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Exchanged communities of abomasal nematodes in cervids with a first report on *Mazamastrongylus dagestanica* in red deer

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Abstract

Introduction: Among large wild game in Poland, the most numerous cervids are red deer and roe deer. Although these species live free, they should be under veterinary supervision because they can transmit infectious agents and parasites to livestock. The aim of this study was to evaluate the biodiversity of the abomasal nematodes which parasitise cervids and present the visual and dimensional characteristics of their spicules. **Material and Methods:** Overall, 2,067 spicules of nematodes derived from nine red deer and five roe deer were measured and microphotographed in order to determine the species. The predominant *Spiculopteragia boehmi* was additionally confirmed molecularly by PCR. The spicule lengths of the most common species found in both hosts simultaneously were compared. **Results:** Fourteen species of abomasal nematode were identified. All examined animals but one were infected. The most prevalent parasites in both host species were *S. boehmi* and *Ostertagia leptospicularis*. The alien *Ashworthius sidemi* was found in both hosts, whereas *Haemonchus contortus* was identified only in red deer. *Mazamastrongylus dagestanica* was noted in red deer for the first time. A 262-base-pair nucleotide sequence of *S. boehmi* was obtained and deposited in GenBank. Significantly longer spicules were found in red deer-derived *O. leptospicularis* and *S. boehmi* and shorter structures were seen in *A. sidemi*. **Conclusion:** The widespread exchange of abomasal nematodes between various ruminant species questions the relevance of their division into specialists and generalists.

Keywords: *Cervus elaphus*, *Capreolus capreolus*, gastric worms, internal transcribe spacer 2 genetic marker.

Introduction

The Regulation of the Polish Minister of Environment on the establishment of the list of wild game species of 11 March 2005 defines five cervid species as large wild game: moose (*Alces alces*), red deer (*Cervus elaphus*), sika deer (*Cervus nippon*), fallow deer (*Dama dama*), and roe deer (*Capreolus capreolus*). Among these, red deer and roe deer are the most numerous, and their population is growing (25). High abundance increases the number of animals susceptible to infection and their contact with livestock, farmed deer and other wild deer species (2). The most common helminthiases of wild ruminants are infections with abomasal nematodes (5, 27). Gastrointestinal nematodes (GINs) belong to two families, namely the Trichostrongylidae Leiper, 1912, containing such genera as *Ostertagia*, *Spiculopteragia*, *Trichostrongylus*, *Haemonchus*, *Ashworthius*, *Teladorsagia* and *Aonchotheca*, and the Molineidae Skrjabin and Schultz, 1937, including the *Nematodirus* genus. Parasitological surveys of red deer in Europe identify the Ostertagiinae as predominant components of the abomasal fauna, which usually include multiple polymorphic species of *Ostertagia* and *Spiculopteragia* (4). So far, 25 species of gastric nematodes have been identified in deer in Poland, including *O. leptospicularis* and *S. boehmi* (5). Among cervid GINs, a particular threat is thought to be posed by blood-sucking parasites of the Haemonchiinae subfamily, such as the invasive alien species *Ashworthius sidemi*, brought to Poland with Asiatic deer, and *Haemonchus contortus*, recognised for its remarkable propensity to develop resistance to the anthelmintics used in its control (8, 17). The risk of the spread of

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highly pathogenic helminths and anthelmintic-resistant parasites between wild and domestic ruminants makes strict veterinary supervision prudent, including parasitological examination of local animal populations (23). The aim of this study was to evaluate the biodiversity of abomasal nematodes of red deer and roe deer and present the visual and dimensional characteristics of the spicules of male parasites of individual species.

Material and Methods

Examined animals and geographical area of the study. The abomasa of nine red deer and five roe deer legally hunted during the 2018 and 2021 hunting seasons were collected in the area of four voivodeships in Poland (Łódźkie, Mazowieckie, Zachodniopomorskie and Warmińsko-Mazurskie). These are respectively located in central, northwest, and northeast Poland. The collected abomasa were transported to the laboratory of the Department of Food Hygiene and Public Health Protection of the Institute of Veterinary Medicine at the Warsaw University of Life Sciences and frozen.

Collection of abomasal nematodes. Each abomasum was defrosted and their content was decanted several times in tap water. Subsequently, small portions of the whole sediment were searched for the presence of abomasal nematodes under a Delta Optical SZ-450 T stereomicroscope (Delta Optical, Mińsk Mazowiecki, Poland). All nematodes found in the whole abomasum content of a single animal were placed in a tube filled with 70% ethanol and marked. Further morphological and molecular analyses were conducted on the preserved specimens.

