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Gastrointestinal nematodes of European roe deer (*Capreolus capreolus*) in Russia

Dmitry N. Kuznetsov*, Natalya B. Romashova & Boris V. Romashov

ABSTRACT. The species composition of gastrointestinal nematodes parasitizing European roe deer *Capreolus capreolus* in Russia was studied. Fourteen individuals of *C. capreolus* from three regions of European Russia (Ryazan', Tver' and Voronezh) were examined at necropsy in the period of 2013–2019 for the nematode infections. Beside this, the species identification of nematodes collected from four individuals of *C. capreolus* in Voronezh State Nature Reserve in 1980s was performed. Fifteen species of nematodes were detected: *Ashworthius sidemi, Bunostomum trigonocephalum, Chabertia ovina, Mazamastrongylus dagestanica, Nematodirus filicollis, Ostertagia antipini* (including minor morph "*Ostertagia lyrataeformis*"), *Ostertagia leptospicularis, Ostertagia ostertagi, Spiculopteragia asymmetrica* (including minor morph "*Spiculopteragia quadrispiculata*"), *Spiculopteragia spiculoptera, Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus vitrinus* and *Trichuris globulosa*. The biggest variety of nematodes (12 species) has been noted in abomasa. Four species (*N. filicollis, T. axei, T. colubriformis* and *T. vitrinus*) were detected both in abomasa and small intestines, but the first one prevailed in small intestines whereas *Trichostrongylus* spp. — in abomasa. This is the first detection of *S. asymmetrica* (as well as its minor morph "*S. quadrispiculata*") in European roe deer in Russia. Asian nematode *A. sidemi* was found in two regions (Tver' and Voronezh) in majority of roe deer individuals studied in 2013–2019, but was not found in the samples collected in 1980s, that confirms the trend for spreading of this parasite, noted in Europe last years.

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Dmitry N. Kuznetsov [dkuznetsov@mail.ru], Center of Parasitology of A.N. Severtsov Institute of Ecology and Evolution RAS, Mytnaya str. 28 / 1, Moscow 119049, Russia; All-Russian Scientific Research Institute of Fundamental and Applied Parasitology of Animals and Plants — a branch of the Federal State Budget Scientific Institution "Federal Scientific Centre VIEV", Bolshaya Cheremushkinskaya str. 28, Moscow 117218, Russia; Natalya B. Romashova [bvnrom@rambler.ru], Voronezh State Nature Biosphere Reserve, Voronezh 394080, Russia; Boris V. Romashov [bvrom@rambler.ru], Voronezh State Agricultural University, Mitchurina str. 1, Voronezh 394087, Russia; Voronezh State Nature Biosphere Reserve, Voronezh 394080, Russia.

Нематоды желудочно-кишечного тракта европейской косули (*Capreolus capreolus*) в России

Д.Н. Кузнецов*, Н.Б. Ромашова, Б.В. Ромашов

РЕЗЮМЕ. Определен видовой состав нематод желудочно-кишечного тракта, обнаруженных у европейской косули *Capreolus capreolus* в России. Нематоды были собраны в 2013–2019 гг. при вскрытиях 14 косуль из Рязанской, Тверской и Воронежской областей. Кроме того, определен видовой состав нематод от четырех косуль из сборов 1980-х гг., хранившихся в Воронежском государственном заповеднике. Обнаружены нематоды 15 видов: *Ashworthius sidemi, Bunostomum trigonocephalum, Chabertia ovina, Mazamastrongylus dagestanica, Nematodirus filicollis, Ostertagia antipini* (в том числе и минорная морфа этого вида — "*Ostertagia lyrataeformis*"), *Ostertagia leptospicularis, Ostertagia ostertagi, Spiculopteragia asymmetrica* (в том числе, минорная морфа — "*Spiculopteragia quadrispiculata*"), *Spiculopteragia spiculoptera, Teladorsagia circumcincta, Trichostrongylus axei, Trichostrongylus colubriformis, Trichostrongylus vitrinus* и *Trichuris globulosa*. Наибольшее количество видов нематод (12 видов) отмечено в сычуге. Четыре вида (*N. filicollis, T. axei, T. colubriformis* и *T. vitrinus*) были обнаружены как в сычуге, так и в тонком кишечнике, однако первый вид превалировал в тонком ки-

* Corresponding author

шечнике, а *Trichostrongylus* spp. — в сычуге. Впервые у европейской косули в России зарегистрирована нематода *S. asymmetrica* (а также минорная морфа этого вида — "*S. quadrispiculata*"). Азиатская нематода *A. sidemi* обнаружена в двух областях (Тверской и Воронежской) у большинства косуль, исследованных в 2013–2019 гг., однако не была обнаружена в сборах 1980-х гг. Это подтверждает тенденцию к распространению этого паразита, отмеченную в последнее время в Европе.

