na	[148]
	Hyalomma asiaticum	Tajikistan	na	na	na	[148]
	Hyalomma scupense	Tajikistan	na	na	na	[148]
	Ixodes sp.	Iran	na	1/1	na	[314]
		Russia	na	na	na	[339]
		Tajikistan	na	na	na	[148]
	Ixodes acuminatus (syn. *I. redikorzevi theodori*)	Israel	na	6/6	1	[311]
	Ixodes canisuga	Hungary	na	4/4	1 N	[293]
	Ixodes hexagonus	Romania	10.1	8/79	2 ♂; 11 ♀; 24 N; 12 L	[294]
	Ixodes ovatus	Nepal	na	1/1	6 ♀	[315]
	Ixodes ricinus	Hungary	na	4/4	3 A	[293]
		Iran	3.5	2/56	na	[48, 295, 296]
		Italy	na	1/1	na	[116]
		Romania	na	1/1	na	[316]
			na	4/4	na	[312]
			26.5	21/79	54 ♂; 45♀; 3 N; 4 L	[294]
		Russia	68.7	103/150	1–62	[53]
		Serbia	55.0	11/20	na	[292]
	Rhipicephalus sp.	Iran	1.7	1/56	na	[48, 295, 296]
		Tajikistan	na	na	na	[148]
	Rhipicephalus annulatus (syn. *Boophilus calcaratus*)	Russia	1.3	2/150	1–2	[53]
	Rhipicephalus cuspidatus	Haute-Volta	na	na	na	[290]
	Rhipicephalus haemaphysaloides	India	na	na	na	[289]
			na	1/1	4 ♂	[306]
			na	2/2	1 ♀; 2 N	[303]
			na	na	na	[300]
		Nepal	na	na	na	[288]
	Rhipicephalus leporis	Iraq	na	na	na	[297]
		Tajikistan	na	na	na	[148]
	Rhipicephalus pumilio	Tajikistan	na	na	na	[148]
		Uzbekistan	na	na	na	[152]
	Rhipicephalus rossicus	Tajikistan	na	na	na	[148]
	Rhipicephalus sanguineus	Algeria	na	2/2	15 ♂; 8 ♀	[317]
		Burma, Ceylon, India	na	na	na	[289]

Table 7 Arthropod parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity (A, ♂, ♀, N, L)[b]	Reference
		India	na	na	na	[300]
		Iran	na	na	na	[314]
		Israel	na	6/6	na	[311]
		Nepal	na	na	na	[288]
		Nigeria[a]	na	3/6	na	[41]
		Romania	na	1/1	na	[316]
			na	4/4	na	[312]
			1.2	1/79	1 ♂	[294]
		Serbia	0.5	1/20	na	[292]
		Tajikistan	na	na	na	[148]
		Turkey	na	na	na	[150]
			na	na	na	[318]
	Rhipicephalus schulzei	Tajikistan	na	na	na	[148]
	Rhipicephalus simus	Kenya	na	na	na	[319]
	Rhipicephalus sulcatus	Haute-Volta	na	na	5 ♂	[290]
	Rhipicephalus turanicus	Iraq	na	na	na	[320]
			100	14/14	na	[297]
		Tajikistan	na	na	na	[148]
		Uzbekistan	na	na	na	[152]
Psoroptidae	*Otodectes cynotis*	Iran	1.7	1/56	na	[48, 295, 296]
Sarcoptidae	*Sarcoptes scabiei*	Israel	na	1/1	na	[287]
Class Insecta						
Boopiidae	*Heterodoxus spiniger*	Africa (North, East); Asia (South); Europe (Southeast)	na	na	na	[321]
		Uganda	na	1/1	1 ♂; 2 ♀	[322]
Ceratophyllidae	*Paraceras melis*	Russia	10.0	15/150	1–12	[53]
			3.3	5/150	na	[323]
Coptopsyllidae	*Coptopsylla lamellifer dubinini*	Uzbekistan	na	na	na	[152]
Hippoboscidae	*Hippobosca longipennis*	Russia	2.7	4/150	1–2	[53]
Linognathidae	*Linognathus setosus*	Afrotropical Region	na	na	na	[324]
		Nepal	na	na	na	[288]
Pediculidae	*Pediculus* sp.	Bangladesh	na	na	na	[118]
Pulicidae	*Pulex irritans*	Afghanistan	na	na	2 ♂; 1 ♀	[325]
		Iran	na	na	3 ♂; 1 ♀	[326]
		Israel	na	6/6	36	[311]
		Russia	24.0	36/150	1–15	[53]
			14.0	21/150	na	[323]
		Tajikistan	na	na	na	[148]
		Uzbekistan	na	na	na	[152]
	Ctenocephalides canis	Afghanistan	na	na	10 ♂; 38 ♀	[325]
		Iran	na	na	13 ♂; 25 ♀	[326]
			10.8	6/56	na	[48, 295, 296]
		Israel	na	4/6	27	[311]

Table 7 Arthropod parasites of the golden jackal, *Canis aureus (Continued)*

Family	Species	Origin	Prevalence (%)	Frequency	Intensity (A, ♂, ♀, N, L)[b]	Reference
		Nigeria[a]	na	1/6	na	[41]
		Russia	48.0	72/150	1–28	[53]
			17.3	30/150	na	[323]
		Tajikistan	na	na	na	[148]
		Turkey	na	na	na	[150]
		Uzbekistan	na	na	na	[152]
	Ctenocephalides felis (syn. *C. felis felis*)	Ethiopia	na	na	na	[327]
		India (as *C. felis felis*)	na	na	na	[328]
		Iran (as *C. felis felis*)	na	na	2 ♂; 2 ♀	[326]
		Israel	50.0	3/6	6	[311]
		Russia	3.3	5/150	1–4	[53]
		Tajikistan	na	na	na	[148]
		Uzbekistan	na	na	na	[152]
	Echidnophaga gallinacea	Afghanistan	na	na	1 ♂	[325]
		Ethiopia	na	na	na	[327]
	Xenopsylla nesokiae	Tajikistan	na	na	na	[148]
Trichodectidae	*Trichodectes canis*	Russia	13.3	20/150	1–34	[53]
		Tajikistan	na	na	na	[148]
Vermipsyllidae	*Chaetopsylla globiceps*	Russia	27.3	41/150	1–19	[53]
			6.7	10/150	na	[323]
Class Maxillopoda						
Linguatulidae	*Linguatula serrata*	Romania	1.3	1/73	1 ♂	Our unpublished data

Abbreviation: na not applicable/not available
[a]Animals kept in captivity
[b]A, adults; ♂, male; ♀, female; N, nymphs; L, larvae

Other arthropods

Although relatively common in most wild carnivores and domestic dogs (Mihalca, personal observation), there is only a single report of *Hippobosca longipennis* in golden jackals (Table 7). This species is an important vector for *Acanthocheilonema dracunculoides*, a filarioid widely distributed in canids across Africa [332]. However, this vector-borne nematode was never reported in golden jackals.

Conclusions

This is the first comprehensive checklist summarizing the data on parasites of golden jackals. The large variety of parasites reported in golden jackals is caused by multiple factors, including their large geographical range, their extensive territorial mobility and wide food spectrum. Moreover, like in other carnivores, the predator behaviour of golden jackals is the cause of common records of pseudoparasites. Nevertheless, even in such cases, although these parasites do not infect jackals, they can be spread and can remain infective for their natural hosts. Considering that jackals share their habitats with domestic dogs and a wide variety of wild carnivores across their distribution range and the high similarity with canine parasites [333], the risk of interspecific transmission among canid species, and the continued spread of the species, is likely to be associated with future territorial expanding of different parasitic diseases. The vast majority of parasites recorded in golden jackals are shared with domestic dogs or even domestic cats. Other parasites of jackals can use a wide variety of other domestic species, including livestock, as intermediate hosts. Hence, jackals are an important source of infection for domestic animals and might be directly or indirectly responsible for economic losses. Probably the most important aspect regarding the parasites of golden jackal is the large number and common occurrence of zoonotic parasites. Among these, several are with high public health impact: *Leishmania*, *Echinococcus*, hookworms, *Toxocara*, and *Trichinella*. Our review brings overwhelming evidence on the importance of *Canis aureus* as wild reservoir of human parasites.

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Authors' contributions
CMG collected the information from databases, systematized information in tables and wrote the manuscript. ADM had the initial idea of this review and critically revised the manuscript for important intellectual content. Both authors read and approved the final manuscript.

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References

1. Viranta S, Atickem A, Werdelin L, Stenseth NC. Rediscovering a forgotten canid species. BMC Zoology. 2017;2(1):6.
2. Jhala YV, Moehlman PD. Golden jackal *Canis aureus* Linnaeus, 1758. Least Concern (2004). In: Sillero-Zubiri C, Hoffman M, DW MD, editors. Status Survey and Conservation Action Plan. Canids: Foxes, Wolves, Jackals and Dogs: Gland: IUCN; 2004. p. 156–61.
3. Koepfli KP, Pollinger J, Godinho R, Robinson J, Lea A, Hendricks S, Schweizer RM, et al. Genome-wide evidence reveals that African and Eurasian golden jackals are distinct species. Curr Biol. 2015;25(16):2158–65.
4. Nagy J. Régibb és újabb adatok a nádifarkasok és a sakálok előfordulásairól. Nimród Vadászlap. 1942;30(35):554–6.
5. Wozencraft WC. Order Carnivora. In: Wilson DE, Reeder DM, editors. Mammal species of the world: a taxonomic and geographic reference. Baltimore: Johns Hopkins University Press; 2005. p. 532–629.
6. Khalaf-Sakerfalke VJ, Taher NABA. *Canis aureus palaestina* Khalaf, 2008: a new golden jackal subspecies from the Gaza strip, Palestine. Gazelle Palestinian Biol Bul. 2008;80:1–13.
7. Rueness EK, Asmyhr MG, Sillero-Zubiri C, Macdonald DW, Bekele A, Atickem A, Stenseth NC. The cryptic African wolf: *Canis aureus lupaster* is not a golden jackal and is not endemic to Egypt. PLoS One. 2011;6(1):e16385.
8. Lindblad-Toh K, Wade CM, Mikkelsen TS, Karlsson EK, Jaffe DB, Kamal M, Clamp M, et al. Genome sequence, comparative analysis and haplotype structure of the domestic dog. Nature. 2005;438:803–19.
9. Sommer R, Benecke N. Late-Pleistocene and early Holocene history of the canid fauna of Europe (Canidae). Mamm Biol. 2005;70:227–41.
10. Trouwborst A, Krofel M, Linnell JDC. Legal implications of range expansions in a terrestrial carnivore: the case of the golden jackal (*Canis aureus*) in Europe. Biodivers Conserv. 2015;24:2593–610.
11. Šálek M, Červinka J, Banea OC, Krofel M, Ćirovic´ D, Selanec I, Penezić A, Grill S, Riegert J. Population densities and habitat use of the golden jackal (Canis aureus) in farmlands across the Balkan peninsula. Eur J Wildl Res. 2014;60:193–200.
12. Giannatos G. Conservation action plan for the golden jackal (*Canis aureus* L., 1758) in Greece. Athens:WWF Greece; 2004.
13. Arnold J, Humer A, Heltai M, Murariu D, Spassov N, Hackländer K. Current status and distribution of golden jackals *Canis aureus* in Europe. Mamm Rev. 2012;42:1–11.
14. Kryštufek B, Tvrtkovič N. Range expansion by Dalmatian jackal population in the twentieth century (*Canis aureus* L., 1758). Folia Zool. 1990;39:291–6.
15. Nowak RM, Paradiso JL. Walker's mammals of the world. 4th ed. Baltimore and London: John Hopkins University Press; 1983.
16. Van Lawick H, Van Lawick-Goodall J. Innocent killers. Boston: Houghton Mifflin; 1970.
17. Kingdon J. East African mammals: an atlas of evolution in Africa, volume 3, Part A: Carnivores. Chicago: University of Chicago Press; 1977.
18. Yakobson B, Manalo DL, Bader K, Perl S, Haber A. An epidemiological retrospective study of rabies diagnosis and control in Israel, 1948–1997. Isr J Vet Med. 1998;53:114–26.
19. Shamir M, Yakobson B, Baneth G, King R, Dar-Verker S, Markovics A, Aroch I. Antibodies to selected canine pathogens and infestation with intestinal helminths in golden jackals (*Canis aureus*) in Israel. Vet J. 2001;162(1):66–72.
20. Aguirre AA. Wild canids as sentinels of ecological health: a conservation medicine perspective. Parasit Vectors. 2009;(Suppl. 1):S7.
21. National Center for Biotechnology Information. U.S. National Library of Medicine, Rockville Pike. 2003. https://www.ncbi.nlm.nih.gov/pubmed. Accessed 20 Sept 2016.
22. Science Direct. Amsterdam: Elsevier B.V.; 1997. http://www.sciencedirect.com/. Accessed 1 Aug 2016.
23. Web of Science. Institute for Scientific Information. New York: Thomson Reuters; 1964. http://apps.webofknowledge.com/. Accessed 1 Sept 2016.
24. Helminthological Abstracts. Wallingford: CABI; 1910. http://www.cabi.org/publishing-products/online-information-resources/helminthological-abstracts/. Accessed 27 July 2016.
25. Biological Abstracts. BIOSIS. New York: Thomson Reuters; 1926. https://www.ebscohost.com/academic/biological-abstracts. Accessed 15 Aug 2016
26. BioOne, Washington, DC. 1999. http://www.bioone.org/. Accessed 15 Sept 2016.
27. Host-Parasite Database. London: Natural History Museum; 1922. http://www.nhm.ac.uk/research-curation/scientific-resources/taxonomy-systematics/host-parasites/database/. Accessed 15 July 2016.
28. Scholar G. Bibliographic database. Mountain View: Google; 2004. https://scholar.google.ro/. Accessed 30 Sept 2016
29. Scientific Electronic Library "Kiberleninka", Moscow. 2012. http://cyberleninka.ru. Accessed 1 July 2016.
30. Scientific Library Earth Papers. http://earthpapers.net/. Accessed 30 June 2016.
31. Adl SM, Simpson AG, Lane CE, Lukeš J, Bass D, Bowser SS, Brown MW, et al. The revised classification of eukaryotes. J Eukaryot Microbiol. 2012;59(5):429–93.
32. Gibson DI, Jones A, Bray RA, editors. Keys to the Trematoda, vol. 1. Wallingford: CAB International; 2002.
33. Jones E, Bray RA, Gibson DI, editors. Keys to the Trematoda, vol. 2. Wallingford: CAB International; 2005.
34. Bray RA, Gibson DI, Jones A, editors. Keys to the Trematoda, vol. 3. Wallingford: CAB International; 2008.
35. Kahlil LF, Jones A, Bray RA, editors. Keys to the cestode parasites of vertebrates. Wallingford: CAB International; 1994.
36. Nakao M, Lavikainen A, Iwaki T, Haukisalmi V, Konyaev S, Oku Y, Okamoto M, Ito A. Molecular phylogeny of the genus *Taenia* (Cestoda: Taeniidae): proposals for the resurrection of *Hydatigera* Lamarck, 1816 and the creation of a new genus *Versteria*. Int J Parasitol. 2013;43(6):427–37.
37. De Ley P, Blaxter M. A new system for Nematoda: combining morphological characters with molecular trees, and translating clades into ranks and taxa. In: Cook R, Hunt DJ, editors. Nematology monographs and perspectives, vol. 2. Leiden: E.J. Brill; 2004. p. 633–53.
38. Amin OM. Classification of the Acanthocephala. Folia Parasitol. 2013;60(4):273–305.
39. Roskov Y, Abucay L, Orrell T, Nicolson D, Flann C, Bailly N, Kirk P, Bourgoin T, DeWalt RE, Decock W, De Wever A. (editors) 2016. Species 2000 & ITIS Catalogue of Life, 28th July 2016. Digital resource at www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands ISSN 2405-8858. Accessed 11 August 2016.
40. Rau MAN. Experimental infection of the jackal (*Canis aureus*) with *Piroplasma canis* Prana & Gallivalerio, 1895. A preliminary note. Indian J Med Res. 1926;14:243–4.
41. Mbaya AW, Aliyu MM, Nwosu CO, Ibrahim UI. Captive wild animals as potential reservoirs of haemo- and ectoparasitic infection of man and domestic animals in the arid-region of northeastern Nigeria. Vet Arhiv. 2008;78:429–40.
42. Mitková B, Hrazdilová K, D'Amico G, Duscher GG, Suchentrunk F, Forejtek P, Gherman CM, et al. Eurasian golden jackal as host of canine vector-borne protists. Parasit Vectors. 2017;10(1):183.
43. Maronpot RR, Guindy E. Preliminary study of *Babesia gibsoni* Patton in wild carnivores and domesticated dogs in Egypt. Am J Vet Res. 1970;31:797–9.
44. Patton WS. Preliminary report on a new piroplasm ("*Piroplasma gibsoni*" sp. nov.) found in the blood of hounds of the madras hunt and subsequently

discovered in the blood of the jackal "*Canis aureus*". Bull Soc Pathol Exot. 1910;3:271–81.
45. Kirkova Z, Raychev E, Georgieva D. Studies on feeding habits and parasitological status of red fox, golden jackal, wild cat and stone marten in Sredna Gora, Bulgaria. J Life Sci. 2011;5:264–70.
46. Bhatia BB, Chauhan PPS, Agrawal RD, Ahluwalia SS. *Eimeria aurei* n. sp. from jackal. Indian J Parasitol. 1979;3:49–50.
47. Latchumikanthan A, Vimalraj PG, Gomathinayagam S, Jayathangaraj MG. Concurrent infection of *Nanophyetus* (*Troglotrema*) *salmincola*, *Ancylostoma* sp. and *Isospora* sp. in a captive jackal (*Canis aureus*). J Vet Parasitol. 2012;26(1):87–8.
48. Razmjoo M, Bahrami AM, Shamsollahi M. Seroepidemiological survey of important parasitic infections of wild carnivores. IJABBR. 2014;2(3):783–92.
49. Ilić T, Becskei Z, Petrović T, Polaček V, Ristić B, Milić S, et al. Endoparasitic fauna of red foxes (*Vulpes vulpes*) and golden jackals (*Canis aureus*) in Serbia. Acta Parasitol. 2016;61(2):389–96.
50. Yakimoff WL, Matikaschwili IL, Rastegaieff EF. Zur Frage über die Coccidien der Schakale, *Eimeria dutoiti* n. sp. Arch Protistenkd. 1933;80:177–8.
51. Glebezdin VS. On the fauna of coccidia wild mammals Southwest Turkmenistan. Izvestiya Akademii Nauk Turkmeniskoi SSR Seriya Biologicheskih Nauk. 1978;3:71–8. (In Russian).
52. Dzerzhinsky VA, Musabekov KS. Findings of coccidians of the genus *Isospora* (Coccidiida) in the Central Asian jackal. Parazitologiya. 1985;19(4):318–20. (In Russian).
53. Tulov AV. Parasitocenoses of jackal (*Canis aureus* L.) in ecosystems of the north-west Caucasus. Krasnodar: Kuban State Agrarian University, Dissertation for the Degree of Candidate of Biological Sciences; 2013. (In Russian).
54. Yakimoff WL, Lewkowitsch EN. *Isospora theileri* n. Sp., coccidie de Schakale. Arch Protistenkd. 1932;77:533–7.
55. Maia JP, Alvares F, Boratyński Z, Brito JC, Leite JV, Harris DJ. Molecular assessment of *Hepatozoon* (Apicomplexa: Adeleorina) infections in wild canids and rodents from north Africa, with implications for transmission dynamics across taxonomic groups. J Wildl Dis. 2014;50(4):837–48.
56. Duscher GG, Kübber-Heiss A, Richter B, Suchentrunk F. A golden jackal (*Canis aureus*) from Austria bearing *Hepatozoon canis* - import due to immigration into a non-endemic area? Ticks Tick Borne Dis. 2013;4(1–2):133–7.
57. Duscher G, Ćirović D, Heltai M, Szabo L, Lanszki J, Bošković I, et al. Hepatozoonosis in golden jackals (*Canis aureus*) from southeastern and central Europe: prevalence data from a first molecular screening. Book of Abstracts of First International Jackal Symposium; Oct 13–16, 2014. Veliko Gradiste; 2014. pp. 70–1.
58. Farkas R, Solymosi N, Takács N, Hornyák Á, Hornok S, Nachum-Biala Y, Baneth G. First molecular evidence of *Hepatozoon canis* infection in red foxes and golden jackals from Hungary. Parasit Vectors. 2014;7:303.
59. Takács A, Szabó L, Juhász L, Takács AA, Lanszki J, Takács PT, Heltai M. Data on the parasitological status of golden jackal (*Canis aureus* L., 1758) in Hungary. Acta Vet Hung. 2014;62(1):33–41.
60. Steinman A, Shpigel NY, Mazar S, King R, Baneth G, Savitsky I, Shkap V. Low seroprevalence of antibodies to *Neospora caninum* in wild canids in Israel. Vet Parasitol. 2006;137(1–2):155–8.
61. Mukherjea AK, Krassner SM. A new species of coccidia (protozoa: Sporozoa) of the genus *Isospora* Schneider, 1881, from the jackal, *Canis aureus* Linnaeus. Proc Zool Soc Calcutta. 1965;18:35–40.
62. Levine ND, Tadros W. Named species and hosts of *Sarcocystis* (protozoa: Apicomplexa: Sarcocystidae). Syst Parasitol. 1980;2:41–59.
63. Ghorbani M, Hafizi A, Shegerfcar MT, Rezaian M, Nadim A, Anwar M, Afshar A. Animal toxoplasmosis in Iran. J Trop Med Hyg. 1983;86(2):736.
64. Namroodi S, Yousefi MR, Milanloo D. *Toxoplasma gondii* serosurvey in golden jackals from Golestan province, Iran. IJMCM. 2014;2:446–50.
65. Radhy AM, Khalaf JM, Faraj AA. Some gastro-intestinal protozoa of zoonotic importance observed in captive animals of al- Zawraa zoo in Baghdad. Int J Sci Nat. 2013;4(3):567–70.
66. Beck R, Sprong H, Lucinger S, Pozio E, Cacciò SM. A large survey of Croatian wild mammals for *Giardia duodenalis* reveals a low prevalence and limited zoonotic potential. Vector Borne Zoonotic Dis. 2011;11(8):1049–55.
67. Chatteriee GC, Roy H, Mitra AN. Notes on *Pentatrichomonas canis-auri* n. sp., found in cecal contents of an Indian jackal (*Canis aureus*), its cultivation in vitro and its method of multiplication. J Dept Sc Calcutta Univ. 1926;8:11.
68. Hamidi AN, Nadim A, Edrissian GH, Tahvildar-Bidruni G, Javadian E. Visceral leishmaniasis of jackals and dogs in northern Iran. Trans R Soc Trop Med Hyg. 1982;76(6):756–7.
69. Ćirović D, Chochlakis D, Tomanović S, Sukara R, Penezić A, Tselentis Y, Psaroulaki A. Presence of *Leishmania* and *Brucella* species in the golden jackal *Canis aureus* in Serbia. Biomed Res Int. 2014;2014:728516.
70. Hervás J, Méndez A, Carrasco L, Gómez-Villamandos JC. Pathological study of visceral leishmaniasis in a jackal (*Canis aureus*). Vet Rec. 1996; 139(12):293–5.
71. Khan MAHNA, Khanm SS, Bashu J, Rima UK, Pervin M, Hossain MZ, et al. Visceral leishmaniasis is endemic in golden jackals of Bangladesh agricultural university campus, a threat for expanding future zoonotic visceral leishmaniasis. Bangl J Vet Med. 2012;10(1&2):101–9.
72. Maruashvili GM, Bardzhadze BG. New natural reservoir of *Leishmania donovani* in Georgia. Med Parazitol. 1980;6:77–8. (In Russian).
73. Musabekov KS, Novak MD, Farizova OM. Jackal as a potential source of leishmaniasis in the south-southeast of Kazakhstan. Math KazSSR Ser Biol Nauk. 1987;4:86–7. (In Russian).
74. Nadim A, Navid-Hamidid A, Javadian E, Bidruni GT, Amini H. Present status of kala-azar in Iran. Am J Trop Med Hyg. 1978;27(1):25–8.
75. Jarallah HM. Dissemination of canine visceral leishmaniasis to different organs of jackals experimentally infected with *Leishmania donovani*. Pak Vet J. 2015;35(1):98–100.
76. Bessad A, Mouloua K, Kherrachi I, Benbetka S, Benikhlef R, Mezai G, Harrat Z. *Leishmania infantum* MON-1 isolated from a golden jackal (*Canis aureus*) in Grande Kabylie (Algeria). Bull Soc Pathol Exot. 2012;105(1):5–7. (In French).
77. Babuadze G, Alvar J, Argaw D, de Koning HP, Iosava M, Kekelidze M, Tsertsvadze N, et al. Epidemiology of visceral leishmaniasis in Georgia. PLoS Negl Trop Dis. 2014;8(3):e2725.
78. Mohebali M, Arzamani K, Zarei Z, Akhoundi B, Hajjaran H, Raeghi S, Heidari Z, et al. Canine visceral leishmaniasis in wild canines (fox, jackal, and wolf) in northeastern Iran using parasitological, serological, and molecular methods. J Arthropod-Borne Dis. 2015;10(4):538–45.
79. Niazi AD. Studies in epidemiology and seroepidemiology of visceral leishmaniasis in Iraq. Doctoral thesis. London: London School of Hygiene Tropical Medicine, 1980. doi:10.17037/PUBS.00682256.
80. Baneth G, Dank G, Keren-Kornblatt E, Sekeles E, Adini I, Eisenberger CL, Schnur LF, King R, Jaffe CL. Emergence of visceral leishmaniasis in central Israel. Am J Trop Med Hyg. 1998;59(5):722–5.
81. Talmi-Frank D, Kedem-Vaanunu N, King R, Bar-Gal GK, Edery N, Jaffe CL, Baneth G. Leishmania tropica infection in golden jackals and red foxes, Israel. Emerg Infect Dis. 2010;16(12):1973–5.
82. Lubova VV. Study of Kzyl-Orda foci. Communication 1 and 2. Voprosy Medizinskykh i Biologicheskykh Issledovaniy, Moskva. 1972;49–52 (In Russian).
83. Latyshev NI, Kryukova AP, Povalishina TP. Essays on the regional parasitology of Middle Asia. I. Leishmaniasis in Tajikistan. Materials for the medical geography of Tadjik SSR. (Results of expeditions in 1945–1+7). Problems of Regional, General and Experimental Parasit Med Zoo., Moscow. 1951;7:35–62. (In Russian).
84. Dursunova SM, Ponirovskii EN, Afanasiev NP. Possible role of wild animals in epidemiology of visceral leishmaniasis in Turkmen SSR. Trudy Ashkhabadskogo Inst Epidem i Gig. 1964;6:157–8. (In Russian).
85. Jarallah HM. Experimental innoculation of jackals with *Leishmania major* and their susceptibility by histopathological lesions. Indian Vet J. 2015b;92(1):90–1.
86. Mircean V, Dumitrache MO, Mircean M, Bolfă P, Györke A, Mihalca AD. Autochthonous canine leishmaniasis in Romania: neglected or (re)emerging? Parasit Vectors. 2014;7:135.
87. Espejo LA, Costard S, Zagmutt FJ. Modelling canine leishmaniasis spread to non-endemic areas of Europe. Epidemiol Infect. 2014;27:1–14.
88. Sasaki M, Omobowale O, Tozuka M, Ohta K, Matsuu A, Nottidge HO, et al. Molecular survey of *Babesia canis* in dogs in Nigeria. J Vet Med Sci. 2007; 69(11):1191–3.
89. Alvarado-Rybak M, Solano-Gallego L, Millán J. A review of piroplasmid infections in wild carnivores worldwide: importance for domestic animal health and wildlife conservation. Parasit Vectors. 2016;9(1):538.
90. Baneth G. Perspectives on canine and feline hepatozoonosis. Vet Parasitol. 2011;181(1):3–11.
91. Duszynski DW, Couch L, Upton SJ. Coccidia (Eimeriidae) of Canidae and Felidae. Supported by NSF-PEET DEB 9521687. 2000. http://biology.unm.edu/coccidia/carniv1.html. Accessed 22 Aug 2017.
92. McAllister MM, Dubey JP, Lindsay DS, Jolley WR, Wills RA, McGuire AM. Dogs are definitive hosts of *Neospora caninum*. Int J Parasitol. 1998;28:1473–8.
93. Gondim LF, McAllister MM, Pitt WC, Zemlicka DE. Coyotes (*Canis latrans*) are definitive hosts of *Neospora caninum*. Int J Parasitol. 2004;34:159–61.