Morphological examination. Male nematode specimens were reserved for morphological study. Their distal body parts were cut off and transferred into a drop of lactophenol on the microscope slide to make the cuticula transparent and the spicules visible (9). The anterior body parts of the examined specimens were transferred individually into an Eppendorf tube filled with 70% ethanol and marked.

The nematode species was determined based on the morphology of the bursa copulatrix of each isolated male using a LAB40 light microscope (OPTA-TECH, Warsaw, Poland) under 100× to 400× magnification (11, 12). Microphotographs of the spicules were taken with a digital camera and measurements were made using OPTA View-15 2019 software (OPTA-TECH).

Statistical analysis. Overall, 2,067 spicules of gastric nematodes were measured. The spicule length of the four most numerous species of gastric nematodes, namely *Ashworthius sidemi*, *Ostertagia kolchida*, *O. leptospicularis* and *Spiculopteragia boehmi*, were compared between roe deer-derived and red deer-derived specimens. Other nematode species were not statistically compared because they were found only in one ungulate species or were in low numbers (four or fewer individuals). The normal distribution of the variables was verified with the Shapiro–Wilk test. If the variables had a normal distribution, the spicula lengths of roe deer-derived and red deer-derived specimens were compared with Student's *t*-test. This was the case for *O. kolchida*. If the variables were not normally distributed, the spiculae length was compared with the Mann-Whitney U test. This test was needed for *A. sidemi*, *O. leptospicularis* and *S. boehmi*.

DNA extraction, amplification and sequencing. Molecular analysis was conducted to verify the morphological determination of the most commonly diagnosed species, *Spiculopteragia boehmi*. Genomic DNA was extracted individually from ethanol-preserved anterior parts of ten male worms (five per host species) using a NucleoSpin Tissue DNA extraction kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's protocol. A partial region of the internal transcribe spacer 2 and large subunit of the ribosomal DNA was amplified by PCR using the following set of primers: forward-NC1 (5′-ACG TCT GGT TCA GGG TTG TT-3′) and reverse-NC2 (5′-TTA GTT TCT TTT CCT CCG CT-3′) (16). PCR was performed in a T100 thermal cycler (Bio-Rad, Hercules, CA, USA) in a volume of 50 µL. Each 50 µL PCR reaction contained 20 µL of Molecular Biology Reagent Water (Sigma-Aldrich, St. Louis, MO, USA), 25 µL of AccuStart II PCR ToughMix (×2 concentration) (Quantabio, Beverly, MA, USA), 1 µL of GelTrack Loading Dye (×50 concentration) (Quantabio), 1 µL of forward primer (20 mM), 1 µL of reverse primer (20 mM), and 2 µL of template DNA. The conditions for PCR were as follows: 94°C for 2 min to denature the DNA, 40 cycles at 94°C for 40 s, 60°C for 60 s, and 72°C for 45 s, and a final extension of 5 min at 72°C to ensure complete amplification. The PCR product was purified with the use of the NucleoSpin Gel and PCR Clean-up kit (Macherey-Nagel), eluted with 30 µL of Molecular Biology Reagent Water (Sigma-Aldrich) and sequenced in both directions by Genomed S.A. (Warsaw, Poland) using the primers used for amplification (5 mM). The sequences were then assembled into contigs using CodonCode Aligner version 8.0 (CodonCode Corporation, Centerville, MA, USA).

Results

Abomasal nematodes. Gastric nematodes (GINs) were presented in all but one animal, with a general prevalence of 92.9% (*i.e.* 13 positive out of 14 studied). The overall prevalence in roe deer was 80% (Table 1), whereas all the examined individuals of red deer were infected (Table 2). The mean intensity of infection with GINs was 466 in roe deer and 698.1 in red deer. In total, 8,147 adult nematodes were collected from the roe deer and red deer combined. In each positive case, mixed infections were observed of two to nine species. In total, seven species of gastric nematodes were found in both

hosts, namely *Ashworthius sidemi* (Fig. 1), *Mazamastrongylus dagestanica* (Fig. 2), *Ostertagia antipini* (Fig. 3), *O. kolchida* (a minor morph of *O. leptospicularis*) (Fig. 4), *O. leptospicularis* (Fig. 5), *Spiculopteragia asymmetrica* (Fig. 6) and *S. boehmi* (Fig. 7). Roe deer were additionally infected with *O. lyrata* (a minor morph of *O. ostertagi*) (Fig. 8), *Trichostrongylus capricola* (Fig. 9) and *T. vitrinus* (Fig. 10). Red deer additionally hosted *Haemonchus contortus* (Fig. 11), *Nematodirus roscidus* (Fig. 12), *S. mathevossiani* (a minor morph of *S. boehmi*) (Fig. 13), and *T. axei* (Fig. 14). The most prevalent gastric nematodes in roe deer were *O. kolchida*, *O. leptospicularis* and *S. boehmi* (prevalence = 60%) (Table 1). *Spiculopteragia boehmi* was the most prevalent species in red deer (prevalence = 100%) and *A. sidemi* and *O. leptospicularis* were the next most frequent (prevalence = 66.7%) (Table 2).