КЛЮЧЕВЫЕ СЛОВА: дикие жвачные, *Capreolus capreolus*, пищеварительный тракт, паразитические нематоды, Европейская Россия, *Ashworthius sidemi*, *Spiculopteragia asymmetrica*.

Intrtoduction

European roe deer (*Capreolus capreolus* Linnaeus, 1758) is one of the most abundant species among wild ruminants in Europe (Grubb, 2005). Russia has a good potential for increasing of population of *C. capreolus*, but currently the population is relatively small and has about 92 thousand individuals (Danilkin, 2014). Helminth infection deteriorates the health conditions of wild ruminants (Stien *et al*., 2002; Irvin *et al*., 2006; Osinska *et al*., 2010) and occupies a significant place among the causes of mortality in roe deer (Aguirre *et al*., 1999). Data on the taxonomic composition of helminths parasitizing in *C. capreolus* in Russia remain insufficient. Some information on the helminths of roe deer from Russia was provided in compilations by Asadov (1960), Pryadko (1976) and Govorka *et al.* (1988). But these data need to be complemented and clarified because of happened changes in wildlife management and fluctuations with population of *C. capreolus* in Russia (Danilkin, 2014). Beside that Russian zoologists (Fleurov, 1952; Heptner *et al*., 1961) used to consider Siberian roe deer (*Capreolus pygargus* Pallas, 1771) as a subspecies of European roe deer. As a result, rather extensive information on helminths of roe deer detected in Russia during XX century (Pryadko, 1976) has been presented without separation between European and Siberian roe deers. Nowadays there was noted that populations of *C. capreolus* and *C. pygargus* overlap in European Russia (Zvychaynaya *et al*., 2011). Therefore, the data on helminths parasitizing *C. capreolus* and *C. pygargus* require the re-evaluation and subdivision. Some new information concerning the helminths of Siberian roe deer was obtained during study conducted in Russian Far East (Kuznetsov *et al*., 2014). In this context a similar study of European roe deer is useful for understanding the differences between the helminth faunas of these ruminants. Our study focused on gastrointestinal nematodes as these helminths are characterized with substantial taxonomic diversity. In addition, gastrointestinal nematodes are admitted as a group of big significance because of high rates of infection (Aguirre *et al*., 1999; Hoberg *et al*., 2001).

Material and methods

Sample collection

Nematodes were collected from 18 European roe deer in three regions of European Russia. The sampling was made in Tver' (56°32' N; 36°35' E), Ryazan' (54°20' N; 40°50' E) and Voronezh (51°51' N; 39°40' E) regions (Fig. 1). Some of the roe deer were shot licensed hunting, other were killed in road accidents or died from natural reasons. Species identification of the roe deer was conducted using morphological traits (Danilkin, 2014). Age of the animals was estimated based on teeth condition (Hoye, 2006). The roe deer were necropsied according to the method of partial helminthological dissection (Ivashkin *et al*., 1971). In each roe deer there were separately examined an abomasum, small and large intestine. These parts of the gastrointestinal tract were ligated at the level of pylorus, ileocecal junction and the rectum and then cut from each other. Then these parts of the gastrointestinal tract were opened and their contents together with washings of mucosa were placed into buckets. Then these matrixes were mixed with tap water (one part of matrix and 5–10 parts of water). When the precipitate has settled the supernatant was decanted. Then the precipitate was washed repeatedly with tap water until the supernatant become transparent. Finally the washed precipitate was conserved with 96% ethanol and then examined in the laboratory by small portions using binocular loupe. The precipitates have been examined in full volume. Detected nematodes were placed in vials with 96% ethanol. Beside this, we conducted the species identification of gastrointestinal nematodes from storage of Voronezh State Nature Reserve (the samples from four European roe deer collected in 1980s).

Taxonomical identification

In most cases an identification of detected nematodes was based on male's morphology due to big similarity of the females. The nematodes were prepared as temporary whole mounts, cleared in glycerol solution (two parts of glycerol and eight parts of water) and then examined using light microscopy at magnification of 40 to 400. The species identification was carried out basing on morphological features presented in literature (Skrjabin *et al*., 1954; Ivashkin *et al*., 1989; Drozdz *et al*., 1995). The main morphological features used for the identification of gastrointestinal strongyles were the peculiarities of male reproductive system, in particular, the shape of spicules and dorsal ray. *Trichuris* nematodes were identified according to Ivashkin *et al.* (1989), Callejon *et al.* (2015) and Yevstafieva *et al.* (2018).