94. King JS, Slapeta J, Jenkins DJ, Al-Qassab SE, Ellis JT, Windsor PA. Australian dingoes are definitive hosts of *Neospora caninum*. Int J Parasitol. 2010;40: 945–50.
95. Dubey JP, Jenkins MC, Rajendran C, Miska K, Ferreira LR, Martins J. Gray wolf (*Canis lupus*) is a natural definitive host for *Neospora caninum*. Vet Parasitol. 2011;181:382–7.
96. Gadzhiev IG, Ataev AM, Gazimagomedov MG. Fauna of helminthes of domestic and wild Canidae in the plane zone of Dagestan. Russian J Parasitol. 2010;4:12–5. (In Russian).
97. Atalay MM. The main helminth infections of wild carnivores and principles of prevention in Dagestan offensive. Vet Pathol Moscow. 2010;2(33):5–10. (In Russian).
98. Hajiyev IG. Helminthiases of dogs in the territory of the Terek-Kuma Lowland and improvement of control measures. Thesis for the degree of candidate of veterinary sciences. Makhachkala 2011, pp. 148.
99. Fataliyev GG. Helminthofauna of wild dogs in Azerbaijan and the ways of its formation. Parasitologyia. 2011;45(2):129–39. (In Russian).
100. Ibrahimova RS, Fataliyev GH. Current state of helminthofauna of canids (Canidae) in Azerbaijan. Proc Azerbaijan Natnl Acad Sci Biological Med Sci. 2015;70(1):35–8. (In Azerbaijani).
101. Trifonov T, Meskov S, Stoimenov K. Helminth fauna of the jackal (*Canis aureus*) in the Strandzha Mountains. Vet Med Nauki. 1970;7(6):51–4. (In Bulgarian).
102. Florijančić T, Ozimec S, Bošković I, Degmečić D, Urošević B, Nekvapil N, et al. Survey on sylvatic parasitosis in Podunavlje Region of Croatia. In: Popović Z, Beuković M, Dordević M, Beuković D, editors. Proceedings of International symposium on hunting "Modern aspects of sustainable management of game population". Zemun: University of Belgrade, Faculty of Agriculture; 2012. p. 118–21.
103. Shahba HK. Hookworm and uncinariosis of carnivores. Ivanovo: Thesis for the degree of candidate of veterinary sciences; 2010 (In Russian).
104. Papadopoulos H, Himonas C, Papazahariadou M, Antoniadou-Sotiriadou K. Helminths of foxes and other wild carnivores from rural areas in Greece. J Helminthol. 1997;71(3):227–31.
105. Dalimi A, Mobedi I. Helminth parasites of carnivores in northern Iran. Ann Trop Med Parasitol. 1992;86(4):395–7.
106. Itin GS, Kravchenko VM. Ecological-faunistic characteristics of helminthcoenosis of jackals (Canis aureus) at the Krasnodar Territory. Theory Pract of Parasitic Dis Animals-Biology. 2011;12:224–7. (In Russian).
107. Kruchkova EN. Helminth fauna of carnivores in the European region of Russia. Theory Pract Parasitic Dis Animals. 2012;13:205–8. (In Russian).
108. Ćirović D, Pavlović I, Penezić A, Kulišić Z, Selaković S. Levels of infection of intestinal helminth species in the golden jackal *Canis aureus* from Serbia. J Helminthol. 2015a;89(1):28–33.
109. Shakarboev EB. The trematodes of vertebrates in Uzbekistan (species composition, ways of circulation and ecological-biological peculiarities). Tashkent: Abstract of Doct thesis; 2009 (In Russian).
110. Sadighian A. Helminth parasites of stray dogs and jackals in Shahsavar area, Caspian region. Iran J Parasitol. 1969;55(2):372–4.
111. Dalimi A, Sattari A, Motamedi G. A study on intestinal helminthes of dogs, foxes and jackals in the western part of Iran. Vet Parasitol. 2006;142(1–2):129–33.
112. Chowdhury N. Indian subcontinent. In: Chowdhury N, Alonso Aguirre A, editors. Helminths of wildlife. Enfield: Science Publishers Inc; 2001. p. 287–371.
113. Wells WH, Randal BH. New hosts for trematodes of the genus *Heterophyes* in Egypt. J Parasitol. 1956;42(3):287–92.
114. Rodonaya TE. On the detection of the USSR fluke *Dexiogonimus ciureanus* Witenberg, 1929. Helminthofauna of animals and plants in Georgia: Collection Institute of Zoology, Tbilisi. 1967;95–97 (In Russian).
115. Massoud J, Jalali H, Reza M. Studies on trematodes of the family Heterophyidae (Odhner, 1914) in Iran: 1. Preliminary epidemiological surveys in man and carnivores in Khuzestan. J Helminthol. 1981;55(4):255–60.
116. Lapini L, Molinari P, Dorigo L, Are G, Beraldo P. Reproduction of the golden jackal (*Canis aureus moreoticus* i. Geoffroy Saint Hilaire, 1835) in Julian pre-Alps, with new data on its range-expansion in the high-Adriatic hinterland (Mammalia, Carnivora, Canidae). Boll Mus Civ St Nat Venezia. 2009;60:169–86.
117. Rao BV. Helminth parasites from an Indian jackal (*Canis aureus narid*): *Ancylostoma braziliense* (Gomez 1910) Leiper 1915, *Rictularia affinis* (Jagerskiold, 1904) (Nematoda): and *Spelotrema narii* n. sp. (Trematoda). Ind J Helminthol. 1965;17:6884.
118. Yousuf MA, Bashu J, Pervin M, Islam MT, Das PM, Khan MAHNA. Identifying diseases of golden jackals of Bangladesh Agricultural University campus, Mymensingh, Bangladesh. Bangl J Vet Med. 2014;12(2):217–24.
119. Vimalraj PG, Latchumikanthan A. *Schistosoma spindale* infection in a captive jackal (*Canis aureus*). J Parasit Dis. 2015;39(1):120–1.
120. Roberts L, Janovy J Jr. Foundations of parasitology. 8th ed. New York: McGraw Hill Publishers; 2009.
121. Olsen OW. Animal parasites: their life cycles and ecology. New York: Dover Publications; 1986.
122. Filimonova LV. Experimental study of the biology of *Nanophyetus schikhobalowi* Skryabin & Podyapolskaya, 1931 (Trematoda, Nanophyetidae). Trudy Gel'mintologicheskoi Laboratorii. 1965;15:172–84. (In Russian).
123. Möhl K, Grosse K, Hamedy A, Wüste T, Kabelitz P, Lücker E. Biology of *Alaria* spp. and human exposition risk to *Alaria* mesocercariae - a review. Parasitol Res. 2009;105(1):1–15.
124. Chai JY, Seo BS, Lee SH, Hong SJ, Sohn WM. Human infections by *Heterophyes heterophyes* and *H. dispar* imported from Saudi Arabia. Korean J Parasitol. 1986;24(1):82–6.
125. Uppal B, Wadhwa V. Rare case of *Metagonimus yokogawai*. Indian J Med Microbiol. 2005;23(1):61–2.
126. Cengiz ZT, Yilmaz H, Dulger AC, Cicek M. Human infection with *Dicrocoelium dendriticum* in Turkey. Ann Saudi Med. 2010;30(2):159–61.
127. Khamidullin Rl, Liubina VS, Khamidullin IR, Medinskiĭ BL. Trematodiases in Tataria. Med Parazitol (Moscow). 1991;2:60–1. (In Russian).
128. Dawes B. The Trematoda, with special reference to British and other European forms. Cambridge: Cambridge University Press; 1968.
129. Gorman AM. Studies on the biology of *Plagiorchis elegans* (Rudolphi, 1802), (Trematoda: Digenea) in its mammalian and molluscan hosts. Leeds: PhD Thesis, University of Leeds. 1980.
130. Sitko J, Bizos J, Sherrard-Smith E, Stanton DW, Komorová P, Heneberg P. Integrative taxonomy of European parasitic flatworms of the genus *Metorchis* Looss, 1899 (Trematoda: Opisthorchiidae). Parasitol Int. 2016; 65(3):258–67.
131. Kumar V. Trematode infections and diseases of man and animals. Dordrecht: Springer Science+Business Media; 1999.
132. Gupta V, Parmar S. On two new species of cestodes of the families Hymenolepididae and Dilepididae from mammals of India. Indian J Helminthol. 1989;40(2):165–71.
133. Gawande P, Baviskar B, Umale N, Gandhe A, Baviskar P, Bawaskar S, Maske DK. Survey of gastrointestinal helminths in captive mammals and birds at Maharajbagh Zoo. Nagpur Zoo's Print J. 2010;25(4):21–2.
134. Shaikh H, Huq MM, Karim MJ, Khan MMM. Indicence of helminth parasites of domestic and wild cats and jackals in Bangladesh. Indian J Parasitol. 1982;6(2):245–7.
135. Niphadkar SM, Narsapur VS, Deshpande VS, Nehete RS. Parasitic infections of zoo animals in Bombay. J Bombay Vet Col. 1989;1(1):37–40.
136. Farahnak A, Mobedi I, Mohamadi F. Study of zoonotic helminths of carnivores in Khuzestan, Iran. Iran J Public Health. 1998;27(3–4):15–20.
137. Litvinov VF, Litvinov VP. Helminths of carnivores in East Azerbaijan. Parazitologiya. 1981;15(3):219–23. (In Russian).
138. Sonsino P. Studi e notizie elmintologiche. Atti Soc Tosc Sc Nat. Proc Verb. 1889;6:224–37.
139. Meshgi B, Eslami A, Bahonar AR, Kharrazian-Moghadam M, Gerami-Sadeghian A. Prevalence of parasitic infections in the red fox (*Vulpes vulpes*) and golden jackal (*Canis aureus*) in Iran. Iran J Vet Res. 2009;10/ 4(29):387–91.
140. Lahmar S, Boufana B, Ben Boubaker S, Landolsi F. Intestinal helminths of golden jackals and red foxes from Tunisia. Vet Parasitol. 2014;204(3–4):297–303.
141. Kirkova Z, Georgieva D, Raychev E. Study on the prevalence of trichurosis in different categories of dogs and wild carnivores. Bulg J Vet Med. 2006;9(2): 141–7.
142. Kamenov Y, Kanchev K, Radev V. Study on the helminth infection in canids in North-West Bulgaria. Sofia: Proceedings of Anniversary Conference of Faculty of Veterinary Medicine, University of Forestry; 2009. p. 298–303.
143. Ray DK, Negi SK, Srivastava PS. Occurrence of helminth parasites of zoonotic nature in wildlife in the Tarai. Indian J Anim Res. 1975;92:75–8.
144. Patel PV, Patel AI, Sabapara RH, Sahu RK, Vyas R. Helminthic infection in wild canids in zoological gardens of Gujarat. Zoo's Print J. 2003;18(4):1084.
145. Arbabi M, Doroudgar A, Hooshyar H, Mobedi I. A survey of cestode infections of carnivores in the Kashan region. A survey on carnivore's cestodes contamination in Kashan region. J Vet Res. 2004;59(3):289–93.
146. Hoida G, Greenberg Z, Furth M, Malsha Y, Craig PS, Schantz PM, Sneir R, El-on J. An epidemiological survey of *Echinococcus granulosus* and other helminths in animal populations in northern Israel. J Helminthol. 1998;72(2):127–31.

147. Musabekov KS. [Diseases and parasites of jackal (*Canis aureus* L., 1758) from South Kazakhstan.] Proceedings of the NAS RK, ser. biological and medical. 2008;3:10–13 (In Russian).
148. Chernyshev VI. [On the ecology and parasites of the jackal in Tadzhikistan.] Trudy Akademii Nauk Tadzhikskoy SSR Dushanbe. 1954;21:151 (In Russian).
149. Lahmar S, Boufana BS, Lahmar S, Inoubli S, Guadraoui M, Dhibi M, et al. *Echinococcus* in the wild carnivores and stray dogs of northern Tunisia: the results of a pilot survey. Ann Trop Med Parasitol. 2009;103(4):323–31.
150. Merdivenci A. Yabanıl Hayvanlarda Pazarizolojik Araştırmalar. Izmir: Proceedings of The 6th National Congress of Biology; 1968. pp 81–101.
151. Irgashev IK. [The helminth fauna of domestic and wild carnivores in the Samarkand region.] Uzbekskii Biologicheskii Zhurnal. 1958;5:39–45 (In Russian).
152. Tariannikov T. [Parasites of the jackal (*Canis aureus aureus*) from the region of middle Syr Daria.] Parazitologiya. 1983;17(6):478–480 (In Russian).
153. Baer JG. The origin of human tapeworms. J Parasitol. 1940;26(2):127–34.
154. Turner JA. Human dipylidiasis (dog tapeworm infection) in the United States. J Pediatr. 1962;61:763–8.
155. Acha PN, Szyfres B. Zoonoses and communicable diseases common to man and animals. Volume 3. Parasitoses. 3rd ed. Washington DC: PAHO (Pan American Health Organization [PAHO]). Scientific and Technical Publication No. 580; 2003.
156. Dhaliwal BBS, Juyal PD. Parasitic zoonoses. New Delhi: Springer; 2013.
157. Deplazes P, Rinaldi L, Alvarez Rojas CA, Torgerson PR, Harandi MF, Romig T, et al. Global distribution of alveolar and cystic echinococcosis. Adv Parasitol. 2017;95:315–493.
158. Romig T, Bilger B, Mackenstedt U. Current spread and epidemiology of *Echinococcus multilocularis*. Dtsch Tierarztl Wochenschr. 1999;106(8):352–7. (In German)
159. Bacigalupo J. Sobre una nueva especie de *Taenia, Taenia infantis*. Semana Med. 1922;26:726.
160. Morishita K, Sawada I. On tapeworms of the genus *Multiceps* hitherto unrecorded from man. Jap J Parasitol. 1966;15:495–501.
161. Spassky AA, Spasskaia LP, Reznik VN. On the biological polyvalence of *Hydatigera taeniaeformis* and its occurrence in man. Med Parazitol Mosk. 1968;37(3):339–43. (In Russian).
162. Sterba J, Barus V. First record of *Strobilocercus fasciolaris* (Taeniidae-larvae) in man. Folia Parasitol. 1976;23:221–6.
163. Hoberg EP, Ebinger W, Render JA. Fatal cysticercosis by *Taenia crassiceps* (Cyclophyllidea: Taeniidae) in a presumed immunocompromised canine host. J Parasitol. 1999;85:1174–8.
164. Slais J. Befunde von fruhen Entwicklungsstadien des *Cysticercus* in der Liber des Menschen. Zbl allg Path path Anat. 1965;108:316–21.
165. Sadykov VM. [Epizootiological and epidemiological significance of *Cysticercus ovis*.] Sb Rab Gel'mintologii Izdatel'stvo "Kolos". 1971;344–350 (In Russian).
166. Beugnet E, Gevrey J, Messouak A. The *Cysticercus ovis* cysticercosis: a non zoonotic muscular cysticercosis. Rev Med Vet. 1996;147(7):547–52.
167. Ing MB, Schantz PM, Turner JA. Human coenurosis in North America: case reports and review. Clinic Infect Dis. 1998;27:519–23.
168. Nguyen MT, Gabriël S, Abatih EN, Dorny P. A systematic review on the global occurrence of *Taenia hydatigena* in pigs and cattle. Vet Parasitol. 2016;226:97–103.
169. Heyneman D. Cestodes. In: Baron S, editor. Medical Microbiology. 4th ed. Galveston: University of Texas Medical Branch at Galveston; 1996. Chapter 89.
170. Yoon KC, Seo MS, Park SW, Park YG. Eyelid sparganosis. Am J Ophthalmol. 2004;2004(138):5–873.
171. Cho JH, Lee KB, Yong TS, Kim BS, Park HB, Ryu KN. Subcutaneous and musculoskeletal sparganosis: imaging characteristics and pathologic correlation. Skelet Radiol. 2000;29:402–8.
172. Dick TA, Nelson PA, Choudhury A. Diphyllobothriasis: update on human cases, foci, patterns and sources of human infections and future considerations. Southeast Asian J Trop Med Public Health. 2001;32(2):59–76.
173. Bowman DB, Hendrix CM, Lindsay DS, Barr SC. Feline clinical parasitology. Ames: Iowa State University Press; 2002.
174. Borkowski J, Zalewski A, Manor R. Diet composition of golden jackals in Israel. Ann Zool Fennici. 2011;48:108–18.
175. Nadeem MS, Naz R, Shah SI, Beg MA. Season- and locality-related changes in the diet of Asiatic jackal (*Canis aureus*) in Potohar, Pakistan. Turk J Zool. 2012;36(6):798–805.
176. Radović A, Kovačić D. Diet composition of the golden jackal (*Canis aureus* L.) on the Peljeac Peninsula, Dalmatia, Croatia. Period Biol. 2010;112(2):219–24.
177. Babaev Y. Data on the helminth fauna of wild mammals in the Karakumskii Canal zone. Izvestiya Akademii Nauk Turkmenskoi SSR. Biol Nauk. 1976;4:68–74.
178. Zariffard MR. Study on helminth parasites of carnivores of East Azerbaijan Province of Iran with focus on *Echinococcus multilocularis* and its importance in public health. Tehran: University of Tehran, Doctor of Philosophy dissertation; 1994.
179. Arbabi M, Droudgar A, Houshyar H, Mobedi E. Prevalence of *Macracanthorhynchus hirudinaceus* in Caniidae in Kashaniran. Vet J (Tehran). 2002;15(2):55–7.
180. Nabavi R, Hajinezhad M, Jamshidian A. Detection of *Pachysentis canicola* (Acanthocephala: Oligacanthorhynchida) in Iranian golden jackal, Sistan, Iran. IJAPBS. 2015;4(8):70–2.
181. Elchuev MS. A first finding of *Centrorhynchus itatsinis* Fukui, 1929 in a jackal in the Azerbaijan SSR. Izvestiya Akademii Nauk Azerbaïdzhanskoï SSR. Biologicheskikh Nauk. 1986;6:47–51. (In Russian).
182. Varadharajan A, Pythal C. Parasites of wildlife - I. A preliminary investigation on the parasites of wild animals at the zoological Farden, Thiruvananthapuram, Kerala. Zoo's Print J. 1999;14(3–12):159–64.
183. Varadharajan A, Kandasamy A. A survey of gastro-intestinal parasites of wild animals in captivity in the V.O.C. Park and mini zoo, Coimbatore. Zoo's Print J. 2000;15(5):257–8.
184. Ghoke SS, Naikwade BS, Thorat KS, Jogdand NK, Kalaskar PS. Incidence of helminthic infection in captive carnivores of Sidhharth municipal zoo, Aurangabad, Maharashtra. Zoos Print J. 2012;27(3):25–6.
185. Javaregowda AK. Studies on prevalence of endo-parasitic infection in wild carnivores maintained under captive state. J Parasit Dis. 2016;40(4):1155–8.
186. Myers BJ, Kuntz RE, Wells WH. Helminth parasites of reptiles, birds, and mammals in Egypt: VII. Check list of nematodes collected from 1948 to 1955. Can J Zool. 1962;40(4):531–8.
187. Baylis MA. Sewell RBS, editor. The fauna of British India, including Ceylon and Burma. In: Nematoda. Vol. II. (Filarioidea, Dioctophymoidea and Trichinelloidea). London: Taylor and Francis; 1939.
188. Gupta NK, Kalia DC. Remarks on two already known nematode parasites from an Indian jackal. Res Bull Panjab Univ Sci. 1988;39(34):227–31.
189. Nashiruddullah N, Chakraborty A. Parasites of captive wild carnivores of Assam state zoo. Intas Polivet. 2001;2:173–81.
190. Singh T, Gupta MP, Singla LD, Singh N, Sharma DR. Prevalance and chemotherapy of gastrointestinal helminthic infections in wild carnivores of Mahendra Choudhury Zoological Park, Punjab. J Vet Parasitol. 2006;20(1):17–23.
191. Gupta NK, Kalia DC. Two new species of *Ancylostoma* (Nematoda) along with a key to the species of the genus possessing three pairs of ventral teeth. Rev Iber Parasitol. 1984;44(4):337–46.
192. Barus V, Kullmann E, Tenora F. Parasitische nematoden aus wirbeltieren Afghanistans. Acta Sci Nat Brno. 1972;6:1–46.
193. Yanchev Y. Morphology, taxonomy and distribution of species of *Uncinaria* (Frölich, 1789) from carnivores in Bulgaria. Khelmintologiya. 1986;22:55–66.
194. Merdivenci A, Buyurman Ü. Turkiye'de çakalda Uncinariasis olgusu. Turk Biol Derg. 1966;15(1–2):52–9.
195. Movsessian SO, Manassian JS, Hovhannissian RL. Specific composition of animal helminths in the Yerevan Zoo. Academy of Sciences of Armenian SSR. Institute of Zoology. Zoological Papers. 1987;21:81–97. (In Russian).
196. Pradhan S, Sharma D, Subba B, Chettri V. Preliminary investigation on the parasites of mammals at Padmaja Naidu Himalayan Zoological Park, Darjeeling. Zoo's Print J. 2011;26(8):11–3.
197. Thawait VK, Maiti SK, Dixit AA. Prevalence of gastro-intestinal parasites in captive wild animals of Nandan Van Zoo, Raipur, Chhattisgarh. Vet World. 2014;7(7):448–51.
198. Kalia DG, Nayital AK. A new subspecies record of *Kalicephalus* Molin, 1861 (Nematoda: Diaphanocephaloidea) from jackal; hitherto a cosmopolitan parasite of snakes. Indian J Parasitol. 1989;13:135–8.
199. Guilhon J. Transmission d'*Angiostrongylus vasorum* (Baillet, 1866) aux canides sauvages. Compt Rend Hebd Seanc Acad Sci Paris. 1965;261(21):4496–7.
200. Panayotova-Pencheva M, Trifonova A, Mirchev R, Movsesyan S. Diversity and morphometric data of blood filarial larvae in carnivorous from Bulgaria. Rossiĭskiĭ Parazitologicheskiĭ Zhurnal. 2014;1:14–23. (In Russian).
201. Nelson GS. *Dipetalonema reconditum* (Grassi, 1889) from the dog with a note on its development in the flea, *Ctenocephalides felis* and the louse, *Heterodoxus spiniger*. J Helminthol. 1962;36(03):297–308.
202. Kirkova Z., Ivanov A., Georgieva D. Dirofilariosis in dogs and wild carnivores in Bulgaria. In: Genchi C, Rinaldi L, Cringoli G, editors. Mappe Parassitologiche 8 "*Dirofilaria immitis* and *D. repens* in dog and cat and human Infections". Zagreb: Rolando Editore; 2007. p. 204.

203. Panayotova-Pencheva MS, Mirchev RL, Trifonova AP. Dirofilaria immitis infection in carnivores from Bulgaria: 2012–2013 update. Bulg J Vet Med. 2016;19(2):153–62.
204. Diakou A, Migli D, Spiridakis G. *Dirofilaria immitis* (heartworm) in a golden jackal (*Canis aureus*) in Greece. In: Poulakakis N, Antoniou A, Karameta E, Psonis N, Vardinoyannis K, editors. Book of abstracts, 13th International Congress on the Zoogeography and Ecology of Greece and Adjacent Regions, University of Crete. Irakleio Hellenic Zoological Society; 2015 p. 27.
205. Tolnai Z, Széll Z, Sproch Á, Szeredi L, Sréter T. *Dirofilaria immitis*: an emerging parasite in dogs, red foxes and golden jackals in Hungary. Vet Parasitol. 2014; 203(3–4):339–42.
206. Rao AT, Acharjyo LN. Incidence of heartworm in captive wild carnivores. Indian J Parasitol. 1993;17:201–2.
207. Meshgi B, Eslami A, Helan JA. Epidemiological survey of blood filariae in rural and urban dogs of Tabriz. J Fac Vet Med Tehran Univ. 2002;57(4):59–63. (In Persian).
208. Heidari Z, Kia EB, Arzamani K, Sharifdini M, Mobedi I, Zarei Z, Kamranrashani B. Morphological and molecular identification of *Dirofilaria immitis* from jackal (*Canis aureus*) in North Khorasan, northeast Iran. J Vector Borne Dis. 2015;52(4):329–33.
209. Ionică AM, Matei IA, D'Amico G, Daskalaki AA, Juránková J, Ionescu DT, et al. Role of golden jackals (*Canis aureus*) as natural reservoirs of *Dirofilaria* spp. in Romania. Parasit Vectors. 2016;9(1):240.
210. Penezić A, Selaković S, Pavlović I, Ćirović D. First findings and prevalence of adult heartworms (*Dirofilaria immitis*) in wild carnivores from Serbia. Parasitol Res. 2014;113(9):3281–5.
211. Agrawal RD, Ahluwalia SS, Chauhan PPS. Occurrence of aortic spirocercosis in jackal. Indian J Anim Sci. 1986;56:402–3.
212. Islam S, Nashiruddullah N. *Spirocerca lupi* in a wild jackal (*Canis aureus*) from Assam. J Vet Parasitol. 2000;14(2):127128.
213. Acharya SK. Incidence of helminth parasites in indigenous dogs and jackals with special reference to hookworms. Indian Vet J. 1939;16:7–9.
214. Varadharajan A, Pythal C, Subramanian H. Investigation on the prevalence of helminth parasites of wild mammals in the Thrissur Zoo, Kerala. Cheiron. 2001;B:12–15.
215. Mihalca AD, Ionică AM, D'Amico G, Daskalaki AA, Deak G, Matei IA, et al. *Thelazia callipaeda* in wild carnivores from Romania: new host and geographical records. Parasit Vectors. 2016;9(1):350.
216. Sadighian A, Amini F. *Dioctophyma renale* (Goeze, 1782) stiles, 1901 in stray dogs and jackals in Shahsavar area, Caspian region. Iran J Parasitol. 1967;53(5):961.
217. Asatrian AM. Spread and specific composition of *Trichinella* in Armenia. Academy of Sciences of Armenian SSR. Institute of Zoology. Zoological Papers. 1987;21:34–40. (In Russian).
218. Bessonov AS. Trichinellosis in the former USSR. Epidemic situation (1988–1992). In: Campbell WC, Pozio E, Bruschi F, editors. Trichinellosis. Rome: Istituto Superiore di Sanità Press; 1994. p. 505–10.
219. Sadikhov IA, Elchuev MS. [The situation for trichinosis in Sheki-Zagatala region of Azerbaijan.] Proceedings of the 7th All-Russia Conf for trichinosis, Moscow. 1996. p. 72–73 (In Russian).
220. Guenov G, Boeva V, Georgieva M, Bankov D, Stoimenov K. Organization and results of trichinellosis control in Bulgaria. In: Kim CW, Ruitenberg EJ, Teppema JS, editors. Proceedings of the fifth international conference on Trichinellosis. Surrey: Reedbooks; 1981. p. 387–9.
221. Georgieva D, Koinarski VT, Ivanov AI, Prelesov PN, Kirkova ZT. Role of wild carnivores in the epizootology and epidemiology of trichinellosis. Bulg J Vet Med. 2000;3(4):199–204.
222. Zelyazkov P, Todev I, Ivanov L, Mirchev R, Lalkovski N. Epizootological studies on trichinelosis (*Trichinella* sp.) among wild carnivores in Bulgaria. In: Proceedings. Traditional and contemporary veterinary medicine, Bulgaria, 2009. Sofia: Lesotekhnicheski Universitet; 2009. p. 335–40. (In Bulgarian).
223. Marian I, Mihalca AD, Gherman CM. Prevalence of *Trichinella* spp. infection in large wild carnivore species from Romania between Jan 2014 and July 2015. Bulletin UASVM Vet Med. 2015;72(2):438–40.
224. Sapunov AY, Andryushchenko VG. Proceedings of the 6th Scientific Conference on Trichinellosis. In: Spreading of trichinellosis among domestic and wild animals in Krasnodar territory. Moscow: Kirov; 1992. p. 177–9.
225. Ozeretskovskaya NN, Mikhailova LG, Sabgaida TP, Dovgalev AS. New trends and clinical patterns of human trichinellosis in Russia at the beginning of the XXI century. Vet Parasitol. 2005;132:167–71.
226. Kushnarev JV. [Epizootology, epidemiology and improvement measures against trichinosis in the Republic of North Ossetia-Alania.] Abstract of the Dissertation for the degree of candidate of Biological Sciences, All-Russian Scientific Research Institute of Helminthology "KN Skryabiia", Moscow; 2007 (In Russian).
227. Odoevskaia IM, Kurnosova OP, Klinkov AV, Bocharova MM. Biological properties of the isolate of *Trichinella* spp. from a jackal in the North Caucasian Region. Med Parazitol (Moscow). 2009;4(3):326. (In Russian).
228. Komardin HK, Didkovskaya LF. Trichinosis in Tajikistan. Health Tajikistan. 1985;6:46–9. (In Russian).
229. Boonthanom P, Nawarat A. The outbreaks of trichinosis at Amphur Mae Sarialng. Bull Pub Health. 1963;33:301–8.
230. Doege TC, Thienprasit P, Headington JT, Pongprot B, Tarawanich S. Trichinosis and raw bear meat in Thailand. Lancet. 1969;1(7592):459–61.
231. Bessonov AS. Epizoology and epidemiology of trichinellosis in the USSR: Prospects for eradication of the infection. In: Kim CW, editor. Trichinellosis. New York: Intext; 1974. p. 557–62.
232. Rukavina J, Brglez J. Trichinellosis of some species of wild animals in Yugoslavia. Wiad Parazytol. 1970;16(1):79.
233. Nezri M, Ruer J, De Bruyne A, Cohen-Valensi R, Pozio E, Dupouy-Camet J. First report of a human case of trichinellosis due to *Trichinella britovi* after jackal (*Canis aureus*) meat consumption in Algeria. Bull Soc Pathol Exot. 2006;99(2):94–5. (In French).
234. Shaikenov BS, Boev SN. Distribution of *Trichinella* species in the Old World. Wiad Parazytol. 1983;29:595–608.
235. Mirjalali H, Rezaei S, Pozio E, Naddaf SR, Salahi-Moghaddam A, Kia EB, et al. *Trichinella britovi* in the jackal *Canis aureus* from south-west Iran. J Helminthol. 2014;88(4):385–8.
236. Deksne G, Segliņa Z, Jahundoviča I, Esīte Z, Bakasejevs E, Bagrade G, et al. High prevalence of *Trichinella* spp. in sylvatic carnivore mammals of Latvia. Vet Parasitol. 2016;231:118–23.
237. Blaga R, Gherman C, Seucom D, Cozma V, Boireau P. First identification of *Trichinella* sp. in golden jackal (*Canis aureus*) in Romania. J Wildl Dis. 2008; 44(2):457–9.
238. Živojinović MŽ. 2013. Epizootiological, serological and molecular investigtions of *Trichinella* species. Belgrade: University of Belgrade, Faculty of Veterinary Medicine. PhD thesis; 2013. doi:10.2298/BG20131225ZIVOJINOVIC. (In Serbian).
239. Živojinović M, Sofronic-Milosavljevic L, Cvetkovic J, Pozio E, Interisano M, Plavsic B, et al. *Trichinella* infections in different host species of an endemic district of Serbia. Vet Parasitol. 2013;194(2–4):136–8.
240. Ćirović D, Teodorović V, Vasilev D, Marković M, Ćosić N, Dimitrijević M, et al. A large-scale study of the *Trichinella* genus in the golden jackal (*Canis aureus*) population in Serbia. Vet Parasitol. 2015b;212(3–4):253–6.
241. Merkushev AV. 100 years of the research on trichinosis in the USSR. Zool Zhurnal. 1965;11(4):229–31. (In Russian).
242. Massoud J, Mahdavi M. Characterization of northern and southern isolates of *Trichinella* in Iran. Iran J Publ Health. 1987;16(1–4):91–100.
243. Kullmann E. Über den ersten Nachweis von *Trichinella spiralis* (Owen) in Afghanistan. Z Parasitenkd. 1965;25:393–8.
244. Feizullaev NA, Litvinov VP, Litvinov VF. *Trichinella spiralis* in predatory mammals at the Kyzyl-Agach reserve. Doklady Akademii Nauk Azerbaidzhanskoi SSR. 1977;33(2):61–2. (In Russian).
245. Matov K, Varadinov A, Genov T. On the distribution of *Trichinella spiralis* (Owen, 1835) in domestic and wild carnivores, rodents and insectivores in Bulgaria. Izvestiya na Tsentralnata Khelmintologichna Laboratoriya. 1960;5: 61–5. (In Bulgarian).
246. Kurashvili BE, Rodonaya TE, Matsaberdize GV, Gurchiani KR, Savvateeva IA, Dzhaparidze LA, Petriashvili LI. Trichinelliasis of animals in the Georgian SSR. Parazitologicheskii Sbornik, Tbilisi. 1971;2:19–48. (In Georgian).
247. Mobedi I, Arfaa F, Madadi H, Movafagh K. Sylvatic focus of trichiniasis in the Caspian region, northern Iran. Am J Trop Med Hyg. 1973;22(6):720–2.
248. Sadighian A, Arfaa F, Movafagh K. *Trichinella spiralis* in carnivores and rodents in Isfahan. J Parasitol. 1973;59(6):986.
249. Hamidi AN, Mobedi I. Sylvatic focus of trichiniasis in Bandar Abbas area south of Iran. Iran J Public Health. 1977;6(1):30–3.
250. Massoud J. Trichinellosis in carnivores in Iran. In: Kim CW, Pawlowski ZS, editors. Trichinellosis. Hanover: University Press of New England; 1978. p. 551–4.
251. Hamidi AN. Trichiniasis among the animals in north eastern Iran, 1969, 1976, 1977. Bull Soc Pathol Exot. 1979;72(3):254–7.
252. Gretillat S. Epidemiologie de la trichinellose sauvage au Senegal. Wiad Parazytol. 1970;16(1):109–10.