Length of spicules in abomasal nematodes of red deer and roe deer. Significant differences in spicule length were observed between roe deer and red deer-derived nematodes in the cases of *A. sidemi*, *O. leptospicularis* and *S. boehmi*. The red deer-derived *O. leptospicularis* and *S. boehmi* had longer spicules than those from roe deer, whereas the roe deer-derived *A. sidemi* had longer spicules than those from red deer (Table 3). Overall, the longest spicules were noted in *N. roscidus*, observed exclusively in red deer, and *A. sidemi*, found in both cervids. The shortest spicules were seen in *T. axei*, found exclusively in red deer, and *T. vitrinus*, occurring exclusively in roe deer (Table 3).

Molecular analysis. The study resulted in 10 homologous nucleotide sequences measuring 262 base pairs (bp) of *S. boehmi*. The sequence was deposited in the GenBank database with accession no. MW839588.

Table 1. Intensity and prevalence of abomasal nematodes of roe deer (*Capreolus capreolus*)

Nematode	Intensity		Prevalence (%)	95% Confidence interval
	X	Range		
Ashworthius sidemi	10	10	20 (1 of 5)	26.1
Mazamastrongylus dagestanica	3	3	20 (1 of 5)	26.1
Ostertagia antipini	2	2	20 (1 of 5)	26.1
O. kolchida	1.7	1–3	60 (3 of 5)	32.0
O. leptospicularis	18.3	2–33	60 (3 of 5)	32.0
O. lyrata	1	1	20 (1 of 5)	26.1
Spiculopteragia asymmetrica	1	1	20 (1 of 5)	26.1
S. boehmi	212.7	17–506	60 (3 of 5)	32.0
Trichostrongylus capricola	4	4	20 (1 of 5)	26.1
T. vitrinus	1	1	20 (1 of 5)	26.1

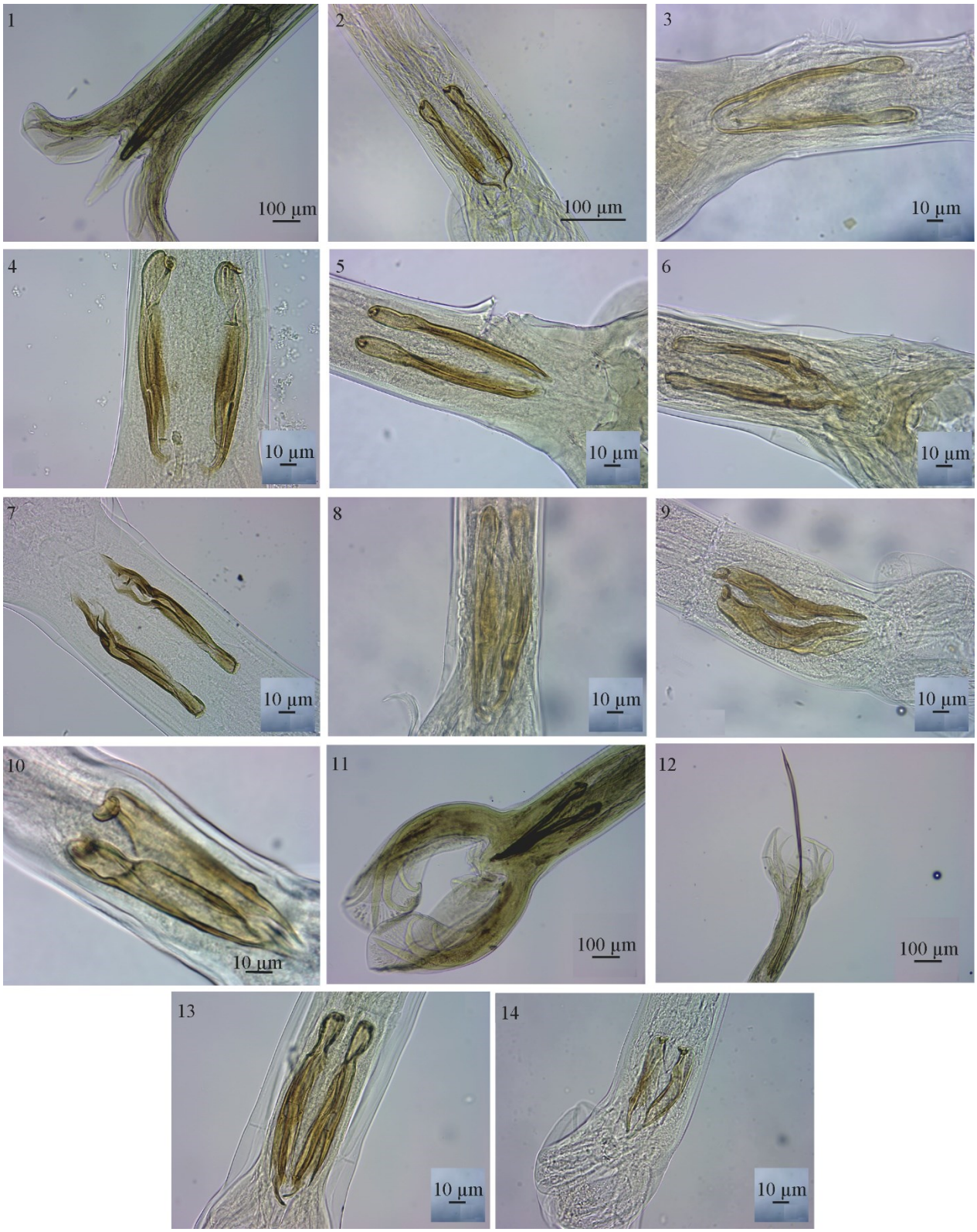
Table 2. Intensity and prevalence of abomasal nematodes of red deer (*Cervus elaphus*)