Molecular analysis

Several nematode specimens collected during the present study were used in molecular analysis. The DNA

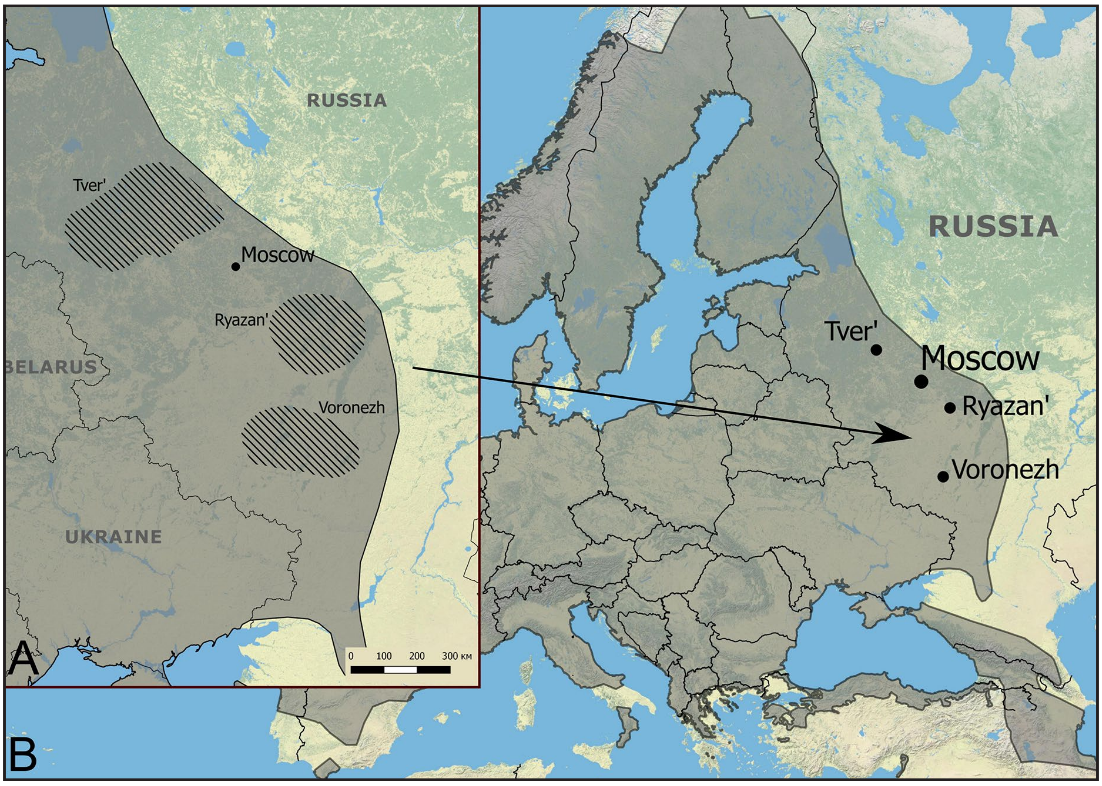

Fig. 1. The area of collection of *C. capreolus* nematodes (hatching) (A) within the distribution range of *C. capreolus* in Europe (B). The distribution range of *C. capreolus* in Europe is given according to IUCN (Lovari *et al*., 2016).

study appeared successful for two males of *Ashworthius sidemi* and two males of *Spiculopteragia spiculoptera*, collected in Voronezh region and previously identified by morphological features. Genomic DNA was isolated from single specimens of nematodes using the procedure as described by Holterman *et al.* (2006). In all cases, DNA was extracted from individual specimens of the nematodes.