253. Cvetkovic J, Teodorovic V, Marucci G, Vasilev D, Vasilev S, Cirovic D, Sofronic-Milosavljevic L. First report of *Trichinella britovi* in Serbia. Acta Parasitol. 2011;56(2):232–5.
254. Petrović J, Pušić I, Apić J, Milanov D, Grgić Ž, Đorđević V, Matekalo-Šverak V. Sylvatic trichinosis - role of wild animals in cycle of spread of trichinosis in Serbia. Vet Glas. 2012;66(3–4):175–83. (In Serbian).
255. Petrović J, Grgić Ž, Živkov-Baloš M. Molecular diagnostics of *Trichinella* species: new data on *Trichinella* life cycle in Vojvodina region. In: Lilić S, Đorđević V, editors. Proceedings of the 57th International Meat Industry Conference: Meat and meat products - perspectives of sustainable production. Belgrade: Institute of Meat Hygiene and Technology; 2013. p. 152–7.
256. Fassbender CP, Mayer P. Uber die Verteilung von *Trichinella spiralis* in der Muskulatur einiger nordafrikanischer Carnivoren. Dtsch Tierarztl Wochenschr. 1974;81:284–7.
257. Anderson RC. Nematode parasites of vertebrates. Their development and transmission, 2nd Ed. New York: CABI Publishing; 2000.
258. Sprent JFA. Notes on *Ascaris* and *Toxascaris*, with a definition of *Baylisascaris* gen. nov. Parasitology. 1968;58(1):185–98.
259. Despommier D. Toxocariasis: clinical aspects, epidemiology, medical ecology, and molecular aspects. Clin Microbiol Rev. 2003;16(2):265–72.
260. Marquardt WC, Demaree RS Jr, Grieve RB. Parasitology and vector biology. 2nd ed. San Diego: Academic Press; 2000.
261. Razmi GHR. Survey of dogs' parasites in Khorasan Razavi Province, Iran. Iran J Parasitol. 2009;4(4):48–54.
262. Rokni MB. The present status of human helminthic diseases in Iran. Ann Trop Med Parasitol. 2008;102(4):283–95.
263. Traversa D, Frangipane di Regalbono A, Di Cesare A, La Torre F, Drake J, Pietrobelli M. Environmental contamination by canine geohelminths. Parasit Vectors. 2014;7:67.
264. Moskvina TV, Ermolenko AV. Helminth infections in domestic dogs from Russia. Vet World. 2016;9(11):1248–58.
265. Popiołek M, Jarnecki H, Łuczyński T. The first record of *Molineus patens* (Dujardin, 1845) (Nematoda, Molineidae) in the ermine (*Mustela erminea* L.) in Poland. Wiad Parazytol. 2009;55(4):433–5.
266. Addison EM, Fraser GA. Life cycle of *Crenosoma petrowi* (Nematoda: Metastrongyloidea) from black bears (*Ursus americanus*). Can J Zool. 1994;72(2):300–2.
267. Ribas A, Milazzo C, Foronda P, Casanova JC. New data on helminths of stone marten, *Martes foina* (Carnivora, Mustelidae), in Italy. Helminthologia. 2004; 41(1):59–61.
268. Merrill JR, Otis J, Logan WD Jr, Davis MB. The dog heartworm (*Dirofilaria immitis*) in man. An epidemic pending or in progress? JAMA 1980;243(10):1066–8.
269. Pampiglione S, Rivasi F. Human dirofilariasis due to *Dirofilaria* (*Nochtiella*) *repens*: an update of world literature from 1995 to 2000. Parassitologia. 2000;42:231–54.
270. Huynh T, Thean J, Maini R. *Dipetalonema reconditum* in the human eye. Br J Ophthalmol. 2001;85:1384.
271. Simón F, Siles-Lucas M, Morchón R, González-Miguel J, Mellado I, Carretón E, Montoya-Alonso JA. Human and animal dirofilariasis: the emergence of a zoonotic mosaic. Clin Microbiol Rev. 2012;25(3):507–44.
272. Burns RP, Helzerman R, Patrick M, Gerhardt N, Beaver PC. Intraocular filiariasis (a motion picture). Trans Sect Ophthalmol Am Acad Ophthalmol Otolaryngol. 1975;79(5):745–8.
273. Beaver PC, Meyer EA, Jarroll EL, Rosenquist RC. *Dipetalonema* from the eye of a man in Oregon, USA. A case report. Am J Trop Med Hyg. 1980;29(3): 369–72.
274. Otranto D, Cantacessi C, Dantas-Torres F, Brianti E, Pfeffer M, Genchi C, et al. The role of wild canids and felids in spreading parasites to dogs and cats in Europe. Part II: helminths and arthropods. Vet Parasitol. 2015;213(1–2):24–37.
275. Campbell B, Little MD. Identification of the eggs of a nematode (*Eucoleus boehmi*) from the nasal mucosa of north American dogs. J Am Vet Med Assoc. 1991;198:1520–3.
276. Cross JH. Intestinal capillariasis. Clin Microbiol Rev. 1992;5(2):120–9.
277. Lalosević D, Lalosević V, Klem I, Stanojev-Jovanović D, Pozio E. Pulmonary capillariasis miming bronchial carcinoma. Am J Trop Med Hyg. 2008;78(1):14–6.
278. Guiliano DB, Oksov Y. *Trichinella* and the nurse cell. In: Schaible UE, Haas A, editors. Intracellular niches of microbes: a pathogens guide through the host cell. Weinheim: Wiley-Blackwell; 2009.
279. Beaver PC, Jung RC, Cupp EW. Clinical parasitology. 9th ed. Philadelphia: Lea and Febiger; 1984.
280. World Health Organization: Dracunculiasis (guinea-worm disease). http://www.who.int/mediacentre/factsheets/fs359/en/ (Updated January 2017). Accessed 10 April 2017.
281. Genis DE. New cases of detection of *Dracunculus medinensis* L. 1758 in domestic animals (cats and dogs) in Kazakhstan. Med Parazitol (Moscow). 1972;41(3):365. (In Russian).
282. Cairncross S, Muller R, Zagaria N. Dracunculiasis (Guinea worm disease) and the eradication initiative. Clin Microbiol Rev. 2002;15(2):223–46.
283. Waikagul J, Diaz Chamacho SP. Gnathostomiasis. In: Murrell KD, Fried B, editors. Food-borne parasitic zoonoses: fish and plant-borne parasites. New York: Springer; 2007. p. 235–61.
284. Ignjatovic I, Stojkovic I, Kutlesic C, Tasic S. Infestation of the human kidney with *Dioctophyma renale*. Urol Int. 2003;70(1):70–3.
285. Traub RJ, Robertson ID, Irwin P, Mencke N, Thompson RC. The role of dogs in transmission of gastrointestinal parasites in a remote tea-growing community in northeastern India. Am J Trop Med Hyg. 2002;67(5):539–45.
286. Radev V, Lalkovski N, Zhelyazkov P, Kostova T, Sabev P, Nedelchev N, Vassileva R. Prevalence of gastrointestinal parasites and *Dirofilaria* spp. in stray dogs from some regions in Bulgaria. Bulg J Vet Med. 2016; 19(1):57–62.
287. Berkovitz A, Waner T, King R, Perl S. Concurrent parasitation with *Sarcoptes* and *Demodex* in a golden jackal. Israel J Vet Med. 2009;64(1):10–1.
288. Mitchell RM. A list of ectoparasites from nepalese mammals, collected during the Nepal ectoparasite program. J Med Entomol. 1979;16(3):227–33.
289. Sharif M. A revision of the Indian Ixodidae with special reference to the collection in the Indian museum. Rec Indian Museum. 1928;30:217–344.
290. Morel PC. Tiques d'animaux sauvages en Haute-Volta. Rev Elev Med Vet Pays Trop. 1978;31(1):69–78.
291. Camicas JL. Contribution a l'étude des tiques du Sénégal (Acarina, Ixodoidea). I. Les larves d'*Amblyomma* Koch et de *Hyalomma* Koch. Acarologia. 1970;12(1):71–102.
292. Stojanov I, Pušić I, Pavlović I, Prodanov Radulović J, Kapetanov M, Ratajac R. Findings of ticks in some species of wild carnivores. In: Mihajla D, editor. Proceedings of International Symposium on Hunting: Modern aspects of sustainable management of game population. Novi Sad: Visio Mundi Academic Media Group; 2014. p. 154–8.
293. Hornok S, Fuente J, Horváth G, Fernández de Mera IG, Wijnveld M, Tánczos B, et al. Molecular evidence of *Ehrlichia canis* and *Rickettsia massiliae* in ixodid ticks of carnivores from South Hungary. Acta Vet Hung. 2013;61(1): 42–50.
294. D'Amico G, Dumitrache MO, Matei IA, Ionică AM, Gherman CM, Sándor AD, et al. Ixodid ticks parasitizing wild carnivores in Romania. Exp Appl Acarol. 2017;71(2):139–49.
295. Razmjoo M, Bahrami AM, Hosseini E. Ectoparasitic species from red fox and jackal in western of Iran. Glob Vet. 2013a;10(6):626–9.
296. Razmjoo M, Bahrami AM, Hosseini E. Infestation diagnosis of ectoparasitism in red fox and jackals in south-west of Iran. Adv Biores. 2013b;4(3):123–6.
297. Shubber HWK, Al-Hassani NA, Mohammad MK. Ixodid ticks diversity in the middle and south of Iraq. Int J Recent Sci Res. 2014;5(9):1518–23.
298. Feldman-Muhsam B. A note on East Mediterranean species of the *Haemophysalis*. Bull Res Counc Isr. 1951;1:96–107.
299. Keysary A, Eremeeva ME, Leitner M, Din AB, Wikswo ME, Mumcuoglu KY, et al. Spotted fever group rickettsiae in ticks collected from wild animals in Israel. Am J Trop Med Hyg. 2011;85(5):919–23.
300. Kaul HN, Dhanda V, Mishra AC. A survey of ixodid ticks in Orissa state, India. Indian J Anim Sci. 1979;49:707–12.
301. Hoogstraal H. Identity, hosts and distribution of *Haemaphysalis* (*Rhipistoma*) *canestrinii* (Supino) (resurrected), the postulated Asian progenitor of the African Leachi complex (Ixodoidea: Ixodidae). J Parasitol. 1971;57(1):161–72.
302. Geevarghese G, Fernandes S, Kulkarni SM. A checklist of Indian ticks (Acari: Ixodoidea). Indian J Anim Sci. 1997;67(5):566–74.
303. Rebello MJ, Reuben R. A report of ticks collected from birds and small mammals in North Arcot and Chittoor districts, South India. J Bombay Natural Hist Soc. 1966;63(2):283–9.
304. Hoogstraal H, Trapido H. *Haemaphysalis kutchensis* sp. n., a common larval and nymphal parasite of birds in northwestern India (Ixodoidea, Ixodidae). J Parasitol. 1963;49(3):489–97.
305. Hoogstraal H. Notes on African *Haemaphysalis* ticks. IV. Description of Egyptian populations of the yellow dog-tick, *H. leachii leachii* (Audouin, 1827) (Ixodoidea, Ixodidae). J Parasitol. 1958;44(5):548–58.

306. Mitchell CJ, Hoogstraal H, Schaller GB, Spillet J. Ectoparasites from mammals in Kanha National Park, Madhya Pradesh, India, and their potential disease relationships. J Med Ent. 1966;3(2):113–24.
307. Hoogstraal H. Identity, distribution, and hosts of *Haemaphysalis* (*Rhipistoma*) *indica* Warburton (resurrected) (Ixodoidea: Ixodidae), a carnivore parasite of the Indian subregion. J Parasitol. 1970;56(5):1013–22.
308. Galli VB. Notes de parasitologie et de technique parasitologique. Zbl Bakteriol. 1909;51:538–45.
309. El Kammah KM, Hoogstraal H, Camicas JL. Notes on African *Haemaphysalis* ticks: XI. *H.* (*Rhipistoma*) *paraleachi* (Ixodoidea: Ixodidae) distribution and hosts of adults. Int J Acarol. 1992;18(3):205–12.
310. Dias Travassos Santos JA. Contribuição para o conhecimento da fauna ixodológica da Índia portuguesa. An Inst Med Trop. 1954;11(2):361–439.
311. Theodor O, Costa M. A survey of the parasites of wild mammals and birds in Israel. Part one. Ectoparasites. The Israel Academy of Sciences and Humanities: Jerusalem; 1967.
312. Mihalca AD, Dumitrache MO, Magdaş C, Gherman CM, Domşa C, Mircean V, et al. Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution. Exp Appl Acarol. 2012;58: 183–206.
313. Abusalimov NS. [Cattle, pigs, wild deer and jackals as hosts of *Hyalomma aegyptium* Linne 1758.] Doklady Akademii Nauk Azerbaijan SSR. 1958;14: 543–545 (In Russian).
314. Abbassian-Lintzen R. A preliminary list of ticks (Acariña: Ixodoidea) occurring in Iran and their distributional data. Acarologia. 1960;2(1):43–61.
315. Hoogstraal H, Clifford CM, Saito Y, Keirans JE. *Ixodes* (*Partipalpiger*) *ovatus* Neumann, subgen. Nov.: identity, hosts, ecology, and distribution (Ixodoidea: Ixodidae). J Med Entomol. 1973;10(2):157–64.
316. Dumitrache MO, Gherman CM, Cozma V, Mircean V, Györke A, Sándor AD, Mihalca AD. Hard ticks (Ixodidae) in Romania: surveillance, host associations, and possible risks for tick-borne diseases. Parasitol Res. 2012;110(5):2067–70.
317. Leulmi H, Aouadi A, Bitam I, Bessas A, Benakhla A, Raoult D, Parola P. Detection of *Bartonella tamiae*, *Coxiella burnetii* and rickettsiae in arthropods and tissues from wild and domestic animals in northeastern Algeria. Parasit Vectors. 2016;9:27.
318. Bursali A, Keskin A, Tekin S. A review of the ticks (Acari: Ixodida) of Turkey: species diversity, hosts and geographical distribution. Exp Appl Acarol. 2012; 57:91–104.
319. Aneurin LE. Some tick investigations in Kenya Colony. Parasitology. 1932; 24(2):175–82.
320. Hubbard CA. Some ticks from Iraq. Ent News. 1955;66(7):189–91.
321. Emerson KC, Price RD. A host-parasite list of the Mallophaga on mammals. Miscell Publ Entomol Soc America Washington. 1981;12:1–72.
322. Plomley NJB. Notes on the systematics of two species of *Heterodoxus* (Mallophaga, Boopidae). Pap Proc Roy Soc Tasmania. 1939;1940:19–26.
323. Tulov AV, Zverjanovskii MI, Zabashta SN. [Associations of consorts in populations of flea at common jackal (*Canis aureus* L.) in the conditions of Krasnodar Region.] Current Issues in Veterinary Biology. 2013;1(17): 31 (In Russian).
324. Pajot FX. Les poux (Insecta, Anoplura) de la région afrotropicale. Paris: IRD. Collection Faune et Flore tropicales; 2000.
325. Lewis RE. Siphonaptera collected during the 1965 Street Expedition to Afghanistan. Fieldiana Zool. 1973;64:1–161.
326. Farhang-Azad A. The flea fauna of Iran. II. A collection of fleas from Esfahan (Central Iran). Ann Mag Nat Hist. 1966;13(9:103–105):343–6.
327. Haeselbarth E, Segerman J, Zumpt Fritz KE. The arthropod parasites of vertebrates in Africa south of the Sahara (Ethiopian region). Volume III (Insecta excl. Phthiraptera). Publ South African Inst Med Res. 1966;13(52):1–283.
328. Kulkarni SM, Bhat HR, Dhanda V. A survey of haematophagous arthropods in western Himalayas, Sikkim and hill districts of West Bengal fleas (Siphonaptera). Indian J Med Res. 1974;62(7):1061–88.
329. Dumitrache MO, Kiss B, Dantas-Torres F, Latrofa MS, D'Amico G, Sándor AD, Mihalca AD. Seasonal dynamics of *Rhipicephalus rossicus* attacking domestic dogs from the steppic region of southeastern Romania. Parasit Vectors. 2014;7:97.
330. Földvári G, Farkas R. Ixodid tick species attaching to dogs in Hungary. Vet Parasitol. 2005;129(1–2):125–31.
331. Walker JB, Keirans JE, Horak IG. The genus *Rhipicephalus* (Acari, Ixodidae): a guide to the brown ticks of the world. New York: Cambridge University Press; 2000.
332. Albrechtová K, Sedlák K, Petrželková KJ, Hlaváč J, Mihalca AD, Lesingirian A, et al. Occurrence of filaria in domestic dogs of Samburu pastoralists in northern Kenya and its associations with canine distemper. Vet Parasitol. 2011;182(2–4):230–8.
333. Taylor MA, Coop RL, Wall RL. Veterinary parasitology. 3rd ed. Oxford: Blackwell Publishing; 2007.
334. Nabavi R, Manouchehri Naeini K, Zebardast N, Hashemi H. Epidemiological study of gastrointestinal helminthes of canids in Chaharmahal and Bakhtiari Province of Iran. Iran J Parasitol. 2014;9(2):276–81.
335. Loos-Frank B. Cestodes of the genus *Mesocestoides* (Mesocestoididae) from carnivores in Israel. Israel J Zool. 1990;37(1):3–13.
336. Zare-Bidaki M, Mobedi I, Sadeghieh Ahari S, Habibizadeh S, Naddaf SR, Siavashi MR. Prevalence of zoonotic intestinal helminths of canids in Moghan plain, northwestern Iran. Iran J Parasitol. 2010;5(2):42–51.
337. Nama HS. Comparative study of the cestodes *Mesocestoides carnivoricolus* and *M. lineatus*. Biology. 1981;3(3):31–4.
338. Gasarov MI, Plieva AM. Helminth fauna of carnivores caught at the territory of the Republic of Ingushetia. Theor Pract Parasit Dis Anim. 2010;11:112–6. (In Russian).
339. Zverzhanovsky MI, Basova NY, Tulov AV. Parasitocenosis of jackals (*Canis aureus* L.) with participation of *Dirofilaria immitis* (Leidy, 1856) in trophic-epizootoloical chains of premountain zone of the Krasnodar Territory. Theor Pract Parasit Dis Animals - Biology. 2011;12:212–5 (In Russian).
340. Mimioğlu MM, Güralp N, Tolgay N, Sayn F. Ankara civarnda tilkilerde (*Vulpes vulpes*) bulduğumuz helmintler. Ankara Univ Vet Fak Derg. 1965;12:164–90.
341. Kornyushin VV, Malyshko (Varodi) EI, Malega AM. The helminths of wild predatory mammals of Ukraine. Cestodes Vestn Zool. 2011;45(6):e1–8.
342. Sadykhov IA. A new cestode species - *Mesocestoides petrowi* nov. sp. - from the intestine of fox (*Vulpes vulpes*). Sb Rab Gel'mintol. 1971:351–3. (In Russian).
343. Chertkova AN, Kosupko GA. Cestodes of the genus *Mesocestoides* found in domestic and wild animals in USSR and some principles of their systematics. Trudy Vsesoyuznogo Instituta Gel'mintologii im KI Skryabina Teoreticheskie Problemy Veterinarnoi Gel'mintologii. 1975;22:193–211. (In Russian).
344. Breyer I, Georgieva D, Kurdova R, Gottstein B. *Echinococcus granulosus* strain typing in Bulgaria: the G1 genotype is predominant in intermediate and definitive wild hosts. Parasitol Res. 2004;93(2):127–30.
345. Dissanaike AS, Paramananthan DC. On the occurrence of *Echinococcus granulosus* (Batsch,1786) in a Ceylon jackal. Ceylon Vet J. 1960;8(3/4):82–7.
346. Troncy P, Graber M. L'échinococcose-hydatidose en Afrique centrale. III. - Teniasis des carnivores à *Echinococcus granulosus* (Bats& 1186 - Rudolphi, 1801). Rev Elev Méd Vet Pays Trop. 1969;22(1):75–84.
347. Yandarhanov HS. Species composition, ecological and biological characteristics and communication biocenotic trematodes and cestodes mining of the Chechen Republic. The South of Russia: ecology, development. 2010;3:94–9. (In Russian).
348. Belyaeva AM. The role of wildlife (carnivores, cloven-hoofed animals and rodents) in the epizootology of echinococcosis. Vet Pathol Moscow. 2006; 2(17):117–9.
349. Rao AT, Acharjyo LN. Diagnosis and classification of common diseases of captive animals at Nandankanan zoo in Orissa (India). Indian J Anim Health. 1984;2:147–52.
350. Zariffard MR. A study on helminthic parasites of wild carnivorous of east Azarbaijan with emphasis on *Echinococcus multilocularis*. Tehran: Tehran University of Medical Sciences. PhD Thesis; 1993.
351. Dalimi A, Motamedi G, Hosseini M, Mohammadian B, Malaki H, Ghamari Z, Ghaffari FF. Echinococcosis/hydatidosis in western Iran. Vet Parasitol. 2002;105(2):161–71.
352. Arbabi M, Hooshyar H. Survey of echinococcosis and hydatidosis in Kashan region, central Iran. Iran J Publ Health. 2006;35(1):75–81.
353. Beiromvand M, Akhlaghi L, Fattahi Massom SH, Mobedi I, Meamar AR, Oormazdi H, et al. Detection of *Echinococcus multilocularis* in carnivores in Razavi Khorasan province, Iran using mitochondrial DNA. PLoS Negl Trop Dis. 2011;5(11):e1379.
354. Mobedi I, Zare-Bidaki M, Siavashi MR, Naddaf SR, Kia EB, Mahmoudi M. Differential detection of *Echinococcus* spp. copro-DNA by nested-PCR in domestic and wild definitive hosts in Moghan plain, Iran. Iran J Parasitol. 2013;8(1):107–13.
355. Gholami S, Jahandar H, Abastabar M, Pagheh A, Mobedi I, Sharbatkhori M. *Echinococcus granulosus sensu stricto* in dogs and jackals from Caspian Sea region, northern Iran. Iran J Parasitol. 2016;11(2):186–94.
356. Panceri P. Due fatti relativi ai Cestodi. Rend dell'Acad Sci Ficici e Mat Napoli. 1868;6:32–4.

357. Macpherson CNL, Karstad L. The role of jackals in the transmission of *Echinococcus granulosus* in the Turkana District of Kenya. In: Karstad L, Nestel B, Graham M, editors. Wildlife disease research and economic development. Ottawa: International Development Research Centre; 1980. p. 53–6.
358. Witenberg G. Zur Kenntnis der Verbreitung von Echinokokkus und Trichinen in Palastina. Arch Schiffs- Tropenhyg. 1933;37:37–41.
359. Iqbal Z, Danso P, Hayat CS, Khan MN. Epidemiology of hydatid disease. Echinococcosis in dogs and jackals in Faisalabad (Pakistan). Indian Vet J. 1996;73(6):620–2.
360. Ulyanov SD. A study on the role of wolves and jackals in spreading larval cestodosis animals. Tr NIVI Kazakh Phil Academy of Agricultural Sciences. 1957;9:402–4. (In Russian).
361. Elkanova ZZ. [Epizootological and epidemiological characteristics of foci of echinococcosis in animals and humans in the ecosystem of the Kabardino-Balkarian Republic.] Moscow: Thesis for the degree of candidate of biological sciences; 2010 (In Russian).
362. Bichieva MM, Atabieva JA, Levchenko NV, Bittirov AM, Shikhaliyev MA, Sarbasheva MM. Epizootological features of Echinococcosis in dogs and wild carnivores in the foothills of the North Caucasus. Vet Pathol. (Moscow). 2011;4(38):103–5. (In Russian).
363. Razikov S, Shodmonov IS, Adylov MH. The role of wild animals (carnivorous, artiodactyl and rodents) in epizootology of echinococcosis in Tadjikistan. Russian Parasitol J. 2010;4:59–63. (In Russian).
364. Boufana B, Lahmar S, Rebaï W, Safta ZB, Jebabli L, Ammar A, et al. Genetic variability and haplotypes of *Echinococcus* isolates from Tunisia. T Roy Soc Trop Med H. 2014;108(11):706–14.
365. Széll Z, Marucci G, Pozio E, Sréter T. *Echinococcus multilocularis* and *Trichinella spiralis* in golden jackals (*Canis aureus*) of Hungary. Vet Parasitol. 2013;197(1–2):393–6.
366. Zariffard MR, Massoud J. Study of *Echinococcus granulosus* and *Echinococcus multilocularis* infections in Canidiae in Ardabile province of Iran. Arch Inst RAZI. 1998;48/49:47–52.
367. Lalošević D, Lalošević V, Simin V, Miljević M, Čabrilo B, Bjelić ČO. Spreading of multilocular echinococcosis in southern Europe: the first record in foxes and jackals in Serbia, Vojvodina Province. Eur J Wildl Res. 2016;62(6):793–6.
368. Kairov IK. [A study of multilocular hydatidosis in Karakalpakiya.] Sovremennoe sostoyanie prirodnykh resursov Karakalpakii. 1977;177–189 (In Russian).
369. Acharjyo LN. Helminthiasis in captive wild carnivores and its control in India. Zoo's Print J. 2004;19(7):1540–3.
370. Polishchuk VI, Dolgov VV. *Multiceps* infection in carnivores and coenuriasis in sheep in southern and central Tadzhikistan, USSR. Trudy Nauchnolssledovatel'skogo Veterinarnogo Instituta Tadzhikskoi SSR. 1979;9:78–80. (In Russian).
371. Zhang L, Hu M, Jones A, Allsopp BA, Beveridge I, Schindler AR, Gasser RB. Characterization of *Taenia madoquae* and *Taenia regis* from carnivores in Kenya using genetic markers in nuclear and mitochondrial DNA, and their relationships with other selected taeniids. Mol Cell Probes. 2007;21(5–6):379–85.
372. Shemshadi B, Ranjbar-Bahadori S, Jahani S. Prevalence and intensity of intestinal helminths in carnivores and primates at Vakilabad zoo in Mashhad, Iran. Comp Clin Pathol. 2014;24(2):387–91.
373. Nelson GS, Pester FRN, Rickman R. The significance of wild animals in the transmission of cestodes of medical importance in Kenya. Trans R Soc Trop Med Hyg. 1965;59(6):507–24.
374. Balkizova ZV, Chilayev SS. Hydatid teniasis of carnivores in the ecosystem of the Central Caucasus region. Bull Krasnoyarsk State Agrarian Univ. 2008;2: 179–82. (In Russian).
375. Kamburov P, Georgieva D. Some data on *Taenia ovis* and *Cysticercus ovis* morphology. Helminthologia. 1984;21(3):195–8.
376. Abuladze KI. [Principles of cestodology. Volume IV. Taeniata. Cestodes of animals and man and the diseases caused by them.] Moscow: Izdatel'stvo 'Nauka'; 1964 (In Russian).
377. Nama HS. Cestode parasites of Indian mammals. New Delhi: Scientific Publishers; 1990.

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SHORT COMMUNICATION

Angiostrongylus chabaudi (Biocca, 1957) in wildcat (*Felis silvestris silvestris*, S) from Romania

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Abstract *Angiostrongylus chabaudi* is a rare cardio-pulmonary nematode infecting felids. Although almost 60 years have passed since the original description of the species in Italy, this parasite has been seldom found in domestic and wildcats in southern Europe. The present study aims to report a new case of patent *A. chabaudi* infection in a road-killed wildcat from Maramureş County in Northern Romania. The necropsy revealed the presence of parasites in the pulmonary arteries and the right ventricle, and the fecal examination showed the presence of L1 larvae. Parasites were morphologically and morphometrically characterized as *A. chabaudi*, showing 100 % nucleotide similarity to an *Angiostrongylus* sp. originating from a wildcat from Germany and 99 % to *A. chabaudi* from Italy. This study reports *A. chabaudi* for the first time in Eastern Europe, expanding knowledge about the distribution range of this species.

Keywords *Angiostrongylus chabaudi* · Wildcat · Romania

Introduction

Except for *Dirofilaria immitis* and *Aelurostrongylus abstrusus*, feline cardio-pulmonary nematodes have been for a long time considered minor parasites, though they have recently gained the interest of veterinarians, due to clinical diseases in domestic cats as well as to the increasing number of case reports in new geographic regions (Brianti et al. 2014a; Traversa and Di Cesare 2013). These nematodes belong to several genera included in the superfamilies Metastrongyloidea, Trichuroidea, and Filarioidea.

The superfamily Metastrongyloidea, also known as "lungworms" comprise the genus *Aelurostrongylus*, with its main species, *A. abstrusus*, parasitizing deeply in the lung tissues of domestic cats (*Felis catus*), the typical definitive hosts (Traversa and Di Cesare 2013). The genus *Troglostrongylus* is represented by four species, namely, *T. troglostrongylus*, *T. subcrenatus*, *T. brevior*, and *T. wilsoni*, which have been sporadically found in wild felids around the world, inhabiting the frontal sinuses, trachea, bronchi, and bronchioles (Brianti et al. 2012, 2014a). The genus *Oslerus*, with the most common species *O. rostratus*, is localized in peribronchial tissues and in the lung parenchyma in wild felid species and in domestic cats (Brianti et al. 2014b). The superfamily Trichuroidea is represented by *Eucoleus aerophilus*, which has a low host specificity, parasitizing the upper respiratory tract of wild and domestic carnivores worldwide (Traversa et al. 2009). Finally, *Dirofilaria immitis* (Filarioidea), the heartworm, parasitizes the heart and pulmonary blood vessels and has a significant clinical importance as it can cause serious life-threatening conditions in domestic and wild felids across the world (McCall et al. 2008). The genus *Angiostrongylus* (Metastrongyloidea) includes 21 known species, affecting various species of mammals, including felids and canids (Spratt 2015). Three species infect domestic and wild felids: *A. felineus*, recently described in the eyra cat, *Puma* (*Herpailurus*) *yagouaroundi*, in Brazil (Vieira et al. 2013); *A. vasorum*, common in dogs, while the cat is considered a permissive host (Dias et al. 2008); and

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A. chabaudi in domestic and wildcats in Europe (Biocca 1957; Varcasia et al. 2014; Diakou et al. 2015). Additionally, sequences of an *Angiostrongylus* sp. recovered from the pulmonary artery of a wildcat in Germany have recently become available in the GenBank [KM409651.1 and KM216825.1].

A. chabaudi is a rare cardio-pulmonary nematode localizing in the right heart and pulmonary arteries, initially recorded in wildcats (*Felis silvestris*) from the forests of the provinces of Rome and Grosseto, Italy (Biocca 1957). It has a rather limited geographical distribution, being identified in domestic cats in Italy (Varcasia et al. 2014; Traversa et al. 2015) and more recently in wildcats from Greece (Diakou et al. 2015; Spratt 2015). The life cycle is unknown (Varcasia et al. 2014).

There are many uncertainties regarding the geographical distribution range, host spectrum, and pathogenicity of angiostrongylids in felids. Due to all these gaps in the current scientific knowledge, it is pivotal to add new data regarding the infection caused by this nematode. In this context, the present paper reports the first case of patent *A. chabaudi* infection in a wildcat in Romania, emphasizing the existence of sylvatic foci throughout Europe.

Materials and methods

Sample origin and collection

A road-killed, young female wildcat (*F. silvestris*) was collected in the area of omcuta Mare village, Maramureş County (47.413595° N, 23.405743° E, 474 m altitude). Based on morphological and morphometric characteristics (Krüger et al. 2009), the animal was identified as a pure European wildcat (*F. s. silvestris*). During the necropsy, 12 slender and roundworms were collected from the right ventricle of the heart ($n = 1$) and pulmonary artery ($n = 11$) (Fig. 1). Parasites were temporarily mounted on microscope slides in saline, examined, photographed, and measured under an optical microscope (Olympus BX51; Soft Imaging solution GMBH LG20, Munster, Germany). The classical Baermann method (Willcox and Coura 1989) was performed on the feces, and the metastrongyloid first-stage larvae (L1) were collected. The morphological and morphometric characteristics of adults and larvae were analyzed, eight parameters being compared to other reports of angiostrongylid parasites in felids.

Molecular analysis and species identification

Genomic DNA was extracted from an adult female using a commercial kit (Isolate II Genomic DNA Kit, Bioline, UK) according to the manufacturer's instructions. PCR reactions were performed to amplify a partial mitochondrial *cytochrome c oxidase* subunit 1 (*cox* 1, ~700 bp) gene and the internal transcribed spacer 2 (ITS2, ~500 bp) of the rRNA gene, using primers and protocols available in literature (Gasser et al. 1993; Caldeira et al. 2003). Amplicons were purified using a commercial kit (QIAquick PCR Purification Kit, QIAGEN) and sequenced through an external service (performed by Macrogen Europe, Amsterdam). The sequences were compared to those available in the GenBank® by Basic Local Alignment Search Tool (BLAST) analysis.

Species identification was based on morphological characteristics, correlated with molecular analysis (Biocca 1957; Costa et al. 2003; Varcasia et al. 2014).

Results

Morphology and morphometry of parasites

Three male and nine female specimens were collected from the pulmonary arteries. The anterior end in both sexes was tapered, with a slightly dilated cuticle appearing as a small cephalic vesicle (Fig. 2). The mouth opening was annular, being surrounded by six labial papillae originally called perityles (Biocca 1957). The posterior end of females was curved, and a small trilobated copulatory bursa was observed in males. The cuticle showed transverse striation, more visible at the posterior extremity and flattened to the anterior end.

The females had a specific barber-pole appearance due to their brownish (blood-filled) intestinal tract that is entwined around the pale uterus. Their size ranged from 18.9- to 23.8-mm length and 0.249- to 0.293-mm width in the mid body. The distance between the nerve ring and cephalic end varied between 265.2 and 297.8 μm. The excretory pore was localized at 396.1–450.9-μm distance from the anterior end. The rhabditiform esophagus was composed of an elongated posterior bulb, followed by an isthmus and a cylindrical corpus, delimitation between these three esophageal components being weakly noticeable. Its size ranged from 313.7 to 355.1 μm. The distances between different posterior structures specific to females, namely from the vulva to the anus, vulva to the caudal end, and anus to the caudal end, ranged as follows: 138.7 to 148.5 μm, 200.3 to 222.9 μm, and 58.6 to 71.7 μm, respectively.