Nematode	Intensity		Prevalence (%)	95% Confidence interval
	X	Range		
Ashworthius sidemi	289.8	1–1.136	66.7 (6 of 9)	30.8
Haemonchus contortus	1.5	1–2	22.2 (2 of 9)	27.2
Mazamastrongylus dagestanica	1	1	11.1 (1 of 9)	20.5
Nematodirus roscidus	1	1	11.1 (1 of 9)	20.5
Ostertagia antipini	3	3	11.1 (1 of 9)	20.5
O. kolchida	3	2–4	33.3 (3 of 9)	30.8
O. leptospicularis	4.2	3–5	66.7 (6 of 9)	30.8
Spiculopteragia asymmetrica	3	3	11.1 (1 of 9)	20.5
S. boehmi	62.4	9–184	100 (9 of 9)	-
S. mathevossiani	3.8	1–10	44.4 (4 of 9)	32.5
Trichostrongylus axei	1	1	11.1 (1 of 9)	20.5

Table 3. Comparison of the spicule length of abomasal nematodes of red deer and roe deer

Species	Red deer			Roe deer			Red deer *vs*. roe deer–derived nematode
	N	X ± SD	Range	N	X ± SD	Range	
Ashworthius sidemi	759	777.8 ± 25.5	675–864	14	780.5 ± 66.0	622–823	P = 0.004*
Haemonchus contortus	6	451.2 ± 22.2	417–469	0	-	-	-
Mazamastrongylus dagestanica	2	198.5 ± 0.7	198–199	4	193.8 ± 7.0	187–202	-
Nematodirus roscidus	1	899	899	0	-	-	-
Ostertagia antipini	4	184.3 ± 2.2	181–186	0	-	-	-
O. kolchida	16	200.3 ± 16.6	169–238	10	192.6 ± 14.8	166–211	P = 0.243
O. leptospicularis	17	182.4 ± 12.8	149–194	52	171.9 ± 7.3	156–187	P < 0.001*
O. lyrata	0	-	-	2	197.5 ± 0.7	197–198	-
Spiculopteragia asymmetrica	4	236 ± 8.0	230–247	2	177 ± 2.8	175–179	-
S. boehmi	502	164.6 ± 8.4	144–213	639	160.9 ± 7.0	140–186	P < 0.001*
S. mathevossiani	24	182.2 ± 16.1	151–211	0	-	-	-
Trichostrongylus axei	2	84 ± 5.7	80–88	0	-	-	-
T. capricola	0	-	-	5	139.4 ± 5.6	134–148	-
T. vitrinus	0	-	-	2	92 ± 1.4	91–93	-