Polymerase chain reaction (PCR) was performed to obtain the ITS-domain of rDNA using a forward primer 18SF3 (5'-GAGAGGACTGCGGACTGCT-GTATCG-3'), proposed by Yamada *et al.* (2012) and a reverse primer NC2 (5'-TTAGTTTCT TTTCCTC-CGCT-3'), proposed by Gasser *et al.* (1993). The PCR was performed using Sileks-M DNA amplification kit (Sileks-M, Russia) in a 25 µl reaction volume according to the manufacturer's protocol. The PCR cycling conditions consisted of an initial denaturation at 95°C for 5 min, followed by 40 cycles (95°C for 30 s, 50°C for 30 s, 72°C for 50 s) with a final extension at 72°C for 10 min. PCR products were visualised in agarose gel and then purified for sequencing using a Wizard SV Gel and PCR Clean-Up System (Promega, USA) according to the manufacturer's protocol. Obtained PCR-products were sequenced using ABI PRISM Big Dye Terminator v.3.1 kit (Applied Biosystems, USA) with an analysis of the reaction products using automatic sequencer Applied Biosystems 3730 DNA Analyzer. Obtained sequences were compared with the NCBI GenBank nucleotide database using the BLASTn 2.8.1+ program (Morgulis *et al*., 2008). Obtained sequences were deposited in GenBank (accession numbers are MT322612 and MT322613 for *A. sidemi*; MT322614 and MT322615 for *S. spiculoptera*).

Results

All studied individuals of *C. capreolus* appeared to be infected with nematodes. Rates of infection intensity and list of detected species are presented in Tab. 1. The species of nematodes are listed in alphabetical order. In total 15 species of gastrointestinal nematodes were found in the present study. Among them 11 species from the family Trichostrongylidae: *Mazamastrongylus dagestanica* (Altaev, 1953); *Ostertagia antipini* Matschulsky, 1950; *Ostertagia leptospicularis* Assadov, 1953; *Ostertagia ostertagi* (Stiles, 1892); *Spiculopteragia asymmetrica* (Ware, 1925); *Spiculopteragia spiculoptera* (Guschanskaja, 1931); *Teladorsagia circumcincta* (Stadelman, 1894); *Trichostrongylus axei* (Cobbold, 1879); *Trichostrongylus colubriformis* (Giles, 1892); *Trichostrongylus vitrinus* Looss, 1905 and *Ashworthius sidemi* Schulz, 1933. For two of the aforementioned species (*S. asymmetrica* and *O. antipini*) their minor morphs ("*S. quadrispiculata*" and "*O. lyrataeformis*" respectively) were also detected. Besides that, there

Table 1. The intensity of infection with gastrointestinal nematodes in studied individuals of *Capreolus capreolus* and the list of detected species. A — abomasum, SI — small intestine, LI — large intestine; major and minor morphs are listed via slash.

Sequence number of the studied European roe deer	Region of sampling	Month and year of sampling	Sex and age of hosts	Number of detected nematodes			Species of detected nematodes, localization and number of males (in brackets)
				Total	Males	Females	
1	Ryazan'	November 2013	male, 2 years	71	31	40	*Bunostomum trigonocephalum* (SI-2), *Chabertia ovina* (LI-1), *Mazamastrongylus dagestanica* (A-24), *Nematodirus filicollis* (A-1, SI-3)
2	Ryazan'	January 2014	male, 4 years	57	22	35	*B. trigonocephalum* (SI-3), *Ch. ovina* (LI-2), *M. dagestanica* (A-12), *N. filicollis* (A-2, SI-3)
3	Ryazan'	January 2019	male, 6 years	1667	795	872	*B. trigonocephalum* (SI-7), *Ch. ovina* (LI-11), *N. filicollis* (A-5, SI-33), *Ostertagia ostertagi* (A-7), *Teladorsagia circumcincta* (A-21), *Trichostrongylus axei* (A-290, SI-27), *Trichostrongylus colubriformis* (A-205, SI-79), *Trichostrongylus vitrinus* (A-99, SI-11)
4	Tver'	October 2016	male, 1 year	491	211	280	*Ashworthius sidemi* (A-7), *B. trigonocephalum* (SI-2), *Ch. ovina* (LI-1), *N. filicollis* (A-3, SI-10), *Ostertagia antipini* (A-28), *Ostertagia leptospicularis* (A-130), *Spiculopteragia asymmetrica* (A-30)
5	Voronezh	March 1985	male, years	392	178	214	*B. trigonocephalum* (SI-10), *M. dagestanica* (A-12), *N. filicollis* (SI-5), *O. antipini* (A-19), *O. leptospicularis* (A-113), *S. asymmetrica* (A-18) / "*S. quadrispiculata"* (A-1)
6	Voronezh	April 1987	male, 3 years	510	208	302	*B. trigonocephalum* (SI -20), *M. dagestanica* (A-16), *N. filicollis* (SI-7), *O. antipini* (A-8), *O. leptospicularis* (A-56), *S. asymmetrica* (A-81) / "*S. quadrispiculata*" (A-20)
7	Voronezh	April 1987	male, 4 years	1541	519	1022	*B. trigonocephalum* (SI -1), *M. dagestanica* (A-12), *N. filicollis* (A-18, SI-266), *O. antipini* (A-129), *O. leptospicularis* (A-44), *S. asymmetrica* (A-38) / "*S. quadrispiculata*" (A-11), *Trichuris globulosa* (LI-1)
8	Voronezh	December 1989	female, 1 year	165	53	112	*B. trigonocephalum* (SI-1), *M. dagestanica* (A-10), *N. filicollis* (SI-2), *O. antipini* (A-9), *O. leptospicularis* (24), *S. asymmetrica* (6) / "*S. quadrispiculata*" (1)
9	Voronezh	February 2017	female, 3 years	143	60	83	*M. dagestanica* (A-1), *N. filicollis* (A-6, SI-11), *O. antipini* (A-34), *O. leptospicularis* (A-5), *S. asymmetrica* (A-2), *Spiculopteragia spiculoptera* (A-1)
10	Voronezh	February 2017	male, 2 years	159	71	88	*A. sidemi* (A-7), *M. dagestanica* (A-1), *N. filicollis* (A-4, SI-11), *O. antipini* (A-32) / "*O. lyrataeformis*" (A-3), *S. asymmetrica* (A-8), *S. spiculoptera* (A-5)
11	Voronezh	January 2017	male, 2 years	233	94	139	*A. sidemi* (A-28), *M. dagestanica* (A-1), *N. filicollis* (A-4, SI-14), *O. antipini* (A-13), *O. leptospicularis* (A-3), *S. asymmetrica* (A-30) / "*S. quadrispiculata*" (A-1)
12	Voronezh	January 2018	male, 4 years	156	54	102	*A. sidemi* (A-10), *B. trigonocephalum* (SI-1), *N. filicollis* (A-4, SI-37), *O. antipini* (A-1), *T. globulosa* (LI-1)