The males had a uniformly colored body with sizes ranging between 14.8- and 15.2-mm length and 0.197 and 0.219-mm width. The distances between the nerve ring and cephalic end varied from 149.2 to 178.8 μm, while the excretory pore was located at 305.1–344.5 μm from the anterior end. The esophagus length ranged between 313.7 and 355.1 μm. The copulatory bursa was small, with two symmetrical lateral lobes and an underdeveloped dorsal lobe. Lateral rays showed a common base, the externolateral being thicker and distant from the mediolateral and posterolateral which are thinner and closer (Fig. 3). The spicules were thin and had unequal lengths; the shorter ranged between 500.2 and 551.0 μm and the longer

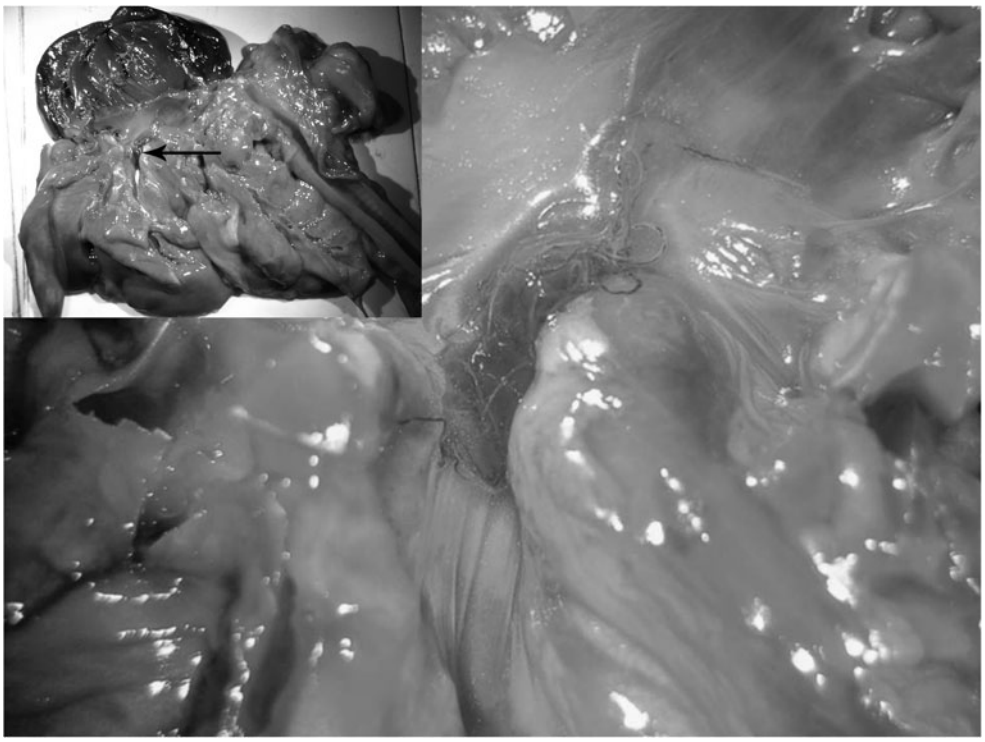

Fig. 1 Location of *A. chabaudi* in the pulmonary arteries (*black arrow*)

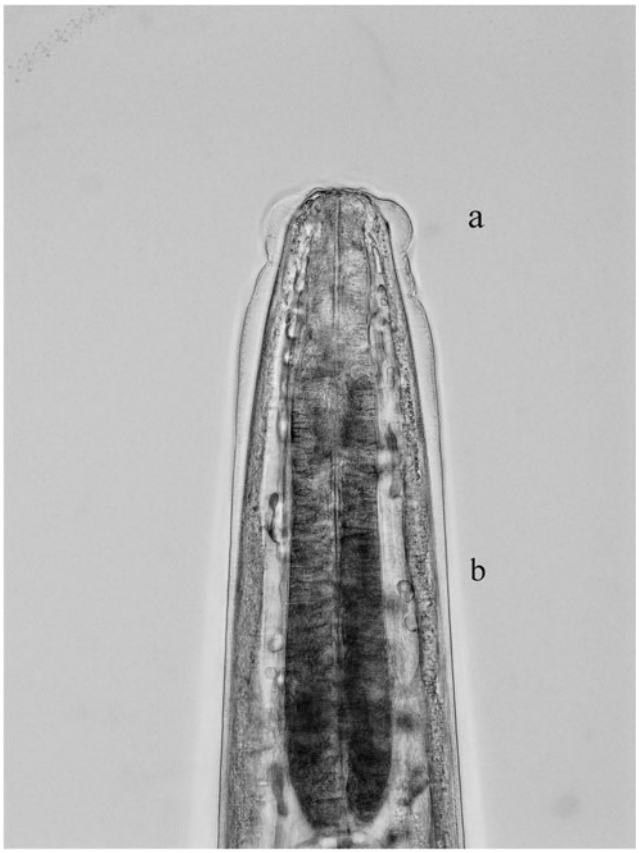

Fig. 2 Anterior end of *A. chabaudi*: (*a*) small cephalic vesicle; (*b*) esophagus

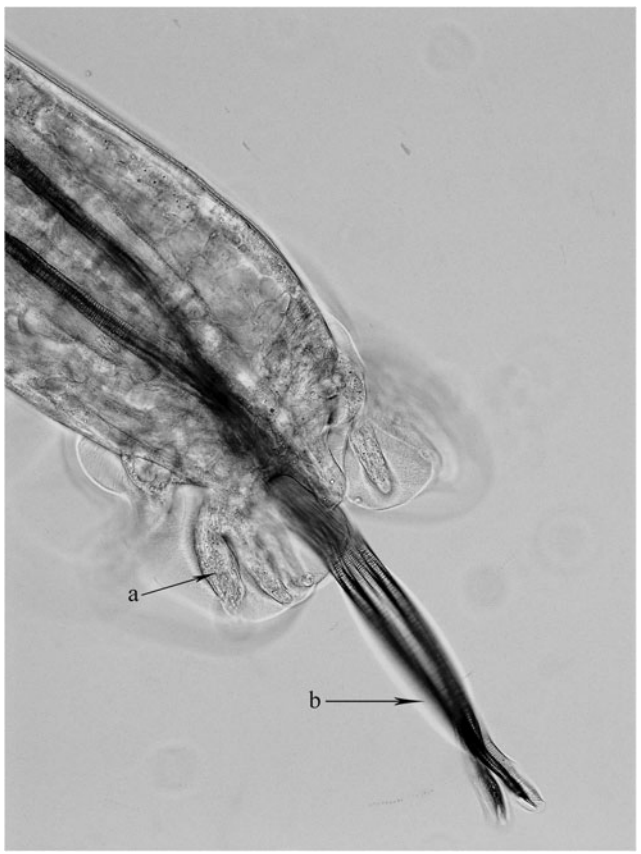

Fig. 3 Copulatory bursa of *A. chabaudi*: (*a*) lateral rays; (*b*) spicules

from 502.35 to 595.74 μm. The spicules showed transverse striations and the tip was corrugated, ending with a yellowish hyaline apex.

The larvae isolated from the feces measured 307.0–419.7 μm in length and 15.3–17.2 μm in width and had a typical metastrongyloid posterior end with a wavy tail and a subterminal spine separated by a notch (Fig. 4).

The morphometry of the isolated parasites was similar to that of *A. chabaudi* recorded in the original description and was compared to the measurements provided by Biocca (1957), Varcasia et al. (2014), Traversa et al. (2015), and Diakou et al. (2015) (Table 1—*A. chabaudi* min/max represent the limits recorded in all reports).

Molecular analysis

The BLAST analysis of ITS2 sequence (accession number KU521522) revealed a 100 % nucleotide similarity to an *Angiostrongylus* sp. recovered from a wildcat from Germany (accession number KM216825) and was 99 % similar to an *A. chabaudi* sequence (accession number KM009115) originated from a domestic cat from Italy. The *cox* 1 gene sequence (accession number KU521521) was 99 % similar to the same *Angiostrongylus* sp. from Germany (accession number KM409651) and showed similarities of 88–90 % to other species of *Angiostrongylus* (e.g., *A. vasorum*—GQ982844; *A. cantonensis*—LK950095; *A. costaricensis*—KR827449).

Discussion

Cardio-pulmonary nematodes of carnivores have been considered for a long time of minor importance, being *A. vasorum* the most common species of the genus, which is diagnosed in domestic and wild canids throughout Europe. Scientific data on the distribution and host range of *A. chabaudi* are minimal, and since its first report in wildcats from Roma and Grosseto provinces in Italy (Biocca 1957), it was diagnosed three times, in a road-killed domestic cat (Varcasia et al. 2014), in a stray cat from Italy (Traversa et al. 2015), and in a road-killed wildcat from Greece (Diakou et al. 2015). Another report of *Angiostrongylus* sp., showing a 99 to 100 % molecular similarity to *A. chabaudi* from Greece and Italy, was recorded in a wildcat from Germany, but this nematode had an undefined morphological similarity to *Angiostrongylus gubernaculatus*, a parasite of mustelids, originally described in North America (Steeb et al. 2014; Dougherty 1946).

The molecular analysis clustered the nematode into the genus *Angiostrongylus*. Indeed, in the present case, both the *cox* 1 and ITS2 sequences were mostly similar to the ones registered in Germany while morphometric data fully supports its conspecificity with *A. chabaudi*. However, the small number of *A. chabaudi* sequences recorded in the GenBank (*cox* 1: accession KM068059; ITS2: accession KM009115) and their relatively short length prevent a thorough molecular analysis. Under these circumstances, only morphometric data can provide the basis for certain species differentiation among the *Angiostrongylus* genus. Morphological parameters of angiostrongylid species reported in carnivores, in Europe, have been herein compared (Table 1). In addition, *A. gubernaculatus*, identified in American badger (*Taxidea taxus*) and striped skunk (*Mephitis mephitis*) in North America, was also considered due to its likely occurrence in wildcat collected from Germany (Steeb et al. 2014). However, a large variability in body length and width (Table 1) was found in adult *Angiostrongylus* species, therefore indicating

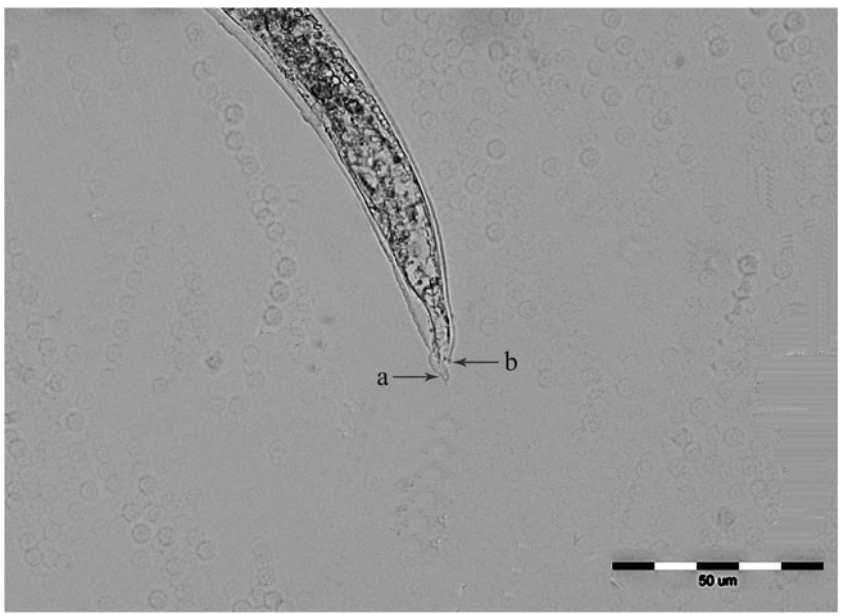

Fig. 4 Posterior end of *A. chabaudi* L1 larva: (*a*) wavy tail; (*b*) subterminal spine

Table 1 Comparative morphometry of adult *Angiostrongylus* species

Species	Present specimens	*A. chabaudi* (Biocca 1957)	*A. chabaudi* (Varcasia et al. 2014)	*A. chabaudi* (Traversa et al. 2015)	*A. chabaudi* (Diakou et al. 2015)	*A. chabaudi* min/max	*A. vasorum* (Costa et al. 2003)	*Angiostrongylus* sp. – fox (Gerrikagoitia et al. 2010)	*Angiostrongylus* sp. – badger (Gerrikagoitia et al. 2010)	*A. daskalovi* (Janchev and Genov 1988)	*A. gubernaculatus* (Dougherty 1946)
Parameters/sex	♂ ($n = 3$)										
Body length (mm)	14.8–15.2	14.6–16.3		9.0	14–18	9.0–18	12.40	13.90 ± 4.75	19.36 ± 7.69	13.36–21.31	18.0–19.5
Body width (μm)	197–219	185.0–225.0		79	193–200	79–225.0	243	221.40 ± 12.13	243.20 ± 19.63	254–306	300
Distance from the nerve ring to the cephalic end (μm)	149.2–178.8	185–230					191				
Distance from the excretory pore to the cephalic end (μm)	305.1–344.5	335.0–405.0			370	305.1–405.0	373	356.5 ± 34.68	409.5 ± 25.50	386–463	
Esophagus length (μm)	313.7–355.1	300.0–345.0		275.0	330	275.0–355.1	220–275	255.33 ± 13.60	333.30 ± 18.24	336–366	300–355
Spicules length (μm) Shorter	500.2–551.0	510.0–555.0		503.3–512.1	576–610	500.2–610.0	410–485	411.06 ± 17.71	345.57 ± 23.95	336–409	510–560
Longer	502.3–595.7										
Parameters/sex	♀ ($n = 9$)										
Body length (mm)	18.9–23.8	19.8–24.1	16.9	19.0	21–22	16.9–24.1	15.57	18.97 ± 10.21	24.76 ± 13.66	14.39–31.12	22.0–24.0
Body width (μm)	249–293	245.0–298.0	195.8	103.7	260–270	103.7–298.0	268	304.14 ± 14.84	344.52 ± 17.95	340–511	350
Distance from the nerve ring to the cephalic end (μm)	265.1–297.8	210–240					215				
Distance from the excretory pore to the cephalic end (μm)	396.1–450.9	395.0–470.0	361.5	370.0	410–430	361.5–470.0	403	391.86 ± 21.44	447.40 ± 101.72	379–636	
Esophagus length (μm)	323.9–375.1	345.0–380.0	276.6	338.0	360	276.6–380.0	240–280	279.88 ± 20.27	368.45 ± 17.00	356–556	335–350
Distance from the vulva to the anus (μm)	138.7–148.5				135–142	135.0–148.5	141	233.63 ± 32.41	295.15 ± 46.25		
Distance from the vulva to the caudal end (μm)	200.3–222.9	170.0–210.0	160.6	264.1	204–210	160.6–222.9	205	275.66 ± 30.75	366.25 ± 44.17	269–412	205–250
Distance from the anus to the caudal end (μm)	58.6–71.7		43.4	64.9	67.0–72.0	43.4–72.0	67	69.70 ± 2.87	78.82 ± 3.45	76–115	75–90

that they cannot be considered as a criterion for a definitive identification.

The length of the spicules discriminated among species of *Angiostrongylus* identified in carnivores in Europe. It ranges between 503.3 and 610.0 μm in *A. chabaudi*, differentiating this species from *A. vasorum*, *A. daskalovi*, and *Angiostrongylus* sp. isolated from a fox and badger in Spain, whose spicules are significantly smaller (410–485 μm, 336–409 μm, 411.06 ± 17.71 μm, and 345.57 ± 23.95 μm). This data confirms early observation by Biocca (1957), on that *A. chabaudi* can be differentiated from *A. vasorum* due to the length of spicules. Nonetheless, the morphology and morphometry of any nematode are affected by the method of preservation (Boag 1984; Naem et al. 2010).

Even though *A. chabaudi* was first collected from wildcats which, in turn, were considered as the primary host for the parasite (Biocca 1957), recent reports indicated the presence of this species equally in domestic and wildcats (Varcasia et al. 2014; Traversa et al. 2015; Diakou et al. 2015; Veronesi et al. 2016).

The paucity of clinical reports of *A. chabaudi* infection depends on the accuracy of the necropsy and coprological exam. For example, the location of the parasite in the right heart and lung vessels requires their careful examination. Regarding the coprological examination, in spite of its correctness, the lack of a thorough diagnosis of *A. chabaudi* L1 and the similarity with other metastrongyloid L1 (a transparent body and sigmoid tail) have created difficulties in species identification (e.g., the morphological description of *A. chabaudi* L1 has been only recently provided (Diakou et al. 2015). The geographical distribution is also affected by the occurrence of intermediate hosts. Though the life cycle of *A. chabaudi* is not known, it is considered that terrestrial mollusks are involved in the parasite transmission, by the similarity with other cardio-pulmonary nematodes of carnivores (Spratt 2015). However, there are numerous cases in which species belonging to the same genus, parasitizing the same host, with the same location, have completely different life cycles.

Temperature and humidity may represent major drivers for the spread of *A. chabaudi*. For example, survival of *A. vasorum* L1 is reduced to 18 % after 66 h in outdoor conditions (Ferdushy and Hasan 2010), therefore needing to rapidly find an intermediate host soon after their shedding with feces. Therefore, the south European distribution of *A. chabaudi* may be related to favorable environmental temperature and humidity values, which ensure a longer survival of the L1 larvae.

Similarly, the survival of L3 larvae in the intermediate hosts may also be important for the spreading of *A. chabaudi*. For instance, in the case of *A. vasorum,* experimental studies have shown that the survival rate and activity of larvae were greater at lower temperatures, having an optimum value of 15 °C in Iberian slugs *Arion lusitanicus* (Dias and Dos Santos Lima 2012; Ferdushy et al. 2010).

The present study confirms the occurrence of *A. chabaudi* in the wildcat in southeastern Europe and provides an exhaustive analysis of certain morphometric criteria, which allows the identification of *A. chabaudi* under the conditions of uncertain results in molecular identification.

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Competing interests The authors declare that they have no competing interests.

References

Biocca E (1957) *Angiostrongylus chabaudi* n. sp. parassita del cuore e dei vasi polmonari del gatto selvatico (*Felis silvestris*). R Accad Naz Lincei 22:526–532

Boag B (1984) Effect of different concentrations of formalin on the preservation of *Trichostrongylus retortaeformis* (Zeder, 1800) (Nematoda). Proc Helminthol Soc Wash 51(2):331

Brianti E, Gaglio G, Giannetto S, Annoscia G, Latrofa MS, Dantas-Torres F, Traversa D, Otranto D (2012) *Troglostrongylus brevior* and *Troglostrongylus subcrenatus* (Strongylida: Crenosomatidae) as agents of broncho-pulmonary infestation in domestic cats. Parasit Vectors 5:178. doi:10.1186/1756-3305-5-178

Brianti E, Giannetto S, Dantas-Torres F, Otranto D (2014a) Lungworms of the genus *Troglostrongylus* (Strongylida: Crenosomatidae): neglected parasites for domestic cats. Vet Parasitol 202(3–4):104–112. doi:10.1016/j.vetpar.2014.01.019

Brianti E, Gaglio G, Napoli E, Falsone L, Giannelli A, Annoscia G, Varcasia A, Giannetto S, Mazzullo G, Otranto D (2014b) Feline lungworm *Oslerus rostratus* (Strongylida: Filaridae) in Italy: first case report and histopathological findings. Parasitol Res 113(10): 3853–3857. doi:10.1007/s00436-014-4053-z

Caldeira RL, Carvalho OS, Mendonça CL, Graeff-Teixeira C, Silva MC, Ben R, Maurer R, Lima WS, Lenzi HL (2003) Molecular differentiation of *Angiostrongylus costaricensis*, *A. cantonensis*, and *A. vasorum* by polymerase chain reaction-restriction fragment length polymorphism. Mem Inst Oswaldo Cruz 98:1039–1043. doi:10.1590/S0074-02762003000800011

Costa JO, De Araújo Costa HM, Guimarães MP (2003) Redescription of *Angiostrongylus vasorum* (Baillet, 1866) and systematic revision of species assigned to the genera *Angiostrongylus* Kamensky, 1905 and *Angiocaulus* Schulz, 1951. Revue Méd Vét 154:9–16

Diakou A, Psalla D, Migli D, Di Cesare A, Youlatos D, Marcer F, Traversa D (2015) First evidence of the European wildcat (*Felis silvestris silvestris*) as definitive host of *Angiostrongylus chabaudi*. Parasitol Res. doi:10.1007/s00436-015-4860-x

Dias SR, Dos Santos Lima W (2012) Effect of temperature on activity of third-stage larvae of *Angiostrongylus vasorum*. Parasitol Res 110(4): 1327–1330. doi:10.1007/s00436-011-2624-9

Dias SRC, Oliveira EL, Viana MH, Lima WS (2008) Permissivity of the domestic cat (*Felis catus*) to infection by *Angiostrongylus vasorum* (Nematoda: Protostrongylidae). Revue Méd Vét 159(2):87–90

Dougherty EC (1946) The genus *Aelurostrongylus* Cameron, 1927 (Nematoda: Metastrongylidae), and its relatives; with descriptions

of *Parafilaroides*, gen. nov., and *Angiostrongylus gubernaculatus*, sp. nov. Proc Helminthol Soc Wash 13:16–26

Ferdushy T, Hasan MT (2010) Survival of first stage larvae (L1) of *Angiostrongylus vasorum* under various conditions of temperature and humidity. Parasitol Res 107(6):1323–1327. doi:10.1007/s00436-010-2004-x

Ferdushy T, Kapel CM, Webster P, Al-Sabi MN, Grønvold JR (2010) The effect of temperature and host age on the infectivity and development of *Angiostrongylus vasorum* in the slug *Arion lusitanicus*. Parasitol Res 107(1):147–151. doi:10.1007/s00436-010-1850-x

Gasser RB, Chilton NB, Hoste H, Beveridge I (1993) Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. Nucleic Acids Res 21:2525–2526

Gerrikagoitia X, Barral M, Juste RA (2010) *Angiostrongylus* species in wild carnivores in the Iberian Peninsula. Vet Parasitol 174:175–180. doi: 10.1016/j.vetpar.2010.07.015

Janchev J, Genov T (1988) *Angiostrongylus daskalovi* sp. n. (Nematoda: Filaroididae) from Mustelidae in Bulgaria. Helminthologia 25:81–88

Krüger M, Hertwig ST, Jetschke G, Fischer MS (2009) Evaluation of anatomical characters and the question of hybridization with domestic cats in the wildcat population of Thuringia, Germany. J Zool Syst Evol Res 47(3):268–282. doi:10.1111/j.1439-0469.2009.00537.x

McCall JW, Genchi C, Kramer LH, Guerrero J, Venco L (2008) Heartworm disease in animals and humans. Adv Parasitol 66:193–285. doi:10.1016/S0065-308X(08)00204-2

Naem S, Pagan C, Nadler SA (2010) Structural restoration of nematodes and acanthocephalans fixed in high percentage alcohol using DESS solution and rehydration. J Parasitol 96(4):809–811. doi:10.1645/GE-2402.1

Spratt DM (2015) Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: a review. Int J Parasitol Parasites Wildl 4(2):178–189. doi:10.1016/j.ijppaw.2015.02.006

Steeb S, Hirzmann J, Eskens U, Volmer K, Bauer C (2014) Lungenwurm-Infektionen bei der Europäischen Wildkatze (in Deutsch). Kompakt Vet 3:9

Traversa D, Di Cesare A (2013) Feline lungworms: what a dilemma. Trends Parasitol 29(9):423–430. doi:10.1016/j.pt.2013.07.004

Traversa D, Di Cesare A, Milillo P, Iorio R, Otranto D (2009) Infection by *Eucoleus aerophilus* in dogs and cats: is another extra-intestinal parasitic nematode of pets emerging in Italy? Res Vet Sci 87(2):270–272. doi:10.1016/j.rvsc.2009.02.006

Traversa D, Lepri E, Veronesi F, Paoletti B, Simonato G, Diaferia M, Di Cesare A (2015) Metastrongyloid infection by *Aelurostrongylus abstrusus*, *Troglostrongylus brevior*, and *Angiostrongylus chabaudi* in a domestic cat. Int J Parasitol 45:685–690. doi:10.1016/j.ijpara.2015.05.005

Varcasia A, Tamponi C, Brianti E, Cabras PA, Boi R, Pipia AP, Giannelli A, Otranto D, Scala A (2014) *Angiostrongylus chabaudi* Biocca, 1957: a new parasite for domestic cats? Parasit Vectors 7:588. doi:10.1186/s13071-014-0588-1

Veronesi F, Di Cesare A, Lepri E, Morganti G, Vercillo F, Grelli D, Cassini R, Marangi M, Iorio R, Ragni B, Traversa D (2016) Occurrence of lungworms in European wildcats (*Felis silvestris silvestris*) of central Italy. J Wild Dis. doi:10.7589/2015-07-187

Vieira FM, Muniz-Pereira LC, Lima SS, Moraes Neto AHA, Guimãraes EV, Luque JL (2013) A new metastrongyloid EAN species (Nematoda) parasitizing pulmonary arteries of *Puma* (*Herpailurus*) *yagouaroundi* (É. Geoffroy, 1803) (Carnivora: Felidae) from Brazil. J Parasitol 99:327–331. doi:10.1645/GE-3171.1

Willcox HP, Coura JR (1989) A new design of the Baermann, Moraes, Coutinho's technique for the isolation of nematode larva (Article in Portuguese). Mem Inst Oswaldo Cruz 84(4):563–565. doi:10.1590/S0074-02761989000400015

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Parasites & Vectors

RESEARCH Open Access

A rare cardiopulmonary parasite of the European badger, *Meles meles*: first description of the larvae, ultrastructure, pathological changes and molecular identification of *Angiostrongylus daskalovi* Janchev & Genov 1988

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Abstract

Background: *Angiostrongylus daskalovi* is a rare cardiopulmonary nematode infecting badgers. The parasite was described in 1988 and, since then, found only once in mustelids in Europe. The present study aims to report new cases of patent *A. daskalovi* infection in badgers from northern Romania and to provide new information on its ultrastructure, molecular diagnosis, and pathology.

Methods: Eight road-killed or hunted badgers originating from Maramureş and Alba counties in Romania were collected and necropsied. Adults and larvae of cardio-pulmonary nematodes were collected and examined by light and scanning electron microscopy (SEM). Genomic DNA was extracted from adults and first-stage larvae (L1). PCR amplification of the internal transcribed spacer 2 (ITS2, ~500 bp) of the rRNA gene was performed. Amplicons were purified, sequenced, and compared to those available in the GenBank database. Histopathological examination of the lungs was performed and lesions described.

Results: The necropsy revealed the presence of nematodes in the pulmonary arteries of three animals. All parasites were mature adults and the coproscopic examination showed the presence of eggs and L1 larvae in all three positive animals. Light microscopy examination confirmed the morphological and morphometric similarity of parasites to *Angiostrongylus daskalovi*. SEM highlighted the typical angiostrongylid structure of the rays of the copulatory bursa and the anterior extremity, with the presence of six sensory papillae surrounding the mouth opening in which a triangular tooth was visible. The first-stage larva (L1) of *A. daskalovi* is described here for the first time. Histopathological examination of the lungs showed chronic interstitial verminous pneumonia due to the presence of adult parasites. Molecular analysis showed 100 % nucleotide similarity to an *Angiostrongylus* sp. isolate originating from a badger from Spain, tentatively identified as *A. daskalovi*.

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Conclusions: Our study unequivocally demonstrates the presence of *A. daskalovi* in European badgers from Romania, provides the first description of the larvae and reveals new data about the ultrastructure of adult parasites and their pathological impact, contributing to the understanding of the phylogenetic relationships with other congeneric species.

Keywords: *Angiostrongylus daskalovi*, Badger, *Meles meles*, Romania, SEM, Histopathology, Molecular analysis

Background

The family Mustelidae is the richest group within the order Carnivora, comprising five subfamilies: Lutrinae (otters), Melinae (European badgers), Mellivorinae (honey badgers), Taxidiinae (American badgers) and Mustelinae (weasels, tayra, wolverines, martens, polecats) [1]. According to recent multigene phylogenetic analysis, the family is split into four major clades and three monotypic lineages [2]. Among all these mustelids, the European badger (*Meles meles*) is spread throughout Europe and in some parts of the Middle East. It is an opportunistic omnivorous species; its diet includes a broad range of animals and plants. This varied diet exposes the badger to the risk of contamination by a wide variety of cysts, eggs, larvae or intermediate hosts of certain parasites. Of these, cardiopulmonary nematodes represent a particular group, several species being reported in mustelids. The genus *Aelurostrongylus* Cameron, 1927 contains two species that are rarely reported in badgers in Europe: *A. falciformis* Schlegel, 1933 in Italy, Germany, Norway and Great Britain [3–6] and *A. pridhami* Anderson, 1962 in Spain. Two other metastrongyloid species belonging to the genus *Angiostrongylus* Kamensky, 1905 have been recorded in badgers. *Angiostrongylus daskalovi* Janchev & Genov, 1988 was described from the pulmonary arteries of the European badger (*M. meles*) in the north-central region of Bulgaria [7] and more recently in Spain [8]. Additionally, *Angiostrongylus vasorum* (Baillet, 1866) was identified in the cardiopulmonary system of badgers in Switzerland, Italy and Spain [9–12]. Another species, *Angiostrongylus gubernaculatus* Dougherty, 1946, was described from the right ventricle of the Californian badger, *Taxidea taxus neglecta* in California and the California Channel Islands, the United States [13, 14].

Apart from these two genera, other lung nematodes have been reported in European badgers including *Crenosoma* sp., *C. vulpis* (Dujardin, 1845) and *C. melesi* Janchev & Genov, 1988, as well as the trichuroid nematode *Capillaria aerophila* Creplin, 1839 [3, 4, 12, 15, 16]. Cardiopulmonary nematodes have also been reported in other mustelids like the stoat (*Mustela erminea*) and weasel (*Mustela nivalis*) infected with *A. vasorum* [17, 18] and the European pine marten (*Martes martes*) and the beech marten (*Martes foina*) infected with *A. daskalovi* [7].

The European badger is considered the typical host for *A. daskalovi* [7]. This nematode has a poorly known geographical distribution, so far being recorded only in Bulgaria and Spain. Moreover, the life-cycle and the host spectrum are incompletely known; the larvae are unknown and the pathological aspects have never been described. Due to these shortcomings, it is important to add new data regarding the infection caused by this nematode species. In this context, the present paper reports the first cases of patent *A. daskalovi* infection in badgers in Romania, emphasizing the ultrastructure of adult parasites and morphology of the L1 larval stage, molecular characterization, and pathological changes.

Methods

Sample origin and collection

Between February 2015 and April 2016, eight European badgers (*Meles meles* L.) were collected in the counties of Maramureș and Alba, in northern and central Romania (Fig. 1). The animals were either road-killed or hunted. Their carcasses were submitted for pathological and parasitological examination within a few hours after the death of the animals and examined immediately. During the necropsy, all nematodes found in the pulmonary arteries were collected in formalin (for morphological examination) and absolute ethanol (for molecular analysis). The classical Baermann method [19] was performed on the lung tissue and faeces, and the metastrongyloid first-stage larvae (L1) were collected. The morphology and morphometry under light microscopy of adults and larvae and SEM characteristics of adults were analysed, ten parameters being compared to other reports of angiostrongylid parasites in mustelids (Table 1).

Pathology

Full necropsy and histological examination were carried out on all badgers included in this study. Selected samples from the right atrium, pulmonary arteries, pulmonary parenchyma and tracheobronchial lymph nodes were collected for histological analysis. Samples were fixed in 10 % phosphate-buffered formalin for 24 h, routinely processed, embedded in paraffin wax, cut into 4 μm sections, and stained with hematoxylin and eosin (H&E).