SD – standard deviation; * – statistically significant difference



Figs 1–7. Male distal body parts of abomasal nematodes found in both red deer and roe deer. 1 – *Ashworthius sidemi* (100×); 2 – *Mazamastrongylus dagestanica* (200×); 3 – *Ostertagia antipini* (400×); 4 – *Ostertagia kolchida* (400×); 5 – *Ostertagia leptospicularis* (400×); 6 – *Spiculopteragia asymmetrica* (400×); 7 – *Spiculopteragia boehmi* (400×)

Figs 8–10. Male distal body parts of abomasal nematodes found exclusively in roe deer. 8 – *Ostertagia lyrata* (400×); 9 – *Trichostrongylus capricola* (400×); 10 – *Trichostrongylus vitrinus* (1,000×)

Figs 11–14. Male distal body parts of abomasal nematodes found exclusively in red deer. 11 – *Haemonchus contortus* (100×); 12 – *Nematodirus roscidus* (100×); 13 – *Spiculopteragia mathevossiani* (400×); 14 – *Trichostrongylus axei* (400×)

Discussion

Previous studies suggest that the host species influences the morphology of generalist abomasal nematodes (30). Our present findings indicate that in all cases, the red deer-derived *O. leptospicularis* and *S. boehmi* had significantly longer spicules than the roe deer-derived specimens, the opposite being true for *A. sidemi*. According to a previous study, the spicules of *O. leptospicularis* varied in size depending on the host species, and those from red deer were slightly smaller than those from roe deer (30); the observation is in agreement with our results obtained for *A. sidemi*.

The red deer and roe deer obtained from various locations in Poland hosted 14 species of gastric nematodes, half of which were identified in both cervids. These nematodes included both those conventionally categorised as generalists, *i.e.* commonly found in more than one host, and those categorised as specialists, *i.e.* mainly parasites of a single host or rarely found in other hosts (3). The most prevalent gastric nematode species in the examined cervids were *S. boehmi* and *O. leptospicularis*, which is in agreement with previous studies (4, 8). *Spiculopteragia boehmi*, *O. leptospicularis* and their minor morphs (*S. mathevossiani* and *O. kolchida*, respectively) are regarded as generalists with a wide host range, as are *H. contortus*, *A. sidemi*, *T. axei* and *T. vitrinus* (7, 12, 29, 30). In addition, specialist species were also identified, including the moose GINs *Mazamastrongylus dagestanica* and *O. antipini* (15), the fallow deer GIN *S. asymmetrica* (20), the small ruminant nematode *T. capricola* (18), and the cattle parasite *O. lyrata*, a minor morph of *O. ostertagi* (10). Interestingly, our findings suggest that *M. dagestanica* has expanded its host range to red deer, as this is the first report of the nematode in the host. Regarding the host specificity of abomasal nematodes, it is worth mentioning that all of these species have also been previously diagnosed in European bison (13, 14, 18, 23). The studied GINs have previously been found to demonstrate a wider than expected host range among ten ruminant species investigated in Austria, with six helminths infecting more than one host species, and four being found in all species (29). In this study, *A. sidemi* was registered in red deer and roe deer for the first time in the Strzałowo Forest. Previously, the species was molecularly confirmed in larval cultures derived from cattle faeces in that area (21). The presence of *Ashworthius sidemi* in the abomasa of both red and roe deer indicates the possibility of rapid spread among different ruminant hosts. The species was first found in Poland in 1997 in the Bieszczady Mountains (south-eastern Poland) in a local population of European bison, red deer and roe deer (12); since then its distribution has expanded to other locations in Poland (7, 20), as well as to neighbouring countries such as the Czech Republic, where it was introduced together with infected European bison and then spread to local game (28). In addition, *A. sidemi* has been also found in fallow deer (20), moose (6) and cattle (21). This raises the question of whether ruminant GINs can be classified into generalist and specialist types.

The occurrence of GINs with a wide host range raises the risk of transmission between wild and domestic ruminants (2, 24). Indeed, it has been demonstrated that *S. spiculoptera* (synonym: *S. boehmi*) can be passed from roe deer to cattle (3). Moreover, infected deer could also potentially spread anthelmintic-resistant nematodes between various hosts; a previous study indicated that an anthelmintic-resistant isolate of *H. contortus* identified in roe deer was able to establish infection in a calf (3). This has serious implications, as both representatives of the Haemonchiinae family, *Ashworthius sidemi* and *H. contortus*, are blood-sucking nematodes with the potential to cause haemorrhagic anaemia (17, 19, 22). In addition, many other trichostrongyles are known to be potentially harmful and become pathogenic at abomasal loads exceeding 8,000 specimens in cervids (1). There is clearly a need for further, more extended studies on the pathogenicity of gastrointestinal nematodes in game and captive cervids.

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