Table 1 (continued)

Sequence number of the studied European roe deer	Region of sampling	Month and year of sampling	Sex and age of hosts	Number of detected nematodes			Species of detected nematodes, localization and number of males (in brackets)
				Total	Males	Females	
13	Voronezh	February 2018	male, 3 years	387	157	230	*A. sidemi* (A-39), *N. filicollis* (A-2, SI-17), *O. antipini* (A-3), *O. leptospicularis* (A-3), *S. asymmetrica* (A-10), *T. colubriformis* (A-70, SI-12), *T. globulosa* (LI-1)
14	Voronezh	March 2018	male, 1 year	4896	2078	2818	*A. sidemi* (A-243), *N. filicollis* (A-65, SI-1710), *O. antipini* (A-5), *O. leptospicularis* (A-3), *T. colubriformis* (A-32, SI-10), *T. globulosa* (LI-10)
15	Voronezh	March 2018	female, 2 years	864	210	654	*N. filicollis* (A-18, SI-138), *O. antipini* (A-30), *O. leptospicularis* (A-16), *T. globulosa* (LI-8)
16	Voronezh	March 2018	male, 4 years	210	49	161	*A. sidemi* (A-2), *N. filicollis* (A-6, SI-17), *O. antipini* (A-15), *O. leptospicularis* (A-5), *T. globulosa* (LI-4)
17	Voronezh	April 2018	female, 3 years	95	33	62	*A. sidemi* (A-2), *N. filicollis* (A-3, SI-11), *O. antipini* (A-14), *O. leptospicularis* (A-3)
18	Voronezh	April 2018	male, 2 years	2578	602	1976	*A. sidemi* (A-501), *N. filicollis* (A-19, SI-65), *O. antipini* (A-10), *O. leptospicularis* (A-4), *T. globulosa* (LI-3)

were detected *Bunostomum trigonocephalum* (Rudolphi, 1808) (family Ancylostomatidae); *Chabertia ovina* (Fabricius, 1794) (Chabertiidae); *Nematodirus filicollis* (Rudolphi, 1802) (Molineidae) and *Trichuris globulosa* (Linstow, 1901) (Trichuridae).

For two of the detected species they taxonomic affiliation was confirmed by molecular analysis. Obtained sequences of *A. sidemi* (790 bp long contained partial 18S, complete ITS1 and 5.8S and partial ITS2 rDNA) and *S. spiculoptera* (908 bp long contained partial 18S, complete ITS1 and 5.8S and partial ITS2 rDNA) were compared with the GenBank nucleotide database using the BLAST. The sequences of *A. sidemi* showed 99.75% identity to *A. sidemi* sequence (accession number EF 467325.1) and sequences of *S. spiculoptera* showed 98.65% identity to *S. spiculoptera* sequence (accession number KP 984759.1). Additionally, before using in molecular analysis, the specimens of *S. spiculoptera* were differentiated from the morphologically close *Spiculopteragia houdemeri.* The last one species has spicules joined at the end (Drozdz, 1965), whereas *S. spiculoptera* has separated spicules.