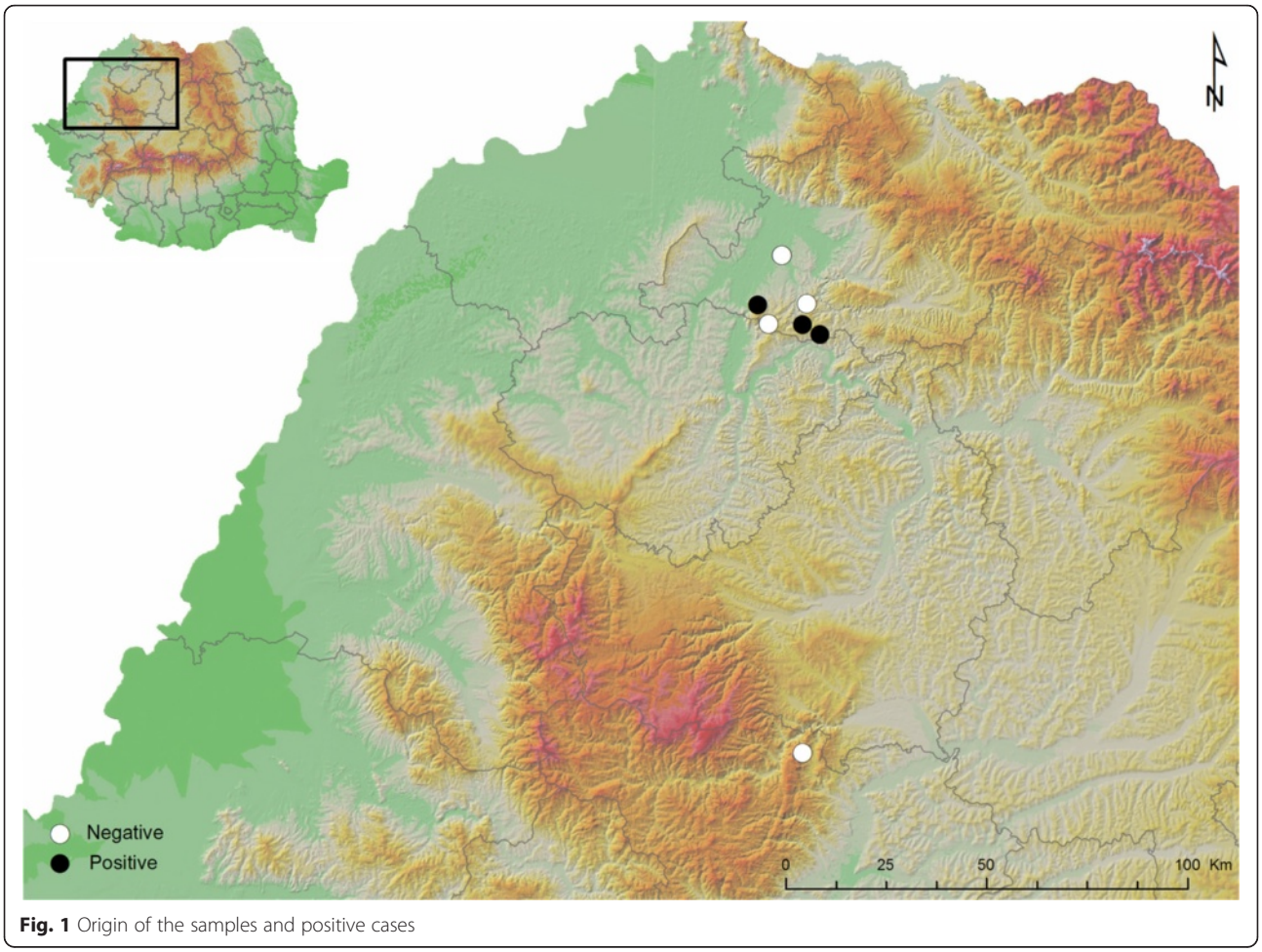

Fig. 1 Origin of the samples and positive cases

Scanning electron and light microscopy

All adult worms were washed in saline, preserved for 24 h in 0.5 % formalin, dehydrated, cleared in lactophenol, mounted in Canada balsam and analyzed by light microscopy using an Olympus BX 61 microscope (Japan). For scanning electron microscopy, some adult parasites were fixed for 2 h at 4 °C in 2.7 % glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) and washed in PBS. Samples were post-fixed for 1 h with 1 % OsO_4. The parasites were dehydrated in an ethanol series (30–100 %), and infiltrated with hexamethyldisilazane, dried, mounted on aluminum stubs coated with a 10 nm gold layer, and examined with a Hitachi SU8230 Scanning Electron Microscope (Japan).

Molecular analyses and species identification

Genomic DNA was extracted from three adult nematodes (one from each positive animal) and 30 L1 stages (a pool of ten from each positive animal) using a commercial kit (Isolate II Genomic DNA Kit, Bioline, London, UK) as stated in the manufacturer's instructions. PCR amplification of the internal transcribed spacer 2 (ITS2, ~500 bp) of the rRNA gene was performed using the NC1/NC2 primer pair as previously described [20]. Amplicons were purified using silica membrane spin columns (QIAquick PCR Purification Kit, Qiagen, Halden, Germany) and externally sequenced by Macrogen Europe (Amsterdam). Sequences were compared to those available in the GenBank database by Basic Local Alignment Search Tool (BLAST) analysis. Phylogenetic analyses were conducted using MEGA6 software [21]. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model [22]. Species identification was based on morphological characteristics, associated with molecular analysis [7, 8].

Results

Morphology and morphometry of *Angiostrongylus daskalovi*

Seven males and 16 females were collected from the pulmonary vessels of the infected badgers (Fig. 2a). Of these, 9 specimens (3 males and 6 females) originated from the first infected badger, two females from the

Table 1 Morphometric features of *A. daskalovi* and comparative data for other *Angiostrongylus* spp. identified in mustelids

Feature/Species		*A. daskalovi*	*A. daskalovi*	*Angiostrongylus* sp. (badger)	*A. vasorum*	*A. gubernaculatus*
Source		Present study	Janchev & Genov [7]	Gerrikagoitia et al. [8]	Costa et al. [23]	Dougherty [13]
Male		(*n* = 7)				
Body length (mm)		15.8–20.5	13.4–21.3	19.4 ± 7.7	12.4	18.0–19.5
Body width (μm)		253–331	254–306	243 ± 20	243	300
Distance from excretory pore to cephalic end (μm)		227–526	386–463	409 ± 25	373	
Oesophagus length (μm)		312–335	336–366	333 ± 18	220–275	300–355
Spicule length (μm)	shorter	322–352	336–409	346 ± 24	410–485	510–560
	longer	337–380				
Female		(*n* = 16)				
Body length (mm)		24.0–34.0	14.4–31.1	25.0 ± 14.0	15.6	22.0–24.0
Body width (μm)		366–852	340–511	345 ± 18	268	350
Distance from excretory pore to cephalic end (μm)		454–618	379–636	447 ± 102	403	
Oesophagus length (μm)		305–456	356–556	368 ± 17	240–280	335–350
Distance from vulva to anus (μm)		203–515		295 ± 46	141	
Distance from vulva to caudal end (μm)		286–612	269–412	366 ± 44	205	205–250
Distance from anus to caudal end (μm)		60–193	76–115	79 ± 3	67	75–90
Vulvar opening (length/width, μm)		38–40/4–5				
Anus (length/width, μm)		8–10/2–3				

second and 12 (4 males and 8 females) were collected from the third animal. Adult worms exhibited a pronounced sexual dimorphism, females being larger than males, see Table 1 for detailed morphometric data for the adult worms.

Both sexes have elongated, cylindrical and slender bodies, slightly tapered at both, anterior and posterior ends (Fig. 2b). The cuticle at the anterior extremity is smooth, more or less dilated, appearing as a small cephalic vesicle (Fig. 3a). Mouth opening, in both sexes, is terminally placed, slightly triangular, being surrounded by six labial papillae each of them having a small protuberance at the top. At the level of the amphids, there are four cephalic papillae. A single rudimentary triangular tooth is visible in the buccal cavity and a small cutting plate in opposite position. Two amphidial pores are present at the anterior extremity (Fig. 3b). The buccal cavity leads into an oesophagus composed of a cylindrical corpus, slightly widened at its posterior part where it forms a bulb (Fig. 3a).

The females have a "barber pole" appearance due to their discolored uterus coiled with the brownish intestinal tract (Fig. 4a), and a slightly curved posterior extremity (Fig. 4b) showing the vulvar and anal openings on the lower curvature (Fig. 4c). The vulvar opening appears as a transverse slit (Fig. 4c). The anus is oval, transversely elongated and smaller than the vulva.

The males had a uniformly coloured body, a small bilobated copulatory bursa, and two unequal spicules. The copulatory bursa had two symmetrical, transparent,

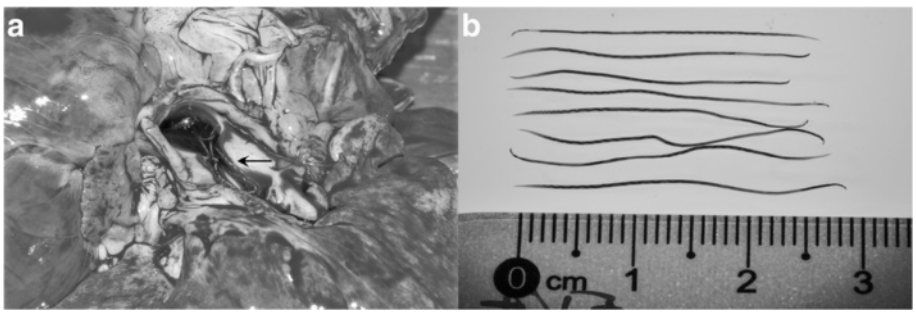

Fig. 2 a Location of *Angiostrongylus daskalovi* in the pulmonary arteries (*arrow*). **b** Size of *A. daskalovi* females

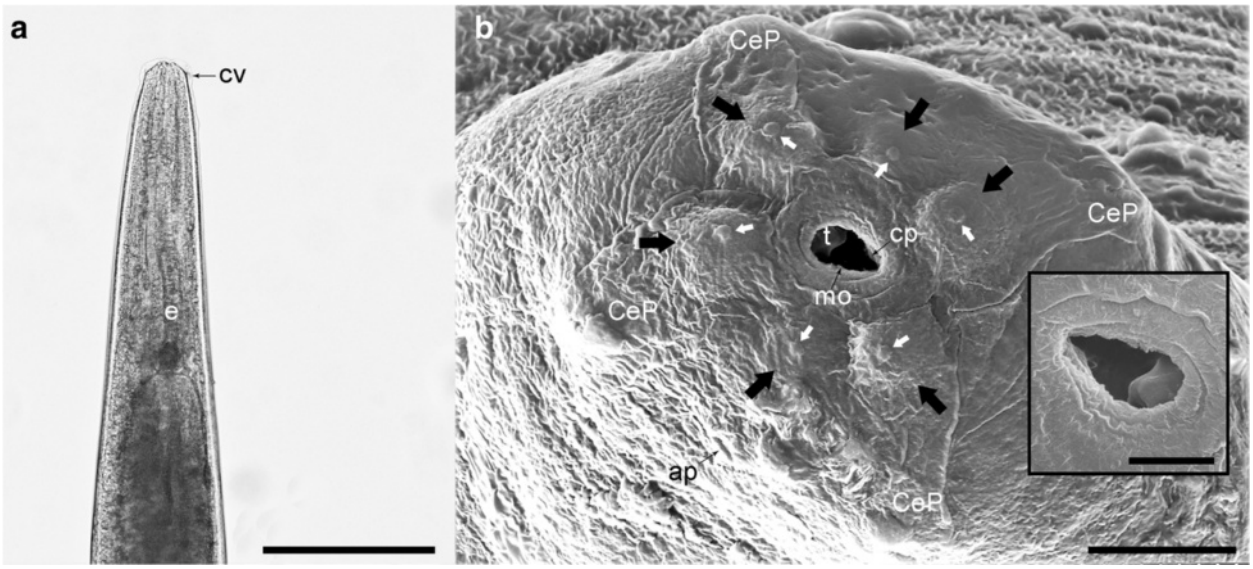

Fig. 3 Light microscopy (**a**) and SEM photomicrographs (**b**) of the anterior extremity of *A. daskalovi*: The tooth and the cutting plate are clearly visible in the inset. Six labial papillae (*black arrows*) and six small protuberances (*white arrows*) are indicated. *Abbreviations*: ap, amphidial pore; CeP, four cephalic papillae; cp, cutting plate; cv, small cephalic vesicle; e, oesophagus; mo, mouth opening; t, tooth. *Scale-bars*: a, 500 μm; b, 10 μm; inset 5 μm

ventro-lateral lobes (Fig. 5), the latter supported by rays with a variable layout and different origin: ventral, lateral, externo-dorsal and median lateral. The ventral ray is distally divided into two branches, ventro-ventral and ventro-lateral, the former being slightly shorter than the latter; ventro-lateral branch ends with a protuberance at the top. The lateral ray is split into three branches: externo-lateral, showing a protuberance at the top, medio-lateral and posterio-lateral, the last two being thinner and separated towards the terminal end. The externo-dorsal ray is straight, undivided, and smaller than the previous two. The median dorsal ray is short, thick, strong and rectangular and has two digitations. The spicules are slender, thin, brownish and unequal, with striated alae. They protrude through the cloacal opening, two papillae (papillae 7) being present behind this orifice; several papillary structures, with a presumptive sensorial role, surround the cloacal opening (Fig. 6a). The spicules show transverse striations and the distal ends are corrugated. When the spicules are joined, they form a channel probably used for semen disposal during fertilization (Fig. 6b).

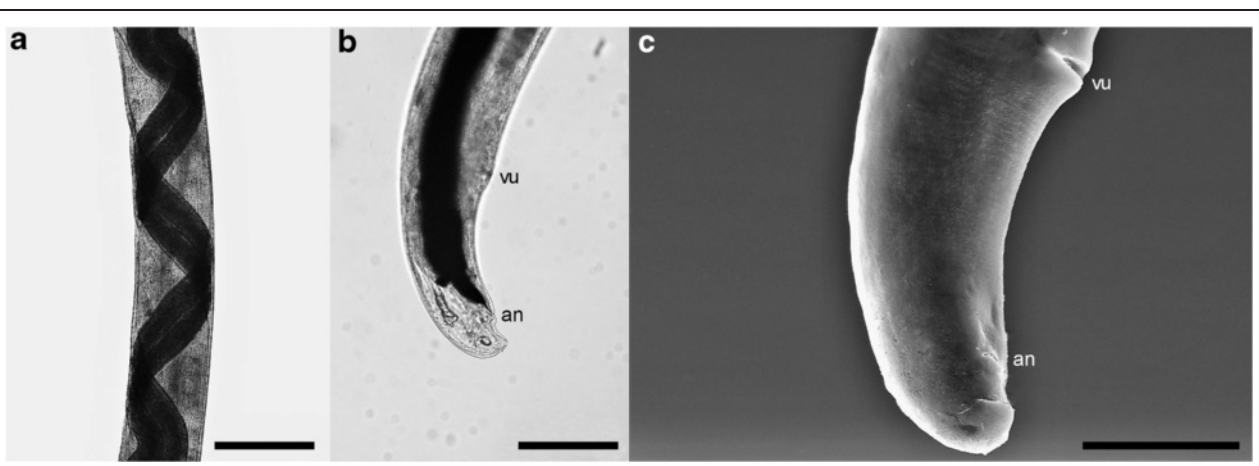

Fig. 4 Light microscopy (**a**, **b**) and SEM photomicrographs (**c**) of *A. daskalovi* female. **a** "Barber-pole" appearance of the body. **b** Slightly curved posterior extremity. **c** Ventral view of the posterior extremity showing the vulva (vu) and the anus (an). *Scale-bars*: a, 500 μm; b, 500 μm; c, 100 μm

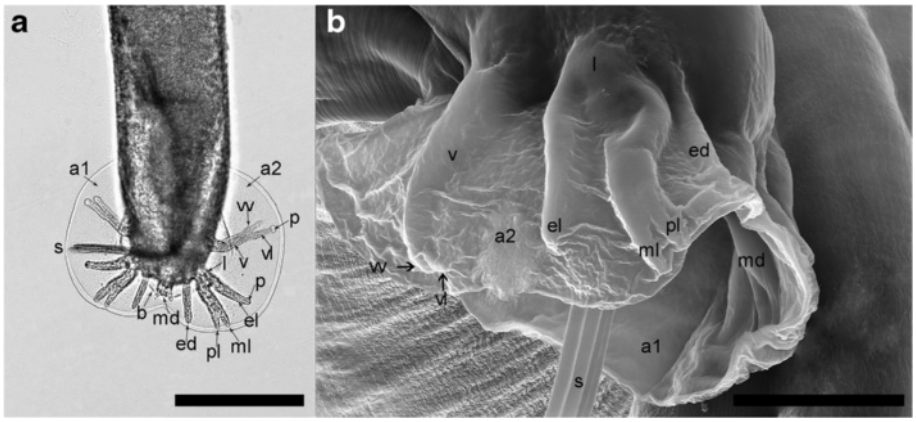

Fig. 5 Light microscopy (**a**) and SEM photomicrographs (**b**) of copulatory bursa of *A. daskalovi*: left ventro-lateral lobe (a1); right ventro-lateral lobe (a2); dorsal lobe (b); ventral ray (v); ventro-ventral branch of ventral ray (vv); ventro-lateral branch of ventral ray (vl); protuberances (p); lateral ray (l); externo-lateral part of lateral ray (el); medio-lateral branch of lateral ray (ml); postero-lateral branch of lateral ray (pl); externo-dorsal ray (ed); median dorsal ray (md); spicules (s). *Scale-bars*: a, 200 μm; b, 50 μm

The measurements of eggs (mean 101 × 79 μm) and larvae (mean 379 × 17 μm) isolated from the lung tissue and faeces are given in Table 2. Stage-two larvae possess the typical metastrongyloid posterior end with a wavy tail, one dorsal spine, and a subterminal notch (Fig. 7a).

Microscopic examination of the Baermann sediment also revealed the presence of some eggs of *A. daskalovi*. These were embryonated, oval, with thin shells (Table 2, Fig. 7b).

Pathology

At necropsy, numerous adult worms were present in the pulmonary arteries and the right atrium, without visible gross morphological changes. The lungs showed diffuse congestion and contained several, variably-sized, firm, gray-red and slightly raised nodules that were randomly distributed within all lung lobes. The tracheobronchial lymph nodes were markedly and diffusely enlarged up to 2–3 times the normal size, irregular in shape, and gray to red on the cross section. The histological findings were consistent with chronic interstitial verminous pneumonia. The fibrous tissue and granulomatous reaction consisting of reactive macrophages, multinucleated giant cells, and fewer neutrophils, eosinophils and plasma cells, occasionally surround the larvae (Fig. 8a).

Larvae were composed of numerous round, basophilic nuclei with scant eosinophilic cytoplasm and a thin amphophilic cuticle (Fig. 8b). Scattered hemorrhagic areas associated with hemosiderin deposits, hemosiderin-laden macrophages (Fig. 8b), and coagulative necrosis were found in the affected parenchyma.

Less affected areas of the lung presented mild congestion, edema, pulmonary atelectasis and alveolar emphysema. The pulmonary arteries exhibited moderate smooth muscle hypertrophy and hyperplasia of the arterial tunica media with mild vacuolar degeneration of the endothelial cells and intimal fibrosis. Multifocal and mild subendocardial fibrosis and minimal mononuclear infiltrates were observed in the right atrium.

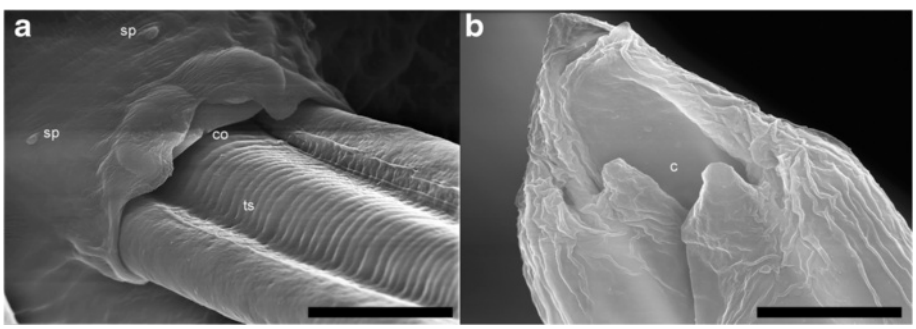

Fig. 6 SEM micrograph of adult male of *A. daskalovi*. **a** Details of the cloacal opening (co) with two papillae (papillae 7) (p), sensory papillae (sp) and transverse striations (ts) of the spicules. **b** Detail of the adjoining arrangement of spicules forming a channel (c). *Scale-bars*: a, 10 μm; b, 5 μm

Table 2 Morphometric data for the eggs and first-stage larvae (L1) of *A. daskalovi*

Species	Stage	Range (μm)		Reference
		Length	Width	
A. daskalovi	Eggs ($n = 10$)[a]	98–105	74–90	Present study
	L1 ($n = 150$)	336–412	14–20	Present study
A. chabaudi	L1	307–420	15–17	[25]
	L1	362–400	15–18	[31]
A. vasorum	L1	310–399	14–16	[24, 32]

[a]Eggs measured in feces. No comparative data for eggs are provided, as the existing data in the literature for *A. chabaudi* and *A. vasorum* refer to measurements of eggs *in utero*

The tracheobronchial lymph nodes showed diffuse reactive hyperplasia, multifocal granulomatous reaction centered on parasitic organisms and numerous aggregates of hemosiderin-laden macrophages (Fig. 8c, d). There were no microscopic lesions in the area of pulmonary arteries where adult parasites were located.

Molecular analysis

All sequences ($n = 6$) obtained from adults and larvae (GenBank accession number KX242346) were identical and showed a 100 % homology to an *Angiostrongylus* sp. recovered from a badger from Spain (accession number GU323341). Phylogenetic analysis clustered *A. daskalovi* within the clade including all other European species *Angiostrongylus* with sequences available in the GenBank database (Fig. 9).

Discussion

This report represents the first comprehensive study of *A. daskalovi* infection in European badgers. The study identified this species based on morphological, morphometric and molecular analyses. Three species of *Angiostrongylus* are described in different badger species across the world, namely *A. vasorum*, *A. daskalovi* and *A. gubernaculatus*. Morphometrically, *A. vasorum* is the smallest species, the length of females ranging between 14.17–17.69 mm and that of males between 11.21–13.91 mm [23]. These values overlap the lower limits of the ranges for length of *A. daskalovi*: 14.39–31.12 mm in females and 13.36–21.31 mm in males [7]. Our data correspond to specific dimensions of *A. daskalovi*, and may differentiate these two species even if the recorded variations could be related to the intervals after infection [24]. The third species, *A. gubernaculatus* is morphometrically very similar to *A. daskalovi*, but host specificity and geographical range differ amongst the two species. Additionally, another congeneric species, *A. chabaudi* was recently found and redescribed in wildcats in the same geographical area from Romania, providing a detailed comparison of the morphometric features [25].

Scanning electron microscopy revealed similar structures of the anterior and posterior ends as described in adult *A. vasorum* [23], but also identified particular structures. At the anterior extremity, the mouth opening is surrounded by six papillae and two amphidal pores, but the small tooth observed in the mouth cavity of *A. daskalovi* is not known in *A. vasorum*. Comparison of the copulatory bursa of *A. vasorum* with that of *A. daskalovi* reveals only a slight difference in the median dorsal ray whose digitations are sometimes separated by a papilla, but none of the males of *A. daskalovi* exhibited this structure.

Despite the morphometric differences, these three species may have a common origin. *Angiocaulus gubernaculatus* which resembles *Angiostrongylus* sp., currently accepted as a synonym [26], may represent a common ancestor for the Brazilian and European populations of *A. vasorum*. Some authors consider that *A. vasorum* is the ancestral species that subsequently spread globally with its carnivore hosts, and evolved into genetically distinct populations in various host species [27]. The phylogenetic analysis performed herein is also supportive of this hypothesis, as *A. daskalovi* clustered with *A. vasorum*.

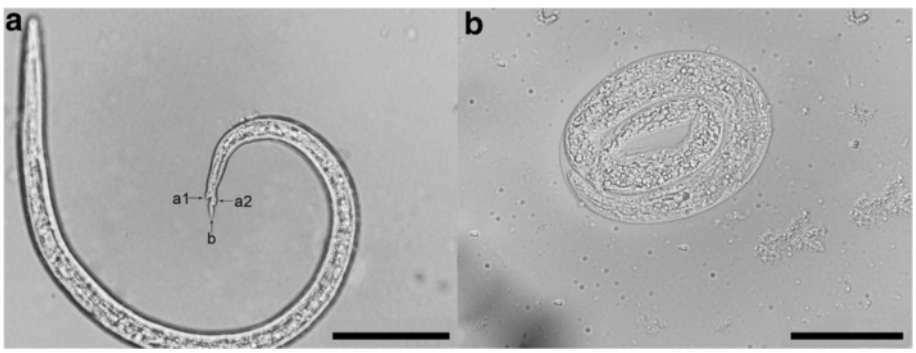

Fig. 7 Light microscopy photomicrographs of *A. daskalovi*. L1 larva. **a** Posterior extremity of L1 larva: dorsal spine (a1); subterminal notch (a2); wavy tail (b). **b** Egg. *Scale-bars*: a, 50 μm; b, 50 μm

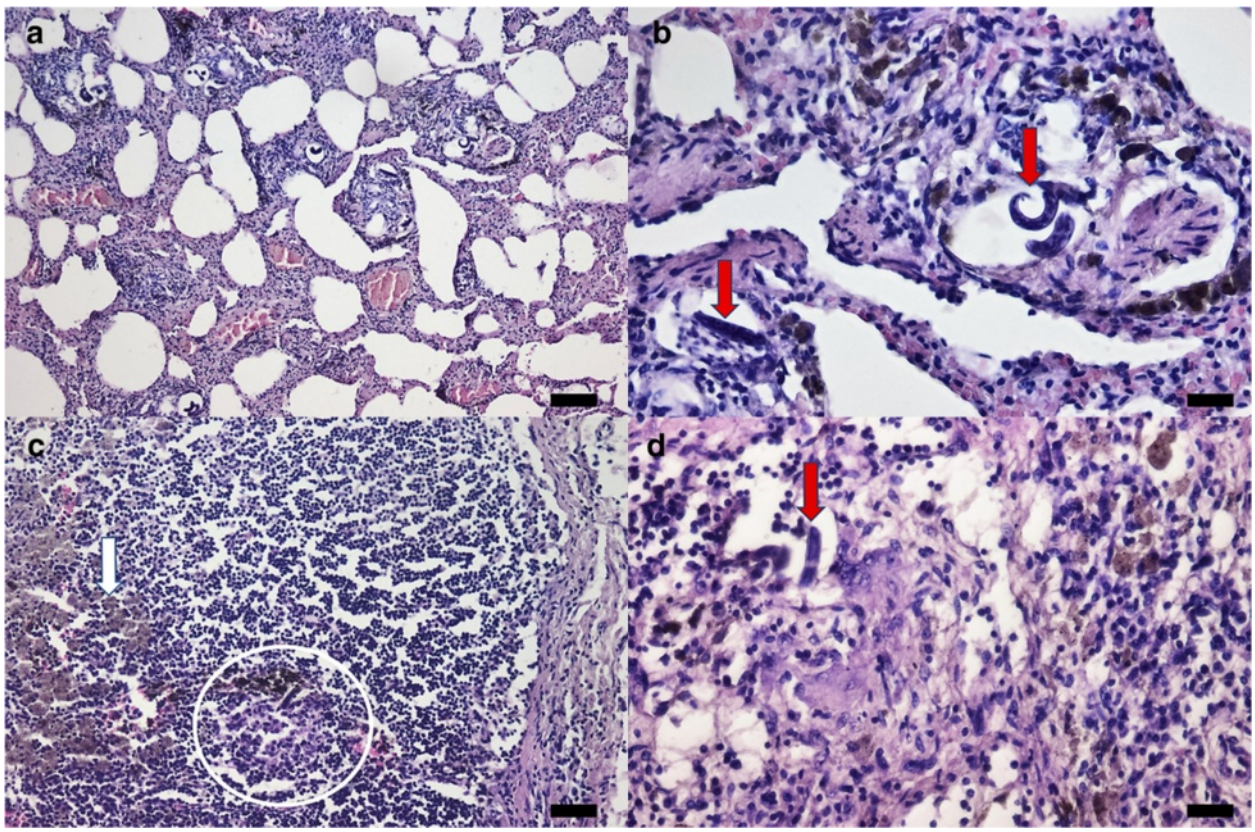

Fig. 8 Histological sections (haematoxylin-eosin staining) of the lung and tracheobronchial lymph nodes of badgers infected with *A. daskalovi*. **a** Diffuse hyperemia, fibrosis and granulomatous reaction of the pulmonary interstitium. **b** Coiled larvae in the bronchial tree and interstitium (*red arrows*). **c** Hemosiderin deposits (*white arrow*) and granulomatous reaction surrounding fragments of parasites (*white circle*) in the tracheobronchial lymph node. **d** Detail of granulomatous reaction centered on larvae (*red arrow*) in the tracheobronchial lymph node. *Scale-bars*: a, 100 μm; b, 20 μm; c, 50 μm; d, 20 μm

To our knowledge, this is the first description of the first-stage larva (L1) of *A. daskalovi*. The differentiation of first-stage larvae of different *Angiostrongylus* species is difficult due to several common characters such as the transparent body, sigmoid tail, the presence of a dorsal spine and a visible notch. Their lengths partly overlap from one species to another: 310–400 μm for *A. vasorum* and 336–412 μm for *A. daskalovi* in the present study (L1s of *A. gubernaculatus* are not described). This does not allow a clear differentiation based on morphological and morphometric criteria. However, the adults and larvae of *A. daskalovi* identified in this study were 100 % similar to adults of *Angiostrongylus* sp. recovered from a badger in Spain and tentatively identified, based on morphology and morphometry, as *A. daskalovi* [8].

The presence of molting larvae in tracheobronchial lymph nodes and eggs in the lung parenchyma provides evidence that the life-cycle of *A. daskalovi* is probably similar to that of *A. vasorum*, following the type II of the known development in the definitive hosts [28]. During their migration, larvae may exert a significant pathogenic action. Hypertrophy and hyperplasia of the arterial tunica media are explainable by pulmonary hypertension due to the presence of nematodes in the lung arteries, being similar to those lesions recorded in dogs naturally infected with *A. vasorum* [29]. The presence of hemosiderin deposits and hemosiderin-laden macrophages, edema, pulmonary atelectasis, alveolar emphysema, diffuse congestion of the lungs and the presence of the nodules randomly distributed within all lung lobes are similar to those produced by *A. vasorum* in dogs [30]. All these pathological alterations of the lungs and the pulmonary arteries confirm that *A. daskalovi* might play an important pathogenic role in infected badgers.

Although the species of *Angiostrongylus* infecting carnivores seem to show a relatively well-defined host specificity, some studies report certain overlaps. As badgers can occasionally be infected with *A. vasorum*, dogs might also be infected occasionally with *A. daskalovi*. Our new molecular data and larval morphology can partly solve possible misdiagnosis problems in European carnivores. Still to be solved is the life-cycle of *A. daskalovi*.