Discussion

Most of the studied individuals of *C. capreolus* showed rather big species diversity of nematodes (Tab. 1). The maximum number of the nematode species registered in one individual was eight, minimum number was four and mean was 5.7. The similar level of the diversity was noted in previous studies of *C. capreolus* conducted in various European countries (Dunn, 1965; Bernard *et al*., 1988; Borgsteede *et al*., 1990; Drozdz *et al*., 1992; Aguirre *et al*., 1999; Cisek *et al*., 2003; Kuzmina *et al*., 2010; Pato *et al*., 2013; Demiaszkiewicz *et al*., 2017). We tend to explain this diversity by the peculiarities of roe deer behavior, such as abilities for long-distance movements and co-pasturing with other ruminants (Danilkin, 2014). The intensity of infection varied from rather low numbers, such as 57 specimens of all detected species (in four-year age male from Ryazan') to significant burden with 4896 specimens (in one-year age male from Voronezh).

Nematodirus filicollis (Rudolphi, 1802) showed the highest rates of the intensity and extensity of infection in the present study (Tabs. 1, 2). Similar data were obtained during studies of *C. capreolus* in Britain (Dunn, 1965), Belgium (Bernard *et al*., 1988), Spain (Pato *et al*., 2013) and Turkey (Bolukbas *et al*., 2012). It worth to mention, that Khrustalev (2009) has revealed, based on revision of *Nematodirus* spp. from Russia, that *N. filicollis* is a typical parasite for roe deer, while in other hosts it was often reported erroneously.

The high prevalence has been detected as well for *Ostertagia antipini* Matschulski, 1950 and *Ostertagia leptospicularis* Assadov, 1953 (Tab. 2). The last one is widespread among wild and domestic ruminants in

Table 2. The extensity of infection with gastrointestinal nematodes in studied individuals (*n*=18) of *Capreolus capreolus*.

Nematode species	Regions of detection	The number of infected animals	Extensity of infection (%)
Ashworthius sidemi	Tver', Voronezh	9	50.0
Bunostomum trigonocephalum	Ryazan', Tver', Voronezh	9	50.0
Chabertia ovina	Ryazan', Tver'	4	22.2
Mazamastrongylus dagestanica	Ryazan', Voronezh	9	50.0
Nematodirus filicollis	Ryazan', Tver', Voronezh	18	100.0
Ostertagia antipini	Tver', Voronezh	15	83.3
Ostertagia leptospicularis	Tver', Voronezh	13	72.2
Ostertagia ostertagi	Ryazan'	1	5.6
Spiculopteragia asymmetrica	Tver', Voronezh	9	50.0
Spiculopteragia spiculoptera	Voronezh	2	11.1
Teladorsagia circumcincta	Ryazan'	1	5.6
Trichostrongylus axei	Ryazan'	1	5.6
Trichostrongylus colubriformis	Ryazan', Voronezh	3	16.7
Trichostrongylus vitrinus	Ryazan'	1	5.6
Trichuris globulosa	Voronezh	7	38.9

Europe (Wyrobisz-Papiewska *et al*., 2018). In contrast with that, the data on occurrence of *O. antipini* limited with small number of countries and host species (Govorka *et al*., 1988; Kuznetsov *et al*., 2014; Wyrobisz-Papiewska *et al*., 2018). We believe that specimens of *O. antipini* and *O. leptospicularis* in some cases cannot be accurately differentiated from each other due to big morphological similarity. We agree with Wyrobisz-Papiewska *et al.* (2018), that *O. leptospicularis* may represent a potential species complex.

Spiculopteragia asymmetrica (Ware, 1925) was found in nine of the studied roe deer from Tver' and Voronezh regions (Tab. 2). In five cases the minor morphs of this species ("*S. quadrispiculata*") were also detected. A small number of specimens of *Spiculopteragia spiculoptera* (Guschanskaja, 1931) were found in two roe deer from Voronezh, in both cases together with *S. asymmetrica* (Tabs. 1, 2). The males of these two species could be easily differentiated from each other basing on morphology of distal parts of the spicules, therefore this detection is not on doubt. Thus, we note a possibility of simultaneous parasitizing of *S. asymmetrica* and *S. spiculoptera.* Zaffaroni *et al.* (2000) consider *S. spiculoptera* along with *O. leptospicularis* as a dominant species in Cervids. A survey by Rossi *et al.* (1997) also showed that *S. spiculoptera* and *O. leptospicularis* were the dominant abomasal species of European roe deer. Similar results have been got by Dunn (1965), Bernard *et al.* (1988), Bolukbas *et al.* (2012) and Pato *et al.* (2013). Our data confirm the high importance of *O. leptospicularis*, but the levels of prevalence and abundance of *S. spiculoptera* were low. On the other hand, we noted quite high prevalence of *S. asymmetrica* registered in two regions (Tver' and Voronezh). In European roe deer *S. asymmetrica* was previously registered in Britain (Dunn, 1965), Belgium (Bernard *et al*., 1988) and Spain (Morrondo *et al*., 2010).

Mazamastrongylus dagestanica (Altaev, 1952) was detected in 50% of the studied roe deer from two regions (Tab. 2). At the same time, the intensity of infection with *M. dagestanica* was rather low (Tab. 1). Apparently, roe deer were infected with *M. dagestanica* when sharing the territory with elk, which is the obligate host for this parasite (Wyrobisz-Papiewska *et al*., 2018). In European roe deer *M. dagestanica* was registered in Poland (Wyrobisz-Papiewska *et al*., 2018). Recently *M. dagestanica* was reported in Siberian roe deer from Russian Far East (Kuznetsov *et al*., 2014).

Ashworthius sidemi Schulz, 1933 was found in two studied regions (Tver' and Voronezh), the intensity of infection ranged from a few specimens to several hundred specimens and the extensity of infection was 50% (Tabs. 1, 2). This species was found in majority of roe deer from Voronezh studied in 2017–2018, but was not found in the samples from Voronezh collected in 1980s. This fact corroborates the spreading of *A. sidemi* in Europe during last decades, noted by several studies **(**Drozdz *et al*., 2003; Hoglund *et al*., 2007; Vadlejch *et al*., 2016; Demiaszkiewicz *et al*., 2017**)**.

Bunostomum trigonocephalum (Rudolphi, 1802) was found in nine of the studied roe deer in all of the explored regions (Tabs. 1, 2). The number of discovered males ranged from 1 to 20, and the number of females ranged from one to seven individuals. This species is widespread in domestic and wild ruminants all over the word, this nematode reported from European roe deer in Netherlands (Borgsteede *et al*., 1990), Poland (Drozdz *et al*., 1992) and Armenia (Movsesyan *et al*., 2019).

We found *Trichuris globulosa* (Linstow, 1901) in roe deer from Voronezh (one sample collected in 1987 and six samples collected in 2018). The number of males ranged from 1 to 10, and the number of females ranged from one to 18 individuals. This species is widespread in domestic and wild ruminants, both in Europe and in other parts of the world (Govorka *et al*., 1988). Yevstafieva *et al.* (2018) reported this nematode in south-eastern regions of Ukraine neighboring with Voronezh region.

Chabertia ovina (Fabricius, 1788) noted in four roe deer from two regions (Tab. 2). The number of males of *C. ovina* ranged from 1 to 11, and the number of females ranged from 2 to 15 individuals. This nematode is known to be quite common in domestic and wild ruminants. In *C. capreolus* this nematode reported in Sweden (Aguirre *et al*., 1999), Britain (Dunn, 1965), Belgium (Bernard *et al*., 1988), Netherlands (Borgsteede *et al*., 1990), Poland (Drozdz *et al*., (1992), Belarus (Shimalov & Shimalov, 2003), Ukraine (Kuzmina *et al*., 2010), Spain (Pato *et al*., 2013), Turkey (Bolukbas *et al*., 2012), Armenia (Movsesyan *et al*., 2019) and Romania (Hora *et al*., 2017).

We found three species of the genus *Trichostrongylus*, in all cases with rather high intensity (Tab. 1). *Trichostrongylus colubriformis* (Giles, 1892) was detected in two roe deer from Voronezh and one from Ryazan' (Tabs. 1, 2). In Voronezh *T. colubriformis* was found only in recently collected samples, but was not found in the samples collected in 1980s. *Trichostrongylus axei* (Cobbold, 1879) and *Trichostrongylus vitrinus* (Looss, 1905) were found in one individual of *C. capreolus* which has been hunted near agricultural area in Ryazan' region. This individual of *C. capreolus* showed the highest species diversity (eight species of nematodes) in the present study. We tend to explain it because of contacts among *C. capreolus* and domestic ruminants. The detected species of *Trichostrongylus* are widespread among domestic and wild ruminants, and have been reported from the areas with different climatic conditions (Skrjabin *et al*., 1954; Govorka *et al*., 1988). In particular, these three species of *Trichostrongylus* reported from *C. capreolus* in Sweden (Aguirre *et al*., 1999), Italy (Zaffaroni *et al*., 2000), Spain (Pato *et al*., 2013), Turkey (Bolukbas *et al*., 2012) and Armenia (Movsesyan *et al*., 2019).