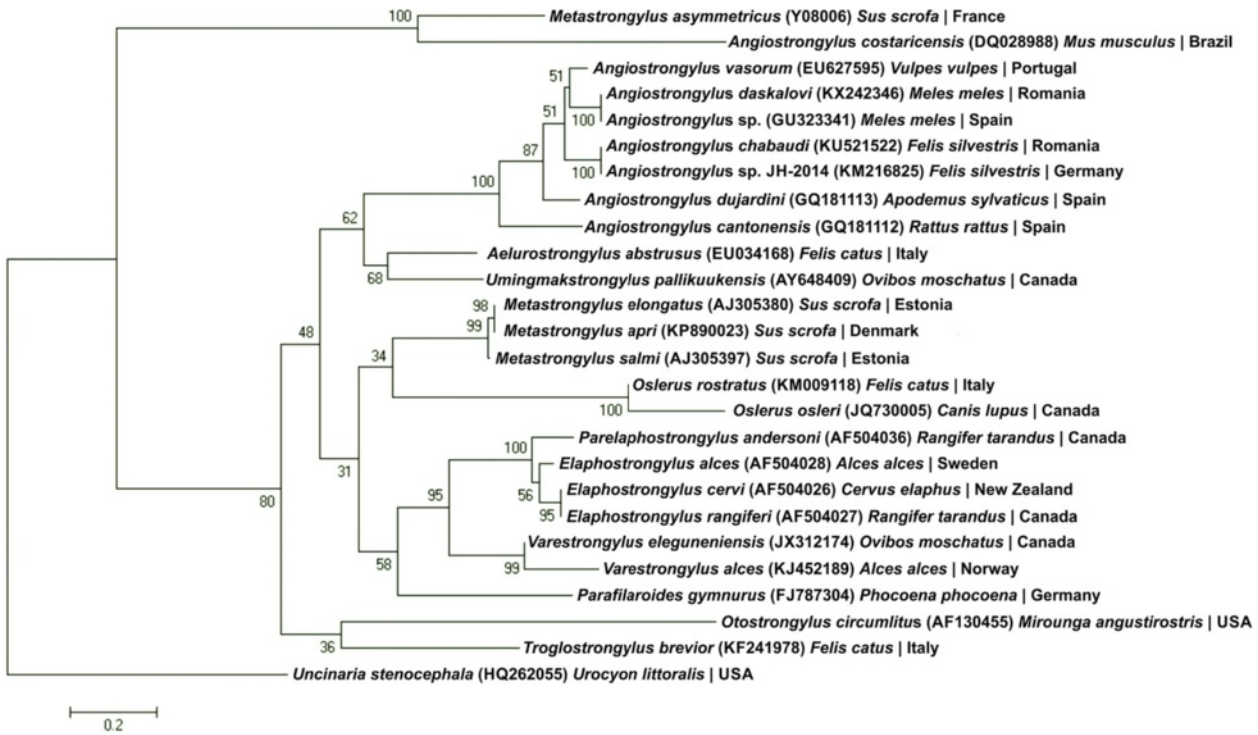

Fig. 9 Phylogenetic tree based on ITS2 sequences for different European species of *Angiostrongylus* retrieved from GenBank. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The tree with the highest log likelihood (-9756.7630) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained by applying the Neighbor-Joining method to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site

Conclusions

The current study confirms the existence of *A. daskalovi* patent infection in badgers from Romania and provides the first description of the larvae, its pathological effect, and its phylogenetic relationships with other congeneric species.

Abbreviations

BLAST, Basic local alignment search tool analysis; DNA, deoxyribonucleic acid; H&E, Hematoxylin and eosin; L1, First larval stage; PCR, polymerase chain reaction; SEM, Scanning electron microscopy

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Availability of data and materials

The data supporting the conclusions of this article are included within the article.

Authors' contributions

CMG, ADM and VC conceived and designed the study. GD and GD'A performed the necropsy. IAM and AMI performed molecular analysis of the parasites. MT conducted the histopathological study. LBT conducted the SEM analysis. AS collected the carcasses of the badgers. CMG wrote the paper. All authors contributed to improve the manuscript and read and approved the final version.

Competing interests

The authors declare that they have no competing interests.

Consent for publication

Not applicable.

Ethics approval and consent to participate

Not applicable.

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References

1. Simpson GG. The principles of classification and a classification of mammals. Bull Am Mus Nat Hist Bulletin. 1945;85:1–350.
2. Koepfli KP, Deere KA, Slater GJ, Begg C, Begg K, Grassman L, et al. Multigene phylogeny of the Mustelidae: resolving relationships, tempo and biogeographic history of a mammalian adaptive radiation. BMC Biol. 2008;6:10.
3. Magi M, Banchi C, Barchetti A, Guberti V. The parasites of the badger (*Meles meles*) in the north of Mugello (Florence, Italy). Parassitologia. 1999;41:533–6.
4. Stubbe M. Zur Biologie der Raubtiere eines abgeschlossenen Waldgebietes. Z Jagdwiss. 1965;11:73–102.

5. Davidson RK, Handeland K, Gjerde B. The first report of *Aelurostrongylus falciformis* in Norwegian badgers (*Meles meles*). Acta Vet Scand. 2006;48:6.
6. Jones GW, Neal C, Harris EA. The helminth parasites of the badger (*Meles meles*) in Cornwall. Mammal Rev. 1980;10:163–4.
7. Janchev J, Genov T. *Angiostrongylus daskalovi* spp. (Nematoda: Filaroididae) from Mustelidae in Bulgaria. Helminthologia (Bratislava). 1988;252:81–8.
8. Gerrikagoitia X, Barral M, Juste RA. *Angiostrongylus* species in wild carnivores in the Iberian Peninsula. Vet Parasitol. 2010;174(1–2):175–80.
9. Miquel J, Feliu C, Torres J, Casanova JC. Corología de las especies de nematodos parásitas de carnívoros silvestres en Cataluña (NE península ibérica). Misc Zool. 1993;17:49–57.
10. Origgi FC, Plattet P, Sattler U, Robert N, Casaubon J, Mavrot F, et al. Emergence of canine distemper virus strains with modified molecular signature and enhanced neuronal tropism leading to high mortality in wild carnivores. Vet Pathol. 2012;49(6):913–29.
11. Magi M, Guardone L, Dell'omodarme M, Prati MC, Mignone W, Torracca B, et al. *Angiostrongylus vasorum* in red foxes (*Vulpes vulpes*) and badgers (*Meles meles*) from central and northern Italy. Hystrix It J Mamm. 2009;20(2):121–6.
12. Torres J, Miquel J, Motjé M. Helminth parasites of the Eurasian badger (*Meles meles* L.) in Spain: a biogeographic approach. Parasitol Res. 2001; 87(4):259–63.
13. Dougherty EC. The genus *Aelurostrongylus* Cameron, 1927 (Nematoda: Metastrongylidae), and its relatives; with descriptions of *Parafilaroides*, gen. nov., and *Angiostrongylus gubernaculatus*, sp. nov. Proc Helminthol Soc Wash. 1946;13:16–26.
14. Faulkner CT, Patton S, Munson L, Johnsont EM, Coonan TJ. *Angiocaulus gubernaculatus* in the island fox (*Urocyon littoralis*) from the California Channel Islands and comments on the diagnosis of angiostrongylidae nematodes in canid and mustelid hosts. J Parasitol. 2001;87(5):1174–6.
15. Millán J, Sevilla I, Gerrikagoitia X, García-Pérez AL, Barral M. Helminth parasites of the Eurasian badger (*Meles meles* L.) in the Basque Country (Spain). Eur J Wildl Res. 2004;50:37–40.
16. Popiołek M, Jarnecki H, Łuczyński T. A record of *Crenosoma vulpis* (Rudolphi, 1819) (Nematoda, Crenosomatidae) from the Eurasian badger (*Meles meles* L.) from Poland. Wiad Parazytol. 2009;55(4):437–9.
17. Simpson V. *Angiostrongylus vasorum* infection in a stoat. Vet Rec. 2010; 166(6):182.
18. Simpson VR, Tomlinson AJ, Stevenson K, McLuckie JA, Benavides J, Dagleish MP. A post-mortem study of respiratory disease in small mustelids in south-west England. BMC Vet Res. 2016;12(1):72.
19. Willcox HP, Coura JR. A new design of the Baermann, Moraes, Coutinho's technique for the isolation of nematode larva (Article in Portuguese). Mem Inst Oswaldo Cruz. 1989;84(4):563–5.
20. Gasser RB, Chilton NB, Hoste H, Beveridge I. Rapid sequencing of rDNA from single worms and eggs of parasitic helminths. Nucleic Acids Res. 1993;21(10):2525–6.
21. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725–9.
22. Tamura K, Nei M. Estimation of the number of nucleotide substitutions in the control region of mitochondrial-DNA in humans and chimpanzees. Mol Biol Evol. 1993;10(3):512–26.
23. Costa JO, De Araújo Costa HM, Guimarães MP. Redescription of *Angiostrongylus vasorum* (Baillet, 1866) and systematic revision of species assigned to the genera *Angiostrongylus* Kamensky, 1905 and *Angiocaulus* Schulz. 1951. Rev Méd Vét. 2003;154:9–16.
24. Rosen L, Ash LR, Wallace GD. Life history of the canine lungworm *Angiostrongylus vasorum* (Baillet). Am J Vet Res. 1970;31:131–43.
25. Gherman CM, Ionică AM, D'Amico G, Otranto D, Mihalca AD. *Angiostrongylus chabaudi* (Biocca, 1957) in wildcat (*Felis silvestris silvestris*) from Romania. Parasitol Res. 2016;115(6):2511–7.
26. Anderson CIH. Keys to genera of the superfamily Metastrongyloidea. In: Anderson RC, editor. Keys to the nematode parasites of vertebrates No. 5. Farnham Royal, Bucks.: Commonwealth Agricultural Bureau; 1978. p. 1–40.
27. Morgan ER, Clare EL, Jefferies R, Stevens JR. Parasite epidemiology in a changing world: can molecular phylogeography help us tell the wood from the trees? Parasitology. 2012;139(14):1924–38.
28. Spratt DM. Species of *Angiostrongylus* (Nematoda: Metastrongyloidea) in wildlife: A review. Int J Parasitol Parasites Wildl. 2015;4(2):178–89.
29. Borgeat K, Sudunagunta S, Kaye B, Stern J, Luis Fuentes V, Connolly DJ. Retrospective evaluation of moderate-to-severe pulmonary hypertension in dogs naturally infected with *Angiostrongylus vasorum*. J Small Anim Pract. 2015;56:196–202.
30. Rinaldi L, Cortese L, Meomartino L, Pagano TB, Pepe P, Cringoli G, Papparella S. *Angiostrongylus vasorum*: epidemiological, clinical and histopathological insights. BMC Vet Res. 2014;10:236.
31. Diakou A, Psalla D, Migli D, Di Cesare A, Youlatos D, Marcer F, Traversa D. First evidence of the European wildcat (*Felis silvestris silvestris*) as definitive host of *Angiostrongylus chabaudi*. Parasitol Res. 2016;115(3):1235–44.
32. Bolt G, Monrad J, Koch J, Jensen AL. Canine angiostrongylosis: a review. The Veterinary Record. 1994;135(19):447.

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CO_2 flagging - an improved method for the collection of questing ticks

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Abstract

Background: Most epidemiological studies on tick-borne pathogens involve collection of ticks from the environment. An efficient collection method is essential for large sample pools. Our main aim was to evaluate the efficacy of a new method, where traditional flagging was enhanced by the use of CO_2 dispersed into the white flannel. The CO_2 was spread through a rubber hose network inserted into the flag blanket. The research was conducted in spring, in March-April 2011 in two locations from Cluj County, Romania.

Methods: The research was conducted in March-April 2011 in two locations from Cluj County, Romania. The flag to be tested contained a fine silicone rubber hose network which dispersed the CO_2 in the shaft. On each collection site n=30 samplings were performed. Each sampling consisted in the simultaneous use of both flags (with and without CO_2) by two persons. The CO_2 concentration level on the flag canvas surface was measured. The efficacy of the method was determined by counting comparatively the total number of ticks and separate developmental stage count.

Results: Using the CO_2 improved flag, 2411 (59%) *Ixodes ricinus* and 100 (53.8%) *Dermacentor marginatus* ticks were captured, while the CO_2-free flag accounted for the collection of 1670 *I. ricinus* (41%) and 86 (46.2%) *D. marginatus* ticks. The addition of CO_2 prompted a concentration difference on the surface of the flag ranging between 756.5 and 1135.0 ppm with a mean value of 848.9 ppm.

Conclusion: The study showed that the CO_2 enhanced sweep flag increased the ability of *I. ricinus* ($p < 0001$) but not of *D. marginatus* to be attracted to the flag blanket.

Keywords: Flagging, Carbon dioxide, Questing ticks, *Ixodes ricinus*

Background

Ticks (suborder Ixodida) are obligate blood-sucking acarines attacking a wide variety of hosts from all tetrapod vertebrate classes [1,2]. Around 700 species of hard ticks are currently recognized as valid species [1]. Most of these species are three-host ticks (i.e. each stage detaches after engorgement) [3,4]. Regardless of the number of hosts, each tick must find a suitable host. In three-host ticks, most of their multiannual life is not spent attached to the host but as free-living organisms. Thus, newly hatched larvae, unfed nymphs and unfed adults are in a permanent host finding state. Host detection and attachment in Ixodidae is achieved through three main alternative behavioral patterns: questing, hunting and tick-host cohabitation (nidiculous ticks) [4].

Most epidemiological studies on tick-borne pathogens involve collection of ticks from the environment [5]. Thus, an efficient collection method is essential for large datasets. Tick collection methods had been reviewed by Gray [6]. He divided these methods into four major categories: (1) flagging or dragging methods; (2) trapping using carbon dioxide baits; (3) collecting from hosts and (4) walking (i.e. on the clothes of the collectors). Despite all these methods are relative and do not estimate density (number per unit area) or absolute size (total number as measured in mark-release-recapture methods) [7], each of these has a variable efficacy depending on several

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factors (i.e. habitat type, tick species, developmental stage etc.). Nevertheless, all methods have been improved over the time in order to increase their efficacy [8].

One of the most important ticks species (regarding its range, abundance, and vectorial importance) in the Palearctic region, with tendency to expand its spread in Northern Europe [9,10], is *Ixodes ricinus* [11]. The host seeking strategy of all developmental stages in *I. ricinus* is questing, when ticks are typically positioned on the vegetation with their legs extended, waiting for a moving host to which they attach [12]. Though, questing is a complex behavioral process, which involves responses to stimuli like host movement, concentration of environmental carbon dioxide and increase of temperature [4]. The most commonly used method for collection of questing ticks is flagging. However, flagging stimulates only the tick sensor for movement, and leaves the other two sensorial components of questing (i.e. carbon dioxide and temperature) unexploited.

The vast majority of ecological and epidemiological studies of tick-borne pathogens involve collection of unfed ticks from the environment. In this view, our main aim was to evaluate the efficacy of a new method, where traditional flagging was enhanced by the use of dispersed CO_2 into the white flannel.

Methods

Sweep and flag design

The sweep consists of a shaft and a flag. The shaft is constructed from a hollow aluminum tube, and the flag from white technical flannel. A JBL 500 g CO_2 bottle with a CO_2 solenoid regulator attached (both aquarium use, JBL Aquarium®, Germany) were fixed with plastic lock seals on the shaft. A silicone rubber hose was attached to the CO_2 solenoid regulator; the hose was introduced through the aluminum shaft and connected to the flag. The rubber hose was pierced (to release CO_2) and attached to the flag by sewing forming a network structure (Figure 1). The hose was made of bending-resistant silicone rubber with the inner diameter of 1 mm and the outer diameter of 2 mm with a wall thickness of 0.5 mm.

The flag surface area was 0.48 m^2 (80 x 60 cm) to allow unrestricted passage across all types of vegetation. Two identical flags were made; one of them with CO_2 and the other without CO_2 (control).

Study area

Two hilly areas were chosen: Vultureni and Faget, both in Cluj county (Figure 2), according to preliminary results of sampling for the evaluation of the presence of tick-borne pathogens in Romania (manuscript under preparation). The habitats consisted in herbaceous vegetation alternating with small shrubs, located at the edge of woods, specific areas for ticks. The climate is moderate continental, influenced by the vicinity of the Apuseni Mountains and Atlantic influences from west of the country, in autumn and winter [13]. The study was conducted in spring, between the end of March and the end of April 2011, as tick abundance is higher in Northwestern Romania in this season [14]. The GPS was used to measure the distance.

Sampling procedure

On each collection site n = 30 samplings were performed (total 60 samplings in the two sites). Each sampling consisted in the simultaneous use of both flags, (with and without CO_2), on 2 m wide adjacent areas, by two persons who had interchanged the sweep every fifty meters for the homogeneity of results. After each 5 m the flags were checked for ticks. All ticks were collected regardless their species and fixed in pure ethanol. Specific identification was performed using morphological keys [15] under a binocular microscope.

Determination of carbon dioxide

The CO_2 concentration level on the flag canvas surface was measured using a portable CIRAS-2 Photosynthesis System equipped with a SRC-1 Soil Respiration Chamber (PP Systems International Inc®, USA). CO_2 level was determined at a single time, but from several points of the flag (n = 20) according to instruction manual and recommendations [16]. The results were expressed in ppm as compared to the standard CO_2 concentration of 393.71 ppm (reference data for 24.04.2011) [16].

Statistical analysis

The efficacy of the method was determined by counting the total number of ticks and separate developmental stage count, attached to the CO_2 flag compared with the control (CO_2-free flag). For statistical analysis, the values were compared in CHI-SQUARE TEST [17]. A p value of <0.05 was considered statistically significant. The relative risk (RR) is a ratio of the probability of the event occurring in the exposed group versus a non-exposed group [18].

Results

A total number of 4267 of ticks belonging to two species were collected in the 60 samplings: *I. ricinus* (n = 4081) and *Dermacentor marginatus* (n = 186).

I. ricinus accounted for 4081 tick captures (adults, nymphs and larvae) were collected in the 60 samplings (Table 1). Of these, 2617 (64.1%) were adults, 1422 (34.9%) nymphs and 42 (1%) larvae. Using CO_2 improved flag were captured 2411 (59%) ticks and 1670 (41%) without CO_2.

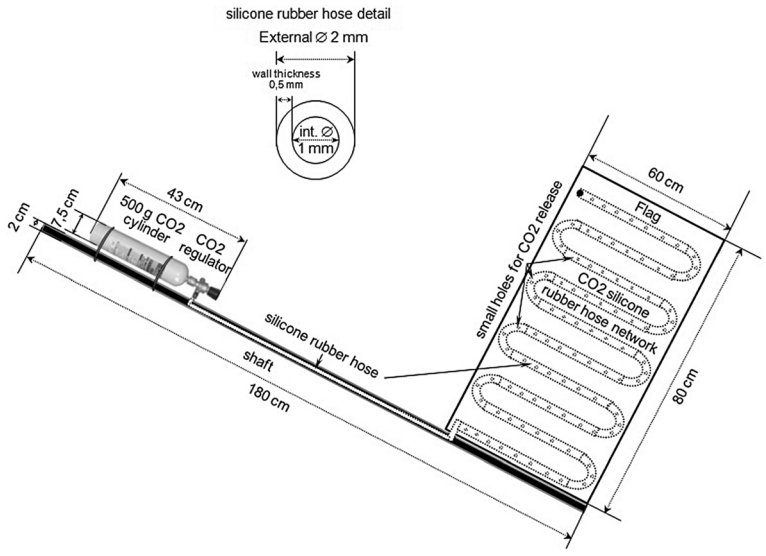

Figure 1 Sweep CO_2 flag diagram.

The statistical analysis revealed highly statistically significant ($p < 0001$) difference between the two variables in adults and nymphs, in both locations and overall; for larvae, the recorded statistical differences were not significant (Table 2). Carbon dioxide flagging was more effective than CO_2-free flagging, with average values of RR ranging between 1.4 and 1.6 for adults and nymphs.

The addition of CO_2 prompted a difference on the surface of the flag ranging between 756.5 and 1135.0 ppm with a mean value of 848.9 ppm.

Discussion

Flagging is the most widespread tick collection method. Over time, several improvements to this method were

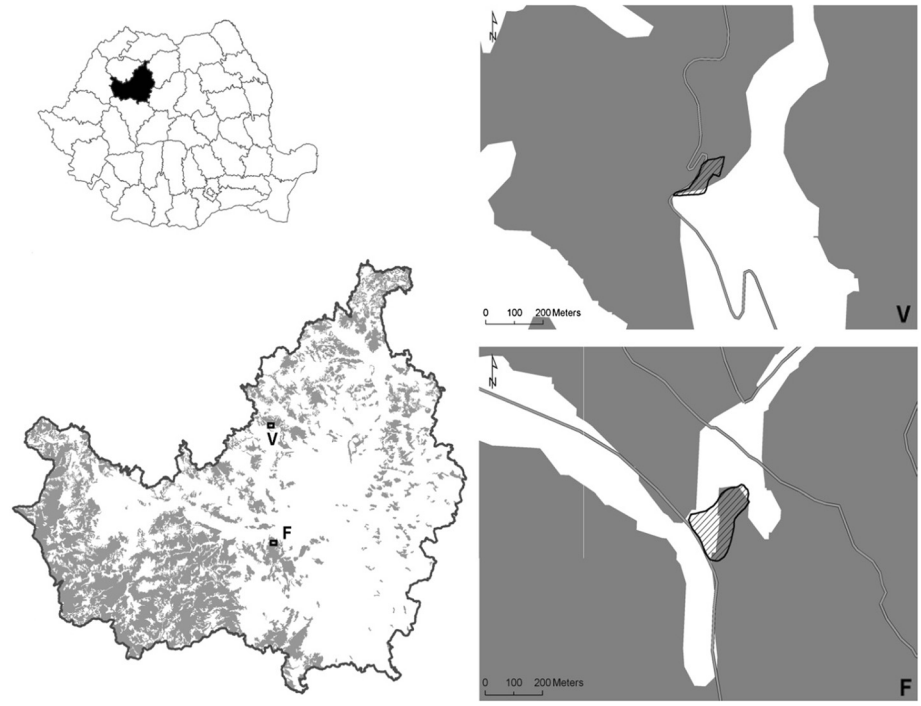

Figure 2 Field studies location (shaded area shows the flagging surface).

Table 1 Number of questing *Ixodes ricinus* ticks collected by flagging with and without CO_2

Location/stage/method		Faget		Vultureni		Total (both locations)	
		IR	DM	IR	DM	IR	DM
Males	CO_2+	456	27	211	22	667	49
	CO_2-	325	21	160	20	485	41
Females	CO_2+	587	26	281	25	868	51
	CO_2-	407	23	190	22	597	45
Nymphs	CO_2+	547	0	304	0	851	0
	CO_2-	353	0	218	0	571	0
Larvae	CO_2+	22	0	3	0	25	0
	CO_2-	16	0	1	0	17	0
TOTAL	CO_2+	1612	53	799	47	2411	100
	CO_2-	1101	44	569	42	1670	86
	Per location	2713	97	1368	89	4081	186

proposed [8] and now there is many types used: double walking flagging and double walking with baited flagging [19], walking flagging with a loose-fitting and white cotton flannel garment worn [20] and strip-flag method [21].

The successful hosts attack in *Ixodes* species expressed as the ability to adhere to a flannel flag is influenced by many factors: light and/or shadow, radiation heat (temperature), mechanical vibration of questing substrate, host odor, and CO_2 concentration [22].

Chemical mediators also known as semiochemicals are as well important for behavioral patterns in ticks. These information-bearing compounds are secreted by animals into the external environment, and when recognized they trigger a specific behavioral response such as food location, sexual partner location or escape [23]. Semiochemicals are categorized into four major categories: (1) pheromones; (2) allomones; (3) kairomones; and (4) synomones [24].

Carbon dioxide acts like an attractant kairomone for ticks [25]. Experimentally, it acted as an attracting agent causing almost immediate activation in the soft ticks *Ornithodoros coriaceus* quiescent ticks [25]. Adults of *Dermacentor andersoni* respond also very well to stimulation with carbon dioxide. Garcia, 1965, described a system based on release of CO_2 for the collection of *D. andersoni*. The system involved a piece of dry ice placed on a wire mesh platform in the desired area. The results indicate that the CO_2 method is more sensitive for detection of adult ticks than is the conventional flagging technique [26]. Carbon dioxide (dry ice) trapping method was demonstrated to be effective in the collection of some tick species: *I. ricinus* [5], *Amblyomma americanum* [27-29] and *A. hebraeum* [30]. Our method is important in Europe as it enhances the capture of *I. ricinus*, the main vector of *Borrelia burgdorferi* s.l. and the tick-borne encephalitis (TBE) virus [31].

Our results are consistent with those cited above. The number of adults and nymphs of *I. ricinus* collected was significantly increased using CO_2 enhanced blanket comparing with flagging without CO_2. The lower number of larvae collected can be explained by the months of sampling, March-April, when larvae may be not fully active and by the quality of blanket, made by technical flannel, material which may not have reached the lower levels of the vegetation where larvae sit to quest.

These data show that the responsiveness to CO_2 is enhanced during host-seeking periods of the life cycle and reduced at other times [32]. However, others [29] did not establish significant differences between flagging method with a strip blanket and CO_2 traps or rabbits scent baits. The response time of ticks to host attachment was shown to be dependent on the tick species and CO_2 concentration in the environment [33]. In *Amblyomma maculatum*, *A. americanum* and *Dermacentor variabilis*,

Table 2 Statistical significance of tick collection efficacy using flagging with and without CO_2

Location		Faget		Vultureni		Total	
Statistical parameter		***I. ricinus***	***D. marginatus***	***I. ricinus***	***D. marginatus***	***I. ricinus***	***D. marginatus***
		P	**P**	**P**	**P**	**P**	**P**
		RR	**RR**	**RR**	**RR**	**RR**	**RR**
Males	**CO_2+**	0.0001	0.31	0.0002	0.83	0.0001	0.30
	CO_2-	1.40 (1.27–1.56)	1.29 (0.86–1.91)	1.32 (1.14–1.53)	1.1 (0.72–1.69)	1.38 (1.27–1.50)	1.20 (0.89–1.61)
Females	**CO_2+**	0.0001	0.69	0.0001	0.68	0.0001	0.47
		1.44	1.13(0.76–1.68)	1.48(1.30–1.67)	1.14(0.76–1.71)	1.45(1.35–1.57)	1.13(0.85–1.51)
	CO_2-	(1.32–1.58)					
Nymphs	**CO_2+**	0.0001		0.0001		0.0001	
	CO_2-	1.6 (1.4–1.7)		1.4 (1.2–1.6)		1.5 (1.4–1.6)	
Larvae	**CO_2+**	0.25		0.48		0.13	
	CO_2-	1.4 (0.9–2.2)		3 (0.5–18)		1.5 (0.9–2.3)	

the groups preconditioned with low ambient CO_2 (422 ppm) always produced response times of longer duration than ticks preconditioned to high ambient CO_2 (956 ppm) [34].

The lack of statistical significance for *D. marginatus* between the two collecting methods compared in the present study might be caused by the major differences in the sample size. The small total number of *D. marginatus* collected (regardless the method used) can be explained by the typical area of this species which prefers biotopes characterized by xerophilic plant communities: dry pasture shrub communities, grazing black locust forests (*Robinietum*), forest-steppe or grikes (*Quercetum pubescentis* and *Cometo-Quercetum*), margins of oak forests and bushy ridges between the fields and field paths [35]. Our study area is characterized by deforested hills and covered with low vegetation; predominant species in forest areas are hornbeam, birch, poplar, hazel, elm, ash and maple. Although *D. marginatus* is almost as widespread in Romania as *I. ricinus*, [36], the population density is significantly lower in most of the sampled localities [37]. Regarding seasonality, in Eastern Europe, *D. marginatus* is most numerous in February and March [38] and most of our sampling was done in April.

Flagging technique seems to work better for other species: *Ixodes dammini* [27], *I. pacificus*, *D. occidentalis* and *D. variabilis* [32], *I. rubicundus* [39] or *I. ricinus* [40]. Four different methods of surveying were tested for *D. variabilis* and *I. banksi* and it was shown that the most successful was flagging, compared with carbon dioxide trapping, nest boxes and collection from hosts [41].

Concerning the temperature, *I. persulcatus* is a more cold-resistant tick than *I. ricinus* and it is more successful both in adhering to the flag and in remaining attached to it at two ranges: 6–10°C and 17–22°C [42]. In general a greater percentage of *I. rubicundus* displayed an appetence response at lower (12, 17 and 21°C) than at high (30°C) temperatures [39]. It is known that all life stages of *I. ricinus* are equipped to sense shifts in light intensity. This allows *I. ricinus* to use onset of darkness to trigger mobility when desiccation risk is reduced in nature [43]. The nymphs are stimulated to walk horizontally by humidity and host scent. When the atmosphere is sufficiently wet they are likely to walk towards odor secreted by host skin [44,45]. The larvae of *I. hirsti* seem to be more sensitive to shade and heat, while they were unresponsive to CO_2 concentration and host odor [46]. Radiation heat and shadowing caused the greatest percentage of *I. rubicundus* to display an appetence response; shadowing and radiation heat had the least effect on *R. punctatus* [22]. A single mechanical perturbation of the substratum caused a mean of 50% of *I. rubicundus* to display an appetence response. Constant mechanical perturbation resulted in a progressive decrease in the proportion of ticks reacting [22]. Host scent is known to initiate questing behavior in *I. persulcatus*, *I. ricinus* and *I. crenulatus*. Both *I. ricinus* and *I. crenulatus* respond strongly to sheep wool [47,48].

Entire-blanket flagging is a better sampling method for *I. ricinus* comparing with others variants of flagging or dragging. Significantly more nymphs and adults were caught by the entire-blanket versus strip-blanket flagging [49]. Flagging was 1.5–1.7 times as effective as dragging; impregnation of the cloths with different substances, like host odor, increased the efficacy by 2.4 (dragging) to 2.8 (flagging) times [39]. From several types of material used (i.e. cotton, woolen flannel, "molleton" - soft thick cotton, and toweling - spongecloth), the last was the best cloth type to optimize the number of ticks collected [36]. However, dry-ice-baited tick-traps is more effective for ticks with increased mobility, like *A. americanum* [27].

Our enhanced technique that combines, for the first time, classical flagging and carbon dioxide trapping methods improved significantly the ability of *I. ricinus* to adhere to flag blanket. By introducing CO_2 in the white blanket we tried to simulate the time approach of a host to the tick. Sudden increase of the CO_2 concentration in the air stimulates its vivacity and questing position, increasing the chance of attachment of ticks to the flag.

Conclusion

The study showed that the CO_2 enhanced sweep flag increased the ability of *I. ricinus* ($p < 0001$) but not of *D. marginatus* to be attracted to the flag blanket.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

GCM wrote the manuscript, performed flagging. MAD concept idea, performed the flagging. DMO identified the ticks. GA statistical analysis. OI measurement of CO_2. SM measurement of CO_2. CV team coordinator. All authors read and approved the final manuscript.

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References

1. Guglielmone AA, Robbins RG, Apanaskevisch DA, Petney TN, Estrada-Pena A, Horak IG, Shao RF, Barker SC: **The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species name.** *Zootaxa* 2010, **2528**:1–28.