Ostertagia ostertagi (Stiles, 1892) as well as *T. circumcincta* (Stadelmann, 1894) was detected in one individual of European roe deer only (Ryazan' region). These species are more typical for Bovidae (Wyrobisz-Papiewska *et al*., 2018) and this roe deer was supposedly infected when sharing pastures with domestic ruminants. Interestingly, that Drozdz *et al.* (1992) in north-eastern Poland found *O. ostertagi* in roe deer in all seasons except winter, but our finding of *O. ostertagi* was made in January.

Thus, all nematode species found in the present study have already been reported from *C. capreolus* in various European countries. Most species of nematodes detected in the present study were also previously noted in other wild ruminants (such as elk and red deer) inhabiting European Russia and neighboring countries (Govorka *et al*., 1988**)**. However, *S. asymmetrica*, as well as its minor morph ("*S. quadrispiculata*") was never previously found in European roe deer in Russia. *Ashworthius sidemi* was detected in *C. capreolus* in Russia for the first time by Kuznetsov *et al.* (2018) based on the necropsies of three roe deer conducted in 2016–2017. In the present study we supplement the data concerning *A. sidemi* with results of necropsies of *C. capreolus* conducted in 2018, and two of the studied roe deer appeared to be infected with rather high intensity numbering several hundred specimens of *A. sidemi*. Four species of gastrointestinal nematodes, which we found in the present study (*T. circumcincta, T. axei*, *T. colubriformis* and *T. vitrinus*) are considered to be zoonotic (Skrjabin *et al*., 1954; Mizani *et al*., 2017).

Four species of gastrointestinal nematodes (*N. filicollis, O. antipini, S. spiculoptera* and *M. dagestanica*) detected in the present study were reported for Siberian roe deer as well (Kuznetsov *et al*., 2014). *M. dagestanica* showed similar infection rates both for *C. capreolus* and *C. pygargus*. *S. spiculoptera* was found in *C. pygargus* with an average of 173 specimens per animal, but in *C. capreolus* we found this nematode in low numbers. On the contrary, *N. filicollis* and *O. antipini* were found in low numbers in Siberian roe deer. In addition to the above mentioned, three other species of gastrointestinal nematodes (*Oesophagostomum venulosum* (Rudolphi, 1809)*, Pygarginema skrjabini* Kadenazii, 1948 and *Spiculopteragia schulzi* (Rajewskaja, 1930)) were reported from Siberian roe deer (Oshmarin & Parukhin, 1963; Kuznetsov *et al*., 2014). Thus, the species composition of gastrointestinal nematodes of *C. pygargus* appears to be less diverse and coincides with that of *C. capreolus* in four species only.

The biggest variety of nematode species we detected in an abomasum (Tab. 1). Here were found twelve species and two of these species (*S. asymmetrica* and *O. antipini*) were presented with their major and minor morphs. Four species (*N. filicollis, T. axei*, *T. colubriformis* and *T. vitrinus*) were detected both in abomasa and small intestines, but the first one prevailed in small intestines whereas *Trichostrongylus* spp. was more abundant in abomasum.

Conclusion

The helminthological study of *C. capreolus* from Russia detected rather high species diversity of gastrointestinal nematodes. Most of the detected nematode species are common both for European roe deer and other aboriginal wild ruminants (elk, red deer) habituating this area. In some cases the helminth fauna of *C. capreolus* was added with species more typical for domestic ruminants. One of the detected species, *Spiculopteragia asymmetrica* (as well as its minor morph "*S. quadrispiculata*"), has been reported in European roe deer in Russia for the first time. The detection of *Ashworthius sidemi* with rather high intensity and extensity of infection confirms the trend of spreading this Asian nematode among aboriginal ruminants in Europe. The presence of zoonotic nematodes (*T. circumcincta, T. axei*, *T. colubriformis* and *T. vitrinus*) in the helminth fauna of *C. capreolus* deserves a special attention.

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