2. Keirans JE: **Systematics of the Ixodida (Argasidae, Ixodidae, Nuttalliellidae): An overview and some problems**. In *Tick Vector Biology, Medical and Veterinary Aspects*. Edited by Fivaz B, Petney T, Horak I. Berlin: Springer Verlag; 1992:1–21.
3. Oliver JH Jr: **Biology and systematics of ticks (Acari: Ixodida).** *Annu Rev Ecol Syst* 1989, **20**:397–430.
4. Petney NT, Robbins RG, Guglielmone AA, Apanaskevich DA, Estrada-Peña A, Horak IG, Shao R: **A Look at the World of Ticks**. In *Progress in Parasitology*. Edited by Mehlhorn H. Berlin: Springer Verlag; 2011:283–296.
5. Capelli G, Ravagnan S, Montarsi F, Ciocchetta S, Cazzin S, Porcellato E, Babiker AM, Cassini R, Salviato A, Cattoli G, Otranto D: **Occurrence and identification of risk areas of Ixodes ricinus-borne pathogens: a costeffectiveness analysis in north-eastern Italy.** *Parasite Vector* 2012, **5**:61.
6. Gray JS: **A carbon dioxide trap for prolonged sampling of *Ixodes ricinus* L. populations.** *Exp Appl Acarol* 1985, **1**:35–44.
7. Braks M, Van der Giessen J, Kretzschmar M, Van Pelt W, Scholte EJ, Reusken C, Zeller H, Van Bortel W, Sprong H: **Towards an integrated approach in surveillance of vector-borne diseases in Europe.** *Parasite Vector* 2011, **4**:192.
8. Carroll JF, Schmidtmann ET: **Tick sweep: modification of the tick drag-flag method for sampling nymphs of the deer tick (Acari: Ixodidae).** *J Med Entomol* 1992, **29**(2):352–355.
9. Jore S, Viljugrein H, Hofshagen M, Brun-Hansen H, Kristoffersen AB, Nygård K, Brun E, Ottesen P, Sævik BK, Ytrehus B: **Multi-source analysis reveals latitudinal and altitudinal shifts in range of Ixodes ricinus at its northern distribution limit.** *Parasite Vector* 2011, **4**:84.
10. Jaenson TG, Jaenson DG, Eisen L, Petersson E, Lindgren E: **Changes in the geographical distribution and abundance of the tick Ixodes ricinus during the past 30 years in Sweden.** *Parasite Vector* 2012, **5**:8.
11. Estrada-Peña A: **Geostatistics as predictive tools to estimate *Ixodes ricinus* (Acari: Ixodidae) habitat suitability in the western Palearctic from AVHRR satellite imagery.** *Exp Appl Acarol* 1999, **23**:337–349.
12. Randolph SE: **Tick ecology: processes and patterns behind the epidemiological risk posed by ixodid ticks as vectors.** *Parasitol* 2004, **129**: S37–S65.
13. Trusca V, Alecu M: **Romania General Facts**. In *Romania's Third National Communication on Climate Change under the United Nations Framework Convention on Climate Change*. Edited by Ministry of Environment and Water Management. Holstebro: Grue & Hornstrup; 2005:21–65.
14. Chiţimia L: *[Ecology of Ixodidae in Southwestern Romania] [in Romanian]. PhD Thesis*. Timişoara: Universitatea de Ştiinţe Agricole şi Medicină Veterinară a Banatului; 2006:283.
15. Feider Z: *[Fauna of the Popular Republic of Romania. Volume 5/2. Acaromorpha, Suprafamily Ixodoidea] [in Romanian]*. Bucureşti: Editura Academiei Republicii Populare Române; 1965.
16. Conway T, Tans P: **NOAA/ESRL.** www.esrl.noaa.gov/gmd/ccgg/trends/. Accessed 30 november 2011.
17. Snedecor GW, Cochran WG: *Statistical Methods*. 8th edition. Ames: Iowa State University Press; 1989.
18. Localio AR, Margolis DJ, Berlin JA: **Relative risks and confidence intervals were easily computed indirectly from multivariable logistic regression.** *J Clin Epidemiol* 2007, **60**(9):874–882.
19. Fernández-Ruvalcaba M, Jesus F, De La Torre P, Cordoba-Juarez G, García-Vazquez Z, Rosario-Cruz R, Saltijeral-Oaxaca J: **Animal bait effect on the recovery of *Boophilus microplus* larvae from experimentally infested grass in Morelos, Mexico.** *Parasitol Latinoam* 2003, **58**:54–58.
20. Chapman ES, Siegle TE: **A novel method for tick retrieval reveals northern migration of *Amblyomma americanum* into Pike County, IL, USA.** *Int J Acarol* 2000, **26**(4):391–393.
21. Gray JS, Lohan G: **The development of a sampling method for the tick Ixodes ricinus and its use in a redwater fever area.** *Ann Appl Biol* 1982, **101**(3):421–427.
22. Fourie LJ, Snyman A, Kok DJ, Horak IG, Van Zyl JM: **The appetence behaviour of two South African paralysis-inducing ixodid ticks.** *Exp Appl Acarol* 1993, **17**:921–930.
23. Sonenshine DE: **Pheromones and other semiochemicals of ticks and their use in tick control.** *Parasitology* 2004, **129**:S405–S425.
24. Evenden ML, Judd GJR, Borden JH: **A synomone imparting distinct sex pheromone communication channels for *Choristonuera rosaceana* (Harris) and Pandemis limitata (Robinson) (Lepidoptera: Tortricidae).** *Chemoecology* 1999, **9**:73–80.
25. Garcia R: **Carbon dioxide as an attractant for certain ticks (Acarina: Argasidae and Ixodidae).** *Ann Entomol Soc Am* 1962, **55**(5):605–606.
26. Garcia R: **Collection of *Dermacentor andersoni* (stiles) with carbon dioxide and its application in studies of Colorado tick fever virus.** *Am J Trop Med Hyg* 1965, **14**(6):1090–1093.
27. Ginsberg HS, Ewing CP: **Comparison of flagging, walking, trapping, and collecting from hosts as sampling methods for northern deer ticks, *Ixodes dammini*, and lone-star ticks, *Amblyomma americanum* (Acari: Ixodidae).** *Exp Appl Acarol* 1989, **7**:313–322.
28. Kinzer DR, Presley SM, Hair JA: **Comparative efficiency of flagging and carbon dioxide-baited sticky traps for collecting the lone star tick, *Amblyomma americanum* (Acarina: Ixodidae).** *J Med Entomol* 1990, **27**(5):750–755.
29. Schulze TL, Jordan RA, Hung RW: **Biases associated with several sampling methods used to estimate abundance of *Ixodes scapularis* and *Amblyomma americanum* (Acari: Ixodidae).** *J Med Entomol* 1997, **34**(6):615–623.
30. Anderson RB, Scrimgeour GJ, Kaufman WR: **Responses of the tick, *Amblyomma hebraeum* (Acari: Ixodidae), to carbon dioxide.** *Exp Appl Acarol* 1998, **22**:667–681.
31. Dautel H, Kahl O: **Ticks (Acari: Ixodoidea) and their medical importance in the urban environment**. In *Proceedings of the Third International Conference on Urban Pests: 19–22 July 1999*. Edited by Robinson WH, Rettich F, Rambo GW. Prague:; 1999:73–82.
32. Li X, Dunley JE: **Optimal sampling and spatial distribution of *Ixodes pacificus*, *Dermacentor occidentalis* and *Dermacentor variabilis* ticks (Acari: Ixodidae).** *Exp Appl Acarol* 1998, **22**:233–248.
33. Gray JS: **Studies on the larval activity of the tick Ixodes ricinus L. in Co. Wicklow, Ireland.** *Exp Appl Acarol* 1985, **1**:307–316.
34. Holscher KH, Gearhart HL, Barker RW: **Electrophysiological responses of three tick species to carbon dioxide in the laboratory and field.** *Ann Entomol Soc Am* 1980, **73**(3):288–292.
35. Nosek J: **The ecology, bionomics, behaviour and public health importance of *Dermacentor marginatus* and *D. reticulatus* ticks.** *Wiad Parazyt* 1972, **XVIII**(4-5-6):721–725.
36. Mihalca AD, Dumitrache MO, Magdaş C, Gherman CM, Domşa C, Mircean V, Ghira IV, Pocora V, Ionescu DT, Sikó Barabási S, Cozma V, Sándor AD: **Synopsis of the hard ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution.** *Exp Appl Acarol* 2012, doi:10.1007/s10493-012-9566-5.
37. Mihalca AD, Gherman CM, Magdas V, Dumitrache MO, Győrke A, Sándor AD, Domşa C, Oltean M, Mircean V, Mărcuţan DI, D'Amico G, Păduraru AO, Cozma V: ***Ixodes ricinus* is the dominant questing tick in forest habitats in Romania: the results from a countrywide dragging campaign.** *Exp Appl Acarol* 2012, doi:10.1007/s10493-012-9568-3.
38. Hornok S: **Allochronic seasonal peak activities of *Dermacentor* and *Haemaphysalis* spp. under continental climate in Hungary.** *Vet Parasitol* 2009, **163**:366–369.
39. Fourie LJ, Van Der Lingen F, Kok DJ: **Improvement of field sampling methods for adult Karoo paralysis ticks, *Ixodes rubicundus* (Acari: Ixodidae), through addition of host odour.** *Exp Appl Acarol* 1995, **19**:93–101.
40. Vassallo M, Pichon B, Cabaret J, Figureau C, Perez-Eid C: **Methodology for sampling questing nymphs of *Ixodes Ricinus* (Acari: Ixodidae), the principal vector of lyme disease in Europe.** *J Med Entomol* 2000, **37**(3):335–339.
41. Keaney P: *The Effectiveness Of Various Trapping Techniques In Collecting Ixodes scapularis and Other Species of Ticks, Bios 569 - Practicum in Aquatic Biology*, 1651 Turtle Creek Ct. Advisor: Dr. Craig. 1994.
42. Uspensky I: **Ability of successful attack in two species of ixodid ticks (Acari Ixodidae) as a manifestation of their aggressiveness.** *Exp Appl Acarol* 1993, **17**:673–683.
43. Perret JL, Guerin PM, Diehl PA, Vlimant M, Gern L: **Darkness induces mobility, and saturation deficit limits questing duration, in the tick *Ixodes ricinus*.** *J Exp Biol* 2003, **206**:1809–1815.
44. Crooks E, Randolph SE: **Walking by *Ixodes ricinus* ticks: intrinsic and extrinsic factors determine the attraction of moisture or host odour.** *J Exp Biol* 2006, **209**:2138–2142.
45. Greenfield BPJ: **Environmental parameters affecting tick (*Ixodes ricinus*) distribution during the summer season in Richmond Park, London.** *Bioscience Horizons* 2011, **0**(0):1–9.

46. Oorebeek M, Sharrad T, Kleindorfer S: **What attracts larval *Ixodes hirsti* (Acari: Ixodidae) to their host?** *Parasitol Res* 2009, **104**:623–628.
47. Lees AD: **The behaviour and physiology of ticks.** *Acarol* 1969, **11**(3):397–410.
48. Elizarov YA, Vasewta AA: **A distant orientation of blood-sucking ixodid ticks to the host's attractant factors. (in Russian).** *Parazit Sb* 1976, **10**:136–141.
49. Tack W, Madder M, De Frenne P, Vanhellemont M, Gruwez R, Verheyen K: **The effects of sampling method and vegetation type on the estimated abundance of *Ixodes ricinus* ticks in forests.** *Exp Appl Acarol* 2011, **54**:285–292.

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CASE REPORT Open Access

First report of *Borrelia burgdorferi* sensu lato in two threatened carnivores: the Marbled polecat, *Vormela peregusna* and the European mink, *Mustela lutreola* (Mammalia: Mustelidae)

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Abstract

Background: Lyme disease is a widespread cosmopolitan zoonosis caused by species belonging to the genus *Borrelia*. It is transmitted from animal reservoir hosts to humans through hard - ticks of genus *Ixodes* which are vectors of the disease.

Case presentation: *Borrelia burgdorferi* sensu lato infection was identified in a marbled polecat, *Vormela peregusna*, and two European minks, *Mustela lutreola*, from Romania, by PCR. RFLP revealed the presence of a single genospecies, *Borrelia burgdorferi* sensu stricto.

Conclusions: This is the first report of the Lyme disease spirochetes in the two mentioned hosts.

Keywords: *Borrelia burgdorferi* s.s, First report, *Mustela lutreola*, *Vormela peregusna*, Romania

Background

Borrelia burgdorferi sensu lato is the causative agent of Lyme borreliosis, the most widespread vector-borne disease in the cool-temperate regions of the Northern hemisphere. The medical importance of this pathogen is generally restricted to humans and few domestic species. However, as for many vector-borne diseases, the key to the understanding of the epidemiology of Lyme borreliosis consists in revealing the ecological relationships that exist between pathogens, vectors and wildlife hosts [1]. All 18 currently recognized genotypes [2] circulate in nature between vectors (several ticks of genus *Ixodes*) and reservoir hosts (various vertebrates) which are able to maintain and transmit the spirochetes [3].

Case presentation

Between 2009 and 2011, 5 specimens belonging to two species of threatened mustelids were brought to the Laboratory of Parasitology and Parasitic Diseases in deep frozen state (Table 1). The dead animals were collected either as road kills (*Vormela peregusna*) or as accidental casualties of live-trapping during field studies (*Mustela lutreola*). The trapping was performed as part of biodiversity and ecology studies in the central part of the Danube Delta using box traps [4]. The accidental death of animals in live traps was caused by extreme morning frost or poor overall health status caused by floods (Kiss JB personal communication). Examination of the fur and skin did not reveal the presence of external parasites.

During the necropsy of these animals, tissue samples (myocardium) were collected and processed for DNA extraction (Qiagen, DNeasy Blood & Tissue Kit). An extraction blank was included in each extraction procedures to control the cross-contamination between extracts. The crude DNA was analyzed by a PCR protocol according to Priem et al. (1997) [5] using primers (Generi Biotech) for *Borrelia burgdoferi* sensu lato (5'-GGGAATAGGTCTAATTTAGCC-3', 5'-CACTAA

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Table 1 The collection localities of threatened Mustelidae specimens used in this study

Sample	Species	Locality (County*)	Coordinates	Date
ML1	European mink (*Mustela lutreola*)	Canal Litcov (TL)	45°08'N 29°19'E	05.03.2010
ML2	European mink (*Mustela lutreola*)	Canal Litcov (TL)	45°08'N 29°18'E	06.03.2010
ML3	European mink (*Mustela lutreola*)	Canal Litcov (TL)	45°08'N 29°18'E	06.03.2010
VP1	Marbled polecat (*Vormela peregusna*)	Sinoe (CT)	44°37'N 28°43'E	30.11.2009
VP2	Marbled polecat (*Vormela peregusna*)	Agighiol (TL)	45°02'N 28°53'E	10.06.2011

* CT: Constanța; TL: Tulcea.

TTGTTAAAGTGGAAGT-3') targeting the OspA gene [6]. Each time the PCR was performed including negative control samples. The positive PCR products were further analyzed by RFLP using two restriction enzymes *Alw*I (BsPI) and *Mse*I (Tru1 I) (Fermentas), according to the manufacturers protocol. PCR analysis revealed positivity to *Borrelia burgdorferi* sensu lato in three samples (ML2, ML3 and VP1) belonging to the two host species: *M. lutreola* and *V. peregusna*.

Analysis of the RFLP pattern of the amplified OspA gene cut, showed bands at 183/134/74 base pairs (bp) for *Mse*I (Tru1 I) (Figure 1A) restriction enzyme and a second pattern at 227/164 bp for *Alw*I (BsPI), which is indicative of *Borrelia burgdorferi* sensu stricto (s.s.) in all three positive samples (Figure 1B).

Discussion

Borrelia burgdorferi is maintained in natural cycles of infection by vector ticks and reservoir hosts. In Western and Central Europe, *Ixodes ricinus* is particularly important in the transmission of the Lyme spirochete. Moreover, *I. hexagonus* has been experimentally confirmed as a vector [7], but was also shown to be important in secondary cycles of *B. burgdorferi* transmission [1,8]. Both of them, together with other congeneric ticks, are well represented in mustelids (Table 2). Although ticks are commonly found on mustelids, *B. burgdorferi* s.l. infection is rarely reported. The scarcity of reports can be explained either by the fact that the infection is rare or, most probably by the lack of studies on this topic.

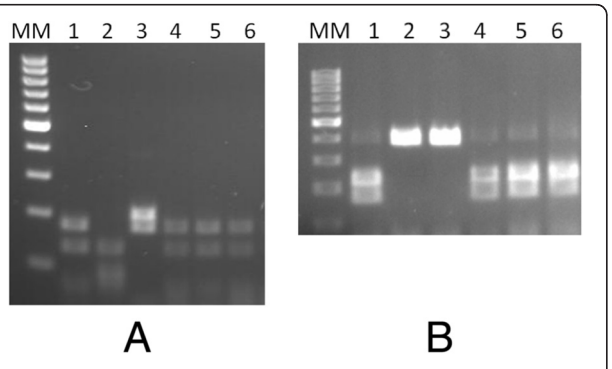

Figure 1 RFLP pattern of the amplified *ospA* gene cut with MseI (TruII) (A) and AlwI (BsPI) (B): M - 100 bp Molecular ladder (Fermentas). 1-3 – positive controls: *B. burgdorferi s.s., B. garinii, B. afzelii*, 4 - *B. burgdorferi s.s.* (*Vormela peregusna*), 5 - *B. burgdorferi s.s.* (*Mustela lutreola*), 6 - *B. burgdorferi s.s.* (*Mustela lutreola*).

Several authors have previously reported the presence of *Borrelia burgdorferi* s.l. in *Ixodes* ticks removed from mustelids: European badger (*Meles meles*) [9], American mink (*Mustela vison*) [10], weasel (*Mustela nivalis*) [11], beach marten (*Martes foina*), European polecat (*Mustela putorius*), and stoat (*Mustela ermina*) [12]. In Italy, none of the *I. hexagonus* and *I. ricinus* collected from beech marten was positive by PCR for *B. burgdorferi* s.l. [13].

An interesting study from Switzerland, where questing ticks were analyzed for the host of the previous feeding stage by Reverse Line Blotting revealed the presence of *Borrelia burgdorferi* s.l. DNA in specimens positive for *Meles meles* and *Mustela putorius* probes. The authors suggest the role of mustelids as reservoir hosts for *Borrelia burgdorferi* [14].

Other authors examined mustelids for the presence of anti-*Borrelia burgdorferi* s.l. antibodies. One least weasel caught in forests of the western part of France was seropositive for *B. burgdorferi* at 1/50 antibody titer [15], however stoats from Canada infested with *I. Scapularis* (the most important vector of Lyme disease in North America) were seronegative [16].

To our knowledge, there is a single study showing the presence of *B. burgdorferi* s.l. DNA in tissues of mustelids. McDonald and Lariviere (2001) [17] found the infection in 10 of 45 stoats from Great Britain. However, no information was provided on the tissues examined or the genospecies identified. Here we report the first identification of *B. burgdorferi* s.l. from the myocardium of a marbled polecat and two European minks. The Marbled polecat, *Vormela peregusna* is widespread from Southeastern Europe to Russia, China and northern Africa [18]. It is listed by IUCN as vulnerable. The European mink, *Mustela lutreola* is a semi-aquatic species of mustelid native to Europe and it is listed by IUCN as Critically Endangered. Its current distribution includes small isolated populations in northern Spain, western France and Eastern Europe (Latvia, Estonia, Belarus, Ukraine, central regions of European Russia, the Danube Delta in Romania and northwestern Bulgaria) [19]. In Romania,

Table 2 Check-list of Ixodid tick species associated with mustelids

Ticks species	Mustelid hosts
I. acuminatus	*Mustela nivalis* [22], *Mustela putorius* [22], *Mustela vison* [23]
I. angustus	*Mustela erminea* [24]
I. cookei	*Mustela frenata* [25], *Mustela nivalis* [26], *Mustela vison* [26,27]
I. crenulatus	*Martes foina* [28], *Meles meles* [24], *Mustela erminea* [29],
	Mustela lutreola [30], *Mustela nivalis* [28], *Mustela putorius* [31], *Mustela vison* [23],
I. gregsoni	*Martes americana* [32], *Martes pennanti* [33], *Mustela* sp. [32], *Mustela vison* [32,33]
I. hexagonus	*Martes foina* [13,31,34-36], *Mustela putorius* [31,35,36],
	Mustela erminea [29,31,35-37], *Mustela nivalis* [31,38],
	M. putorius furo [31,39], *Meles meles* [34-36], *Mustela vison* [23]
I. muris	*Mustela vison* [40]
I. nipponensis	*Mustela sibirica* [41]
I. pacificus	*Mustela frenata* [42], *Taxidea taxus* [42]
I. persulcatus	*Martes flavigula* [37], *Martes martes* [37], *Meles meles* [37], *Mustela erminea* [37], *Mustela nivalis* [37], *Mustela putorius* [37], *Mustela sibirica* [37]
I. ricinus	*Martes foina* [13,22,35,37], *Martes martes* [37], *Meles meles* [14,35,37], *Mustela erminea* [14,29], ***Mustela lutreola*** [37],
	Mustela nivalis [37], *Mustela putorius* [14,37], *Mustela vison* [23]
I. rugicollis	*Martes* sp. [43]
I. scapularis	*Mustela erminea* [16], *Mustela frenata* [25]
I. tanuki	*Martes flavigula* [44]
I. texanus	*Mustela frenata* [25]
I. ventalloi	*Mustela nivalis* [38]

both species are present in the Southeastern part of the country, Dobrogea [20,21].

Conclusions

Detection of Lyme spirochete is relatively rare in mustelids, suggesting the limited role of these hosts in natural foci. The detection of pathogens in tissues or in ticks feeding on various vertebrates does not confer to these hosts the status of reservoir host, but rather carrier hosts. Hence, although it has been suggested that mustelids are reservoir hosts for *B. burgdorferi* s.l., experimental transmission is required to clarify this aspect.

Competing interests

All authors have seen and approved the manuscript and declare that they have no competing interest.

Authors' contributions

CMG conceived the study and drafted the manuscript. ADS made major contribution to the study design, identified the mustelid species and contributed to the writing of the manuscript. JK performed PCR and RFLP. MM had trapped the mustelids. ADM contributed to the writing of the manuscript. All authors read and approved the final manuscript.

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References

1. Gern L, Humair P: **Ecology of *Borrelia burgdorferi* sensu lato in Europe**. In *Lyme Borreliosis: Biology, Epidemiology and Control*. Edited by Gray JS KO, Lane RS, Stanek G. New York: CABI Publishing; 2002:149–174.
2. Stanek G, Reiter M: **The expanding Lyme Borrelia complex-clinical significance of genomic species?** *Clin Microbiol Infect* 2011, **17**(4):487–493.
3. Kahl O, Gern L, Eisen L, Lane RS: **Ecological research on *Borrelia burgdorferi* sensu lato: terminology and some methodological pitfalls**. In *Lyme Borreliosis: Biology, Epidemiology and Control*. Edited by Gray JS KO, Lane RS, Stanek G. New York: CABI Publishing; 2002:29–46.
4. Kranz A, Toman A, Marinov M Jr, Kiss JB: **European mink research: results of the spring 2004 expedition.** *Sci Ann Danube Delta Inst Res Develop* 2005, **11:**42–44.
5. Priem S, Rittig MG, Kamradt T, Burmester GR, Krause A: **An optimized PCR leads to rapid and highly sensitive detection of *Borrelia burgdorferi* in patients with Lyme Borreliosis.** *J Clin Microbiol* 1997, **35**(3):685–690.
6. Floris R, Menardi G, Bressan R, Trevisan G, Ortenzio S, Rorai E, Cinco M: **Evaluation of a genotyping method based on the ospA gene to detect *Borrelia burgdorferi* sensu lato in multiple samples of Lyme borreliosis patients.** *New Microbiol* 2007, **30:**399–410.
7. Gern L, Toutoungi LN, Hu CM, Aeschlimann A: ***Ixodes* (*Pholeoixodes*) *hexagonus*, an efficient vector of *Borrelia burgdorferi* in the laboratory.** *Med Vet Entomol* 1991, **5:**431–435.
8. Gern L, Rouvinez E, Toutoungi LN, Godfroid E: **Transmission cycles of *Borrelia burgdorferi* sensu lato involving *Ixodes ricinus* and/or *I. hexagonus* ticks and the European hedgehog, *Erinaceus europaeus*, in suburban and urban areas in Switzerland.** *Folia Parasitol* 1997, **44:**309–314.
9. Gern L, Sell K: **Isolation of *Borrelia burgdorferi* sensu lato from the skin of the European badger (*Meles meles*) in Switzerland.** *Vector Borne Zoonotic Dis* 2009, **9**(2):207–208.
10. Magnarelli LA, Swihart RK: **Spotted fever group Rickettsiae or *Borrelia burgdorferi* in *Ixodes cookei* (Ixodidae) in Connecticut.** *J Clin Microbiol* 1991, **29**(7):1520–1522.
11. Gassner F, Verbaarschot P, Smallegange RC, Spitzen J, Van Wieren SE, Takken W: **Variations in *Ixodes ricinus* density and *Borrelia* infections associated with cattle introduced into a woodland in The Netherlands.** *Appl Environ Microbiol* 2008, **74**(23):7138.
12. Piesman J, Gern L: **Lyme borreliosis in Europe and North America.** *Parasitol* 2004, **129**(Suppl1):S191–S220.
13. Lorusso V, Lia RP, Dantas-Torres F, Mallia E, Ravagnan S, Capelli G, Otranto D: **Ixodid ticks of road-killed wildlife species in southern Italy: new tick-host associations and locality records.** *Exp Appl Acarol* 2011, **55:**293–300.
14. Morán Cadenas F, RAIS O, Humair PF, Douet V, Moret J, Gern L: **Identification of host bloodmeal source and *Borrelia burgdorferi* Sensu Lato in field-collected *Ixodes ricinus* ticks in Chaumon (Switzerland).** *J Med Entomol* 2007, **44**(6):1109–1117.
15. Doby JM, Betremieux C, Lambert MC, Lorvelec O, Rolland C, Costil C: **Les micromammiferes forestiers reservoirs de germes pour *Borrelia***

burgdorferi agent de la borreliose de Lyme ? Etude serologique de 296 animaux dans l'ouest de la France. *Rev Med Vet* 1991, **142**(10):737–742.
16. Bouchard C, Beauchamp G, Nguon S, Trudel L, Milord F, Lindsay LR, Bélanger D, Ogden NH: **Associations between *Ixodes scapularis* ticks and small mammal hosts in a newly endemic zone in southeastern Canada: Implications for *Borrelia burgdorferi* transmission.** *Ticks Tick Borne Dis* 2011, **2:**183–190.
17. McDonald RA, Lariviere S: **Diseases and pathogens of Mustela spp., with special reference to the biological control of introduced stoat *Mustela erminea* populations in New Zealand.** *J Royal Soc New Zealand* 2001, **31**(4):721–744.
18. Schreiber A, Wirth R, Riffel M, Van Rompaey H: *Weasels, Civets, Mongooses and their relatives: an action plan for the conservation of mustelids and viverrids*. Broadview: Kelvyn Press Inc; 1989.
19. Maran T, Skumatov D, Palazón S, Gomez A, Põdra M, Saveljev A, Kranz A, Libois R, Aulagnier S: ***Mustela lutreola**. In IUCN 2011. IUCN Red List of Threatened Species. Version 2011.2. <www.iucnredlist.org> Downloaded on 04 April 2012*; 2012.
20. Călinescu R: **[Mammals of Romania. Distribution and biogeographical-economic problems].** *Buletinul Ministerului Agriculturii Domeniilor* 1931, **251:**19–81 [in Romanian].
21. Cuzic M, Murariu D: **[Data on the presence of the Marbled Polecat (*Vormela peregusna* Güld. 1770) in south-east Romania].** *Studii Cercetări Științele Naturii Muzeologie* 2006, **3:**193–198.
22. Santos-Silva MM, Beati L, Santos AS, De Sousa R, Núncio MS, Melo P, Santos-Reis M, Fonseca C, Formosinho P, Vilela C, Bacellar F: **The hard-tick fauna of mainland Portugal (Acari: Ixodidae): an update on geographical distribution and known associations with hosts and pathogens.** *Exp Appl Acarol* 2011, **55:**85–121.
23. Page RJC, Langton SD: **The occurrence of Ixodid ticks on wild mink *Mustela vison* in England and Wales.** *Med Vet Entomol* 1996, **10:**359–364.
24. Murrell BP, Durden LA, Cook JA: **Host associations of the tick, *Ixodes angustus* (Acari: Ixodidae), on Alaskan Mammals.** *J Med Entomol* 2003, **40**(5):682–685.
25. Carey AB, Krinsky WL, Main J: ***Ixodes dammini* (Acari: Ixodidae) and associated did ticks in south-central Connecticut, USA.** *J Med Entomol* 1980, **17:**89–99.
26. Whitaker JO Jr, Goff R: **Ectoparasites of wild carnivora of Indiana.** *J Med Entomol* 1979, **15**(5–6):425–430.
27. Kollars TM Jr, Oliver JH Jr: **Host associations and seasonal occurrence of *Haemaphysalis leporispalustris*, *Ixodes brunneus*, *I. cookei*, *I. dentatus*, and *I. texanus* (Acari: Ixodidae) in southeastern Missouri.** *J Med Entomol* 2003, **40**(1):103–107.
28. Caeiro V, Santos-Silva MM, Simões AL, Núncio MS: ***Ixodes* (Pholeoixodes) *canisuga* Johnston, 1949 uma espécie nova na ixodofauna em Portugal.** *Rev Port Cinc Vet* 1997, **92:**120–123.
29. Sleeman DP: **Ectoparasites of the Irish stoat.** *Med Vet Entomol* 1989, **3:**213–218.
30. Corbet GB, Harris S: *The Handbook of British Mammals*. Oxford: Blackell Scientific Publications; 1991.
31. Von Liebisch A, Walter G: **Untersuchungen von Zecken bei Haus- und Wildtieren in Deutschland: Zum Vorkommen und zur Biologie der Igelzecke (*Ixodes hexagonus*) und der Fuchszecke (*Ixodes canisuga*).** *Dtsch Tierarztl Wschr* 1986, **93:**377–464.
32. Lindquist EE, Wu KW, Redner JH: **A new species of the tick genus *Ixodes* (Acari: Ixodidae) parasitic on mustelids (Mammalia: Carnivora) in Canada.** *Can Entomol* 1999, **131:**151–170.
33. Lubelczyka CB, Hanson T, Lacombe EH, Holman MS, Keirans JE: **First U.S. Record of the Hard Tick *Ixodes* (Pholeoixodes) *gregsoni* Lindquist, Wu, and Redner.** *J Parasitol* 2007, **93**(3):718–719.
34. Dias JATS, Núncio MS, Gongalves ACB: **Contribuição para a elaboração de um inventário da fauna ixodoideológica (Acarina-Ixodoidea) de Portugal.** *Garcia Orta Sér Zool* 1994, **20:**49–68.
35. Dominguez G: **North Spain (Burgos) wild mammals ectoparasites.** *Parasite* 2004, **11:**267–272.
36. Toutoungi LN, Gern L, Aeschlimann A, Debrot S: **A propos du genre *Pholeoixodes*, parasite des carnivores en Suisse.** *Acarologia* 1991, **32**(4):311–328.
37. Anderson JF, Magnarelli LA: **Epizootiology of Lyme Disease-Causing Borreliae.** *Clin Dermatol* 1993, **11:**339–351.
38. Dias JATS, Santos-Reis M: **A carraqa *Ixodes ventalloi* Gil Collado, 1936 como principal ectoparasita de uma populao de doninhas (*Mustela nivalis* Linnaeus, 1766) em Portugal.** *Garcia Orta Sér Zool* 1989, **14:**35–50.
39. Beichel E, Petney TN, Hassler D, Brückner M, Maiwald M: **Tick infestation patterns and prevalence of *Borrelia burgdorferi* in ticks collected at a veterinary clinic in Germany.** *Vet Parasitol* 1996, **65**(1–2):147–155.
40. Walker ED, Stobierski MG, Poplar ML, Smith TW, Murphy AJ, Smith PC, Schmitt SM, Cooley TM, Kramer CM: **Geographic distribution of ticks (Acari: Ixodidae) in Michigan, with special emphasis on *Ixodes scapularis* and *Borrelia burgdorferi*.** *J Med Entomol* 1998, **35:**872–882.
41. Kim HC, Han SH, Chong ST, Klein TA, Choi CY, Nam HY, Chae HY, Lee H, Ko S, Kang JG, Chae JS: **Ticks collected from selected mammalian hosts surveyed in the Republic of Korea during 2008–2009.** *Korean J Parasitol* 2011, **49**(3):331–335.
42. Furman DE, Loomis EC: **The ticks of California (Acari: Ixodida).** *Bull Calif Insect Surv* 1984, **25:**1–239.
43. Kolonin GV: *World distribution of ixodid ticks (genus Ixodes)*. Nauka: Moscow; 1981 [In Russian].
44. Kitaoka S, Suzuki H: **Studies on the parasite fauna of Thailand. 5. Parasitic ticks on mammals and description of *Ixodes siamensis* sp. n. and *Rhipicephalus tetracornus* sp. n. (Acarina: Ixodidae).** *Trop Med* 1983, **25:**205–219.

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Parasites & Vectors

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Tick parasites of rodents in Romania: host preferences, community structure and geographical distribution

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Abstract

Background: Ticks are among the most important vectors of zoonotic diseases in temperate regions of Europe, with widespread distribution and high densities, posing an important medical risk. Most ticks feed on a variety of progressively larger hosts, with a large number of small mammal species typically harbouring primarily the immature stages. However, there are certain Ixodidae that characteristically attack micromammals also during their adult stage. Rodents are widespread hosts of ticks, important vectors and competent reservoirs of tick-borne pathogens. Micromammal-tick associations have been poorly studied in Romania, and our manuscript shows the results of a large scale study on tick infestation epidemiology in rodents from Romania.

Methods: Rodents were caught using snap-traps in a variety of habitats in Romania, between May 2010 and November 2011. Ticks were individually collected from these rodents and identified to species and development stage. Frequency, mean intensity, prevalence and its 95% confidence intervals were calculated using the EpiInfo 2000 software. A p value of <0.05 was considered statistically significant.

Results: We examined 423 rodents (12 species) collected from six counties in Romania for the presence of ticks. Each collected tick was identified to species level and the following epidemiological parameters were calculated: prevalence, mean intensity and mean abundance. The total number of ticks collected from rodents was 483, with eight species identified: *Ixodes ricinus*, *I. redikorzevi*, *I. apronophorus*, *I. trianguliceps*, *I. laguri*, *Dermacentor marginatus*, *Rhipicephalus sanguineus* and *Haemaphysalis sulcata*. The overall prevalence of tick infestation was 29.55%, with a mean intensity of 3.86 and a mean abundance of 1.14. Only two polyspecific infestations were found: *I. ricinus* + *I. redikorzevi* and *I. ricinus* + *D. marginatus*.

Conclusions: Our study showed a relatively high diversity of ticks parasitizing rodents in Romania. The most common tick in rodents was *I. ricinus*, followed by *I. redikorzevi*. Certain rodents seem to host a significantly higher number of tick species than others, the most important within this view being *Apodemus flavicollis* and *Microtus arvalis*. The same applies for the overall prevalence of tick parasitism, with some species more commonly infected (*M. arvalis*, *A. uralensis*, *A. flavicollis* and *M. glareolus*) than others. Two rodent species (*Mus musculus*, *Rattus norvegicus*) did not harbour ticks at all. Based on our results we may assert that rodents generally can act as good indicators for assessing the distribution of certain tick species.

Keywords: Hard-ticks, Ixodidae, Rodents, Micromammals, Romania

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Background

Rodents (Order Rodentia) are usually small-sized mammals with a worldwide distribution, accounting for over 40% of all mammal species. Rodents are both widespread and abundant, as are their associated ticks. Thus, mainly from a human health perspective, the rodent-tick associations have a huge importance in most ecosystems [1]. Besides their role as tick hosts, rodents serve as reservoirs of tick-borne pathogens, hence increasing their importance in the eco-epidemiology of diseases like Lyme borreliosis, rickettsiosis, babesiosis, ehrlichiosis or tularaemia [1-3].

Most of the hard ticks feeding on rodents follow a three-host life cycle (i.e. each of the active stages - larva, nymph and adult - feeds on a different host individual). Usually, these ticks feed on a variety of progressively larger hosts, meaning that a large number of small mammal species typically harbour the immature stages [1]. On the other hand, there are certain Ixodidae that characteristically attack micromammals also during their adult stage. One of the most comprehensive reviews on micromammal-tick associations [1] lists 14 species of adult Ixodidae parasitic on rodents (*Anomalohimalaya cricetuli, A. lama, A. lotozskyi, Haemaphysalis verticalis, Ixodes angustus, I. apronophorus, I. crenulatus, I. laguri, I. nipponensis, I. occultus, I. pomerantzevi*, *I. redikorzevi, I. trianguliceps, Rhipicephalus fulvus*). However, the variety of species parasitizing rodents as immature stages is much higher [1].

The importance of hard-ticks in the epidemiology of several human vector-borne infections has received considerable attention in recent years and will certainly offer an opportunity for new studies in the years to come. The ecology of tick-borne infections is a popular field in parasitology and besides the research focused on the molecular epidemiology of tick-borne pathogens, studies on host preferences, seasonal variation and community structure are nevertheless important. From their reservoir-host perspective, rodents are known to act as key ecological links in the very complex transmission chains of tick-borne diseases as Lyme borreliosis or viral encephalitis [1,4].

Romania has an outstanding position in terms of biodiversity, being the only European country with five ecoregions on its territory [5]. This unique situation created a wide range of habitats and is mirrored by the number of mammal species present (112 species) [6]. Moreover, Romania not only holds this high biodiversity (especially among rodents [7]), but has nearly half of its human population living and working in rural areas and maintaining close contacts with nature [8], creating an interesting situation for epidemiological processes. Thirty-two species of wild rodents are known to occur in Romania [6]. Both this habitat variety and available host diversity [9] account for relatively high tick species diversity in Romania (25 species) [10], as compared to neighbouring countries [11]. However, micromammal-tick associations have been poorly studied in Romania despite the importance of each in the ecology of public pathogens. In this context, our manuscript shows the results of a study of tick infestation epidemiology in rodents from Romania.

Table 1 Rodent species collected (total number, number by county and by month)

Species	By County	By Month
Apodemus agrarius (n=94)	Buzău (n=2) Cluj (n=72) Constanța (n=3) Mureş (n=17)	April (n=5) May (n=4) August (n=3) September (n=27) October (n=47) December (n=8)
Apodemus flavicollis (n=51)	Bacău (n=1) Cluj (n=17) Mureş (n=28) Tulcea (n=5)	April (n=4) May (n=8) August (n=12) September (n=6) October (n=15)
Apodemus sylvaticus (n=22)	Cluj (n=8) Constanța (n=10) Mureş (n=3) Tulcea (n=1)	April (n=3) May (n=3) June (n=1) September (n=2) October (n=10) December (n=3)
Apodemus uralensis (n=24)	Constanța (n=18) Harghita (n=2) Mureş (n=2) Tulcea (n=2)	April (n=5) May (n=2) October (n=17)
Myodes glareolus (n=32)	Cluj (n=6) Mureş (n=26)	May (n=2) August (n=7) October (n=23)
Micromys minutus (n=11)	Cluj (n=7) Constanța (n=3) Tulcea (n=1)	April (n=1) July (n=1) October (n=8) December (n=1)
Microtus arvalis (n=54)	Cluj (n=5) Constanța (n=39) Mureş (n=10)	April (n=1) May (n=4) June (n=2) August (n=3) September (n=1) October (n=41) November (n=1) December (n=1)
Microtus subterraneus (n=49)	Cluj (n=44) Harghita (n=1) Mureş (n=4)	May (n=5) June (n=1) August (n=1) September (n=21) October (n=18) December (n=5)
Mus musculus (n=53)	Cluj (n=47) Harghita (n=5) Mureş (n=1)	Aprilie (n=3) May (n=2) June (n=1) August (n=2) September (n=25) October (n=15) November (n=5)
Mus spicilegus (n=8)	Bacău (n=1) Cluj (n=1) Constanța (n=1) Tulcea (n=5)	April (n=2) July (n=5) September (n=1)
Rattus norvegicus (n=12)	Cluj (n=10) Harghita (n=1) Mureş (n=1)	April (n=1) June (n=1) July (n=1) September (n=1) October (n=5) November (n=3)
Spermophilus citellus (n=13)	Constanța (n=1) Tulcea (n=12)	

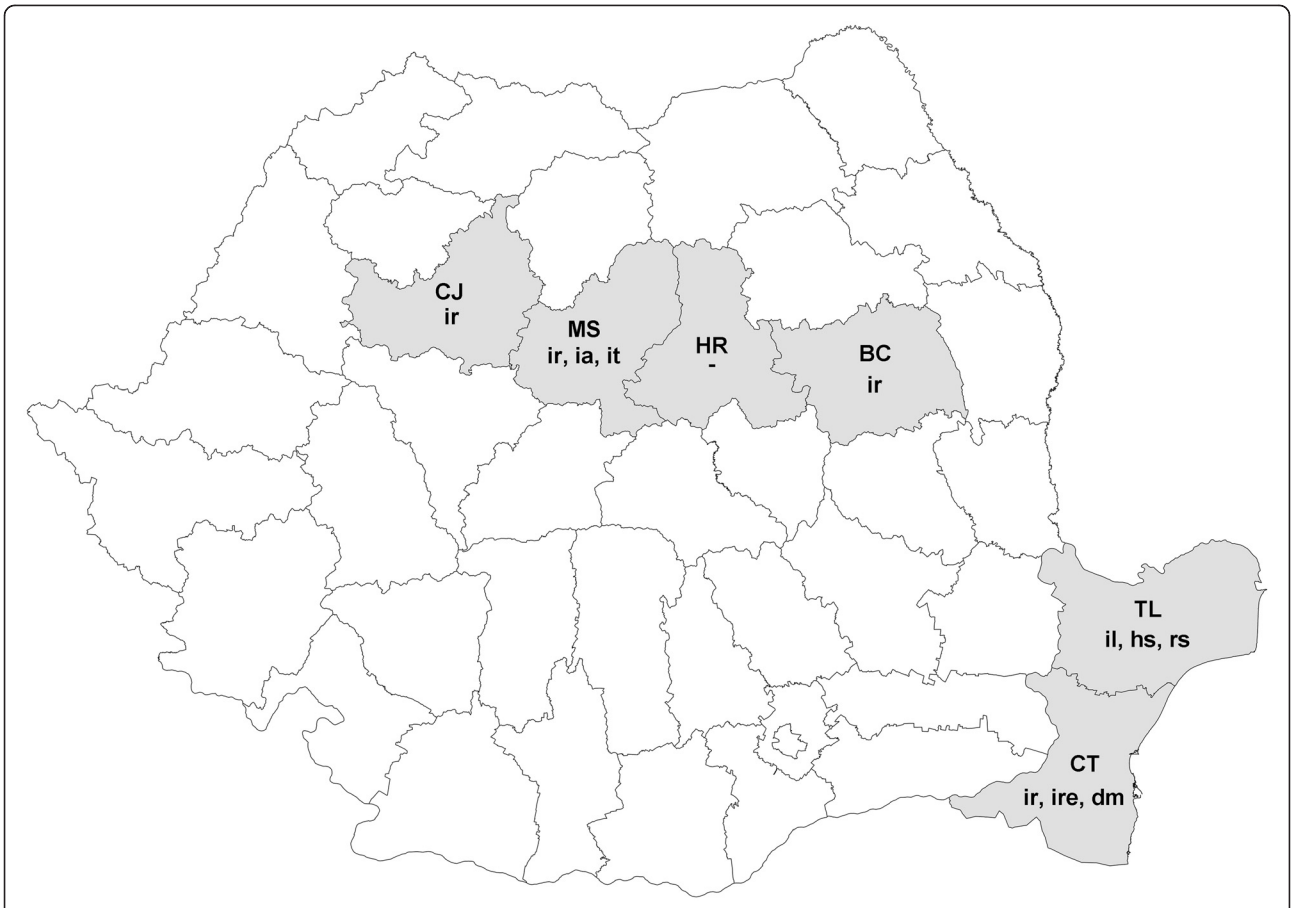

Figure 1 Geographical distribution of ticks collected from rodents (county names: BC - Bacău, CJ - Cluj, CT - Constanța, HR - Harghita, MS - Mureș, TL - Tulcea; tick species: dm - *Dermacentor marginatus*, hs - *Haemaphysalis sulcata*, ia - *Ixodes apronophorus*, il - *Ixodes laguri*, ire - *Ixodes redikorzevi*, ir - *Ixodes ricinus*, it - *Ixodes trianguliceps*, rs - *Rhipicephalus sanguineus*).

Methods

423 rodents from 12 species (Table 1) were collected from a variety of habitats in Romania between May 2010 and November 2011 (Figure 1). Rodents were caught using overnight snap-traps with peanut butter or chocolate bait. The traps were controlled early in the morning and the captured animals were immediately transferred to individual plastic zip bags and frozen. Each individual rodent was carefully checked for the presence of ectoparasites under a dissection microscope in the laboratory. All collected ticks were fixed in 70% ethanol for subsequent examination. Identification to species level was done according to morphological keys [12,13]. Identification of rodent species was carried out according to Aulaigner *et al*. 2009 [14]. Digital maps were created using ArcGis/ArcMap 9.2 (ESRI, © 1999–2006). The following epidemiological parameters were calculated: prevalence (per cent of infested animals from the total number of examined animals), mean intensity (total number of ticks collected per total number of infested animals) and mean abundance (total number of ticks collected per total number of examined animals) [15]. Frequency, prevalence and its 95% confidence intervals were calculated using the EpiInfo 2000 software. A p value of <0.05 was considered statistically significant.

Results

From the total of 423 examined animals, 125 (29.55%) harboured ticks with a mean intensity of 3.86 and a mean abundance of 1.14 (Table 2). The highest prevalence of tick infestation was found in *Microtus arvalis* (70.37%) while two species did not harbour ticks at all (*Mus musculus*, *Rattus norvegicus*). The highest intensity was found in *Apodemus agrarius* (7.10) and the highest mean abundance in *M. arvalis* (2.87).

The total number of ticks collected from rodents was 483, with eight species identified (Table 3). The dominant species was *I. ricinus* (71.01%), followed by *I. redikorzevi* (23.60%) and *I. apronophorus* (2.48%). The other 5 species accounted each for less than 1.5% from the total of the collected ticks. The majority of *I. ricinus* collected were

Table 2 Prevalence, intensity and abundance of hard-tick parasitism in rodents by host species

Host	Examined (n)	With ticks (n)	Prevalence (%)	Intensity (range; mean±sd)	Abundance (mean±sd)
Apodemus agrarius	94	21	22.34	1-67; 7.10±14.16	1.59±7.21
Apodemus flavicollis	51	26	50.98	1-12; 3.65±3.24	1.86±2.94
Apodemus sylvaticus	22	4	18.18	1-5; 2.50±1.91	0.45±1.22
Apodemus uralensis	24	13	54.17	1-6; 2.69±1.97	1.46±1.98
Myodes glareolus	32	16	50.00	1-4; 1.69±1.01	0.84±1.11
Micromys minutus	11	2	18.18	1; 1.00±0.00	0.18±0.40
Microtus arvalis	54	38	70.37	1-25; 4.08±4.25	2.87±4.01
Microtus subterraneus	49	2	4.08	2; 2.00±0.00	0.08±0.40
Mus musculus	53	0	0.00	-	-
Mus spicilegus	8	1	12.50	1; 1.00±0.00	0.13±0.35
Rattus norvegicus	12	0	0.00	-	-
Spermophilus citellus	13	2	15.38	1-4; 2.50±2.12	0.38±1.12
Total	**423**	**125**	**29.55**	**1-67; 3.86±6.58**	**1.14±3.98**

larvae (76.97%), while in case of *I. redikorzevi,* nymphs were predominant (82.46%).

The highest overall prevalence was recorded for *I. ricinus* (20.57% of rodents infested) followed by *I. redikorzevi* (7.09%). All other ticks species had prevalences below 0.5% (Table 4). Only two hosts had polyspecific parasitism, with *I. ricinus* + *I. redikorzevi* and *I. ricinus* + *Dermacentor marginatus* respectively.

The highest number of host species was recorded for *I. ricinus* (8 host species) followed by *I. redikorzevi* (3 host species) and *Rhipicephalus sanguineus* (2 host species). All the other tick species were found only on a single host species (Table 5). Adult ticks (regardless of the species) were found on 5 host species, nymphs on 6 host species and larvae on 7 species (Table 5).

Table 3 Developmental stage distribution of ticks feeding on rodents in Romania (number and percentage of all collected)

Tick species	Total number of ticks	Adults	Nymphs	Larvae
Ixodes ricinus	343 (71.01)	16 (4.66)	63 (18.37)	264 (76.97)
Ixodes redikorzevi	114 (23.60)	20 (17.54)	94 (82.46)	0 (0.00)
Ixodes laguri	1 (0.21)	1 (100)	0 (0.00)	0 (0.00)
Ixodes apronophorus	12 (2.48)	0 (0.00)	0 (0.00)	12 (100)
Ixodes trianguliceps	2 (0.41)	1 (50.00)	0 (0.00)	1 (50.00)
Dermacentor marginatus	1 (0.21)	1 (100)	0 (0.00)	0 (0.00)
Rhipicephalus sanguineus	6 (1.24)	0 (0.00)	2 (33.33)	4 (66.67)
Haemaphysalis sulcata	4 (0.83)	0 (0.00)	0 (0.00)	4 (100)
Total	483 (100)	39 (8.07)	159 (32.92)	285 (59.01)

The regional distribution of ticks parasitizing rodents shows that certain species were found in both examined regions (i.e. *I. ricinus* central and south-eastern Romania), while others were restricted to the central part (*I. apronophorus*, *I. trianguliceps*) or the south-eastern part (*I. laguri*, *Haemaphysalis sulcata*, *R. sanguineus*, *I. redikorzevi*) (Figure 1).

Discussion

Host preferences

In the case of Lyme borreliosis, small mammals are the vertebrate group that has been the most extensively investigated up to now, mainly because they can be easily captured in large numbers, handled and maintained

Table 4 Prevalence of developmental stages by tick species (number and percentage of all collected)

Tick species	Number of rodents infested	Host with adults	Host with nymphs	Host with larvae
Ixodes ricinus	87 (20.57)	6 (6.90)	28 (32.18)	64 (73.56)
Ixodes redikorzevi	30 (7.09)	12 (40.00)	23 (76.67)	0 (0.00)
Ixodes laguri	1 (0.24)	1 (100.0)	0 (0.00)	0 (0.00)
Ixodes apronophorus	2 (0.47)	0 (0.00)	0 (0.00)	2 (100)
Ixodes trianguliceps	1 (0.24)	1 (100)	0 (0.00)	1 (100)
Dermacentor marginatus	1 (0.24)	1 (100)	0 (0.00)	0 (0.00)
Rhipicephalus sanguineus	2 (0.47)	0 (0.00)	2 (100)	1 (50.00)
Haemaphysalis sulcata	1 (0.24)	0 (0.00)	0 (0.00)	1 (100)
Total	**125 (29.55)***	**21 (16.80)**	**53 (42.40)**	**69 (55.20)**

*2 animals with polyspecific infestation.

Table 5 Tick-rodent associations in Romania

Tick species	Hosts for adults	Hosts for nymphs	Hosts for larvae	Host species
Ixodes ricinus	*Aa, Mm, Ma*	*Aa, Af, As, Au, Ma*	*Aa, Af, As, Au, Mg, Ma, Msu*	*Aa, Af, As, Au, Ma, Mg, Mm, Msu*
Ixodes redikorzevi	*Au, Ma, Mm*	*Au, Ma*	-	*Au, Ma, Mm*
Ixodes laguri	*Sc*	-	-	*Sc*
Ixodes apronophorus	-	-	*Af*	*Af*
Ixodes trianguliceps	*Msu*	-	*Msu*	*Msu*
Dermacentor marginatus	*Ma*	-	-	*Ma*
Rhipicephalus sanguineus	-	*Af, Msp*	*Af*	*Af, Msp*
Haemaphysalis sulcata	-	-	*Sc*	*Sc*
Total	*Aa, Mm, Ma, Msu, Sc*	*Aa, Af, As, Au, Ma, Msp*	*Aa, Af, As, Au, Mg, Ma, Msu*	

Aa - Apodemus agrarius; Af - Apodemus flavicollis; As - Apodemus sylvaticus; Au - Apodemus uralensis; Mg - Myodes glareolus; Mm - Micromys minutus; Ma - Microtus arvalis; Msu - Microtus subterraneus; Msp - Mus spicilegus; Sc - Spermophilus citellus.

in the laboratory [2]. The main reservoir hosts for *Borrelia burgdorferi sensu lato* (s.l.) in Europe are *A. agrarius*, *A. flavicollis*, *A. sylvaticus* and *Myodes glareolus*. Moreover, certain genospecies of this pathogen (i.e. *Borrelia afzelii*) are cycled almost exclusively by rodents [2]. The ecological importance of reservoir hosts is greater if they are also common hosts to competent vector ticks. For instance, several vertebrate species were experimentally demonstrated to be competent reservoir hosts but their role as hosts to competent vector ticks is less important (i.e. *R. norvegicus*, *R. rattus*, *Sciurus vulgaris*, *Glis glis* [2]. Our study suggests that certain rodent species are more prone to be attacked by ticks than others. In species like *M. arvalis*, *A. uralensis*, *A. flavicollis* and *M. glareolus* the overall prevalence of parasitism with hard ticks was more than 50%. On the other hand, we found lower prevalence in *A. agrarius*, *A. sylvaticus*, *Micromys minutus*, *Mus spicilegus* and *Spermophilus citellus* even if sympatric with other infested hosts species. Interestingly, very abundant synanthropic rodent species like *M. musculus* and *R. norvegicus* were not harbouring ticks at all.

In a similar study from France, the overall prevalence of tick burden in micromammals was 25.19%, with *I. ricinus* being the dominant tick-parasite [16]. The authors found the highest prevalence in *M. arvalis* (31.58%), followed by *A. sylvaticus* (22.73%), *M. agrestis* (16.13%) and *M. glareolus* (14.16%). In the Netherlands [17], variable prevalences (19-56%) of tick parasitism in *A. sylvaticus* were reported during spring and summer and the only tick species found was *I. ricinus*. It seems also that the most important reservoir hosts for the Lyme borreliosis agent are usually infested with a higher number of ticks than other rodent species. Higher mean intensity and abundance were found in *A. agrarius*, *A. flavicollis*, *A. sylvaticus*, *A. uralensis* and *M. arvalis* while in other host species these parameters were lower (i.e. *Mus spicilegus*, *Micromys minutus*).

Community and population structure

Another important aspect is the tick species diversity found in our study. Most published data on ticks of rodents from Europe report few species. A survey on 799 micromammals in France revealed the presence of only two tick species: *I. ricinus* and *I. trianguliceps* [16]. In the Netherlands, only *I. ricinus* was reported from rodents [16], while in rodents from Russia four tick species were found [18]. In a multinational study (Germany, Slovakia and Romania) on the epidemiology of TBE virus, the authors reported only *I. ricinus* on *A. flavicollis*, *A. sylvaticus*, *A. uralensis* and *M. glareolus* and *I. trianguliceps* on *Microtus subterraneus* [19]. In a study from Germany, out of 11,680 ticks collected from rodents (*A. flavicollis*, *A. sylvaticus* and *M. glareolus*), 97.9% were *I. ricinus, while* the rest were *I. trianguliceps* [20].

All these data, together with other nation-wide surveys [21] add new evidence that the principal tick infesting rodents in Europe is mainly *I. ricinus*. *Ixodes ricinus* is also the most common tick feeding on humans [22], which may confer to rodents an important status as reservoir hosts for human diseases [23].

The host sharing by different tick species is important mainly for the bridging of microbial pathogens through the reservoir hosts. Although ticks specifically feeding on rodents (i.e. *I. apronophorus*, *I. redikorzevi*, *I. trianguliceps*) are attacking humans only exceptionally [24], they may maintain the infection cycle of their rodent host with certain pathogens. Subsequently, a more generalist tick (usually *I. ricinus*) can bridge the pathogens from these rodents to humans. Examples include *B. burgdorferi* s.l. isolated from *I. trianguliceps* [25] and *I. redikorzevi* [26] or the Omsk virus isolated from *I. apronophorus* [27], all in Russia.

Assessing the age structure of tick populations infesting rodents, using the prevalence of each developmental stage showed a skewed age ratio towards immatures. In Germany, a study of the population structure of *I.*

ricinus on three rodent species showed that 97.9% of all ticks were larvae, 2.0% nymphs, and 0.1% females [20]. A multinational study focusing on rodents' ticks in Central Europe found only larvae and nymphs [19]. In the case of *I. ricinus*, our study confirmed other general observations [13], according to which rodents are important hosts mainly for the immature stages of this tick. Although in our study we found adults of *I. ricinus* on 1.4% of the examined animals, interestingly, the majority of them were collected from *M. arvalis*. From 54 examined animals, four (7.4%) harboured adults of *I. ricinus*. This suggests that certain rodent species can act also as more common hosts for *I. ricinus*.

Geographical distribution

According to a recent review [10], a number of tick species found in the present study have a widespread distribution in Romania (*I. ricinus*, *D. marginatus*), while others are restricted to the southern regions (*I. laguri*, *H. sulcata*, *R. sanguineus*). The results of tick community structures from rodents analysed in accordance with general distribution maps [10] show that rodents are a good marker for assessing the distribution of certain tick species, but more heterogeneous seasonal collection campaigns are required to draw reliable conclusions.

Conclusions

Our study showed a relatively high diversity of ticks parasitizing rodents in Romania. The most common tick in rodents was *I. ricinus*, followed by *I. redikorzevi*. Certain rodents seem to host a significantly higher number of tick species than others, the most important within this view being *Apodemus flavicollis* and *Microtus arvalis*. The same applies for the overall prevalence of tick parasitism, with some species more commonly infected (*M. arvalis*, *A. uralensis*, *A. flavicollis* and *M. glareolus*) than others. Two rodent species (*Mus musculus*, *Rattus norvegicus*) did not harbour ticks at all. Based on our results we may assert that rodents generally can act as good indicators for assessing the distribution of certain tick species.

Competing interests

All authors have seen and approved the manuscript and declare that they have no competing interest.

Authors' contributions

MAD conceived the study and drafted the manuscript. DMA and MC identified the ticks. SDA contributed to study design and identified the small mammals. OM, MIA and IA examined the rodents and collected the ticks. GA performed the data analysis. DG collected the samples in the field. CV is the team coordinator, while GCM designed the study and coordinated the research grant. All authors read and approved the final manuscript.

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References

1. Durden LA: *Taxonomy, host associations, life cycles and vectorial importance of ticks parasitizing small mammals*. Micromammals and Macroparasites From Evolutionary Ecology to Management, Springer-Verlag Tokyo: In S. Morand, B.R. Krasnov, R. Poulin (Eds.); 2006:91–102.
2. Gern L, Humair PF: *Ecology of Borrelia burgdorferi sensu lato in Europe*. CABI: In Lyme Borreliosis: Biology, Epidemiology and Control (Gray JS, Kahl O, Lane RS, Stanek G, editors); 2002:149–174.
3. Dantas-Torres F, Latrofa MS, Otranto D: **Quantification of Leishmania infantum DNA in females, eggs and larvae of *Rhipicephalus sanguineus*.** *Parasit Vectors* 2011, **4:**56.
4. Kurtenbach K, Schäfer SM, de Michelis S, Etti S, Sewell HS: *Borrelia burgdorferi sensu lato in the vertebrate host*. CABI: In Lyme Borreliosis: Biology, Epidemiology and Control (Gray JS, Kahl O, Lane RS, Stanek G, eds); 2002:117–148.
5. Cogălniceanu D, Cogălniceanu CG: **An enlarged European Union challenges priority settings in conservation.** *Biodiv Conserv* 2010, **19:**1471–1483.
6. Popescu A, Murariu D: *[Fauna of Romania: Mammalia Vol. XVI., Fasc. 2: Rodentia]*. [in Romanian]: Editura Academiei; 2001.
7. Krystufek B, Griffiths HI: **Species richness and rarity in European rodents.** *Ecography* 2002, **25:**120–128.
8. Vincze M, Kerekes K: *Impact of CAP's Pillars on Romanian Rural Employment*. Debrecen: Proceedings of the Aspects and Visions of Applied Economics and Informatics Conference; 2009:1338–1351.
9. Doniță N, Popescu A, Paucă-Comănescu M, Mihăilescu S, Biriș IA: *Habitats from Romania*. București: Editura Tehnică Silvică; 2005 [in Romanian].
10. Mihalca AD, Dumitrache MO, Magdaş C, Gherman CM, Domşa C, Mircean V, Ghira IV, Pocora V, Ionescu DT: **Sikó Barabási S, Cozma V, Sándor AD: Synopsis of the hard-ticks (Acari: Ixodidae) of Romania with update on host associations and geographical distribution.** *Exp Appl Acarol* 2012, **58:**183–206.
11. Kolonin GV: *Fauna of ixodid ticks of the world (Acari, Ixodidae)*; 2009. http://www.kolonin.org.
12. Feider Z: *[Fauna of the Popular Republic of Romania. Volume 5/2. Acaromorpha, Suprafamily Ixodoidea]*. [in Romanian]: Editura Academiei Republicii Populare Române, Bucuresti; 1965.
13. Nosek J, Sixl W: **Central-European ticks (Ixodoidea).** *Mitt Abt Zool Landesmus Joanneum* 1972, **1:**61–92.
14. Aulagnier S, Haffner P, Mitchell-Jones AJ, Moutou F, Zima J: *Mammals of Europe*. London: North Africa and the Middle East. A&C Black; 2009.
15. Rózsa L, Reiczigel J, Majoros G: **Quantifying parasites in samples of hosts.** *J Parasitol* 2000, **86:**228–232.
16. L'Hostis M, Dumon H, Fusade A, Lazareff S, Gorenflot A: **Seasonal incidence of *Ixodes ricinus* ticks (Acari: Ixodidae) on rodents in western France.** *Exp Appl Acarol* 1996, **20:**359–356.
17. de Boer R, Hovius KE, Nohlmans MK, Gray JS: **The woodmouse (*Apodemus sylvaticus*) as a reservoir of tick-transmitted spirochetes (*Borrelia burgdorferi*) in the Netherlands.** *Zentralbl Bakteriol* 1993, **279:**404–416.
18. Panina TV, Katelina AF: *Ixodid ticks as parasites of the common red-backed vole (Clethrionomys glareolus)*. Tula: In Problems of Natural Focal Infections and Medical Geography Conference (Demianov AG et al. eds.); 1993:69–72.
19. de Mendonça PG, Benedek AM, Jurčovičová M: **Molecular screening of European wild rodents for tick-borne encephalitis virus.** *Acta Zool Bulgar* 2011, **63**(2):195–197.
20. Kurtenbach K, Kampen H, Dizij A, Arndt S, Seitz HM, Schaible UE, Simon MM: **Infestations of rodents with larval *Ixodes ricinus* (Acari, Ixodidae) is an important factor in the transmission cycle of *Borrelia burgdorferi* s.l. in German woodlands.** *J Med Entomol* 1995, **32:**807–817.
21. Mihalca AD, Gherman CM, Magdaş C, Dumitrache MO, Györke A, Sándor AD, Domşa C, Oltean M, Mircean V, Mărcuțan DI, D'Amico G, Păduraru AO, Cozma V: ***Ixodes ricinus* is the dominant questing tick in forest habitats**

from Romania: the results from a countrywide dragging campaign. *Exp Appl Acarol* 2012, **58**(2):175–182.
22. Briciu VT, Titilincu A, Ţăţulescu DF, Cârstina D, Lefkaditis M, Mihalca AD: **First survey on hard ticks (Ixodidae) collected from humans in Romania: possible risks for tick-borne diseases.** *Exp Appl Acarol* 2011, **54**(2):199–204.
23. Schorn S, Pfister K, Reulen H, Mahling M, Silaghi C: **Occurrence of *Babesia* spp., *Rickettsia* spp. and *Bartonella* spp. in *Ixodes ricinus* in Bavarian public parks, Germany.** *Parasit Vectors* 2011, **4**:135.
24. Bursali A, Tekin S, Orhan M, Keskin A, Ozkan M: **Ixodid ticks (Acari: Ixodidae) infesting humans in Tokat Province of Turkey: species diversity and seasonal activity.** *J Vector Ecol* 2010, **35**:180–186.
25. Gorelova NB, Korenberg EI, Kovalevskii YV, Postic D, Baranton G: **Isolation of Borrelia from the tick *Ixodes trianguliceps* (Ixodidae) and the significance of this species in epizootiology of ixodid tick-borne borreliosis.** *Parazitologiya* 1996, **30**:13–18 [in Russian].
26. Rigó K, Miklós G, Tóth AG, Földvari G: **Detection of *Borrelia burgdorferi* sensu lato and *Anaplasma phagocytophilum* in small mammals and ectoparasites in Hungary.** *Vector-Borne Zoonot* 2011, **11**:1499–1501.
27. Eldridge BF, Scott TW, Day JF, Tabachnick WJ: *Arbovirus Diseases*. Kluwer Academic Publishers: In Medical Entomology - A Textbook on Public Health and Veterinary Problems Caused by Arthropods, (Eldridge BF, Edman JD Eds.); 2004:415–460.

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