

Infection of terrestrial snails with larvae of *Elaphostrongylus cervi* (Nematoda, Protostrongylidae) in Białowieża National Park

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Summary

Species composition of intermediate hosts of *Elaphostrongylus cervi* – terrestrial snails, prevalence and intensity of their infection in natural invasions have been determined. The intermediate hosts of *E. cervi* are *Succinea putris*, *Bradybena fruticum*, *Perforatella bidentata* and *Zonitoides nitidus*. From June to August the percentage of snails *S. putris* infected with *E. cervi* larvae has been on constant level and in September and October decreased. Snails *B. fruticum* the highest prevalence of invasion showed on July and later it successively lowered. In the case of *P. bidentata* prevalence of *E. cervi* infection was the lowest in August, and than raised up to the highest value in October. For the first time the highest extensiveness of spontaneous infection has been found in *P. bidentata*. Till now as the most infected species of snail always has been noted *S. putris*. The highest intensity of infection - 242 larvae was found in *B. fruticum*, and little lower - 202 in *S. putris*. In two other species intensiveness ranged from 1 to 23 larvae. The most important role in spreading of *E. cervi* in Białowieża Forest fulfil *S. putris* and *B. fruticum* because of their high number in environment and *P. bidens* because of the highest intensiveness of infection.

Keywords: *Elaphostrongylus cervi*; Cervides; Protostrongylidae; Nematodes; intermediate host

Introduction

Elaphostrongylus cervi Cameron, 1931 is the nematode from the family Protostrongylidae, parasite of intermuscular connective tissue and central nervous system. This parasite occurs in red deer in Eurasia. Adult nematodes situated in the intermuscular connective tissue cause sub-clinical elaphostrongylosis, while those occurring in the central nervous system may produce grave clinical symptoms leading even to death of the deer. Many species of terrestrial snails can play the role of their intermediate

hosts. In the external environment L_1 get out faeces of a deer and actively penetrate body integument of a snail. In the muscles of foot L_1 feed themselves intensively, grow up, molt twice and in the period of 20 - 40 days reach invasive stadium L_{III} (Demiaszkiewicz, 1987b). Red deer infect themselves with these nematodes eating together with grass and vegetable creeping on them infected with invasive larvae small terrestrial snails.

Material and methods

Snails were collected in section No 314 in centrum of restricted part of Białowieża National Park Natural Reserve, along Orłówka river, in red deer watering place.

Table 1. Results of snails collecting in 2000 – 2004 year

Snails	2000	2001	2002	2003	2004
<i>Succinea putris</i>	257	1393	158	327	105
<i>Bradybaena fruticum</i>	97	433	24	277	162
<i>Perforatella bidens</i>	17	87	21	38	3
<i>Zonitoides nitidus</i>	2	41	46	2	6
<i>Valonia costata</i>					26
<i>Cochlicopa lubrica</i>				6	15
<i>Limax sp.</i>					9
<i>Arion subfuscus</i>		7			1
<i>Clausilia pumila</i>					3
<i>Laciniaria sp</i>			3		
<i>Limax cinereo-niger</i>		1	1		
<i>Trichia hispida</i>		2			
<i>Vitrina pellucida</i>		2			
total	373	1966	253	650	330

Snails were collected on the surface and 10 cm deep early morning with dew, when the snails were active. Results of snails collecting are presented in Tab.1.

To examine the infection of the snails by larvae of *E. cervi*, snails were necropsied and examined in compressor using stereomicroscope with 40x magnification. After fragmentation snails were digested in 1 % pepsin in 1 molar hydrochloric acid to compare with results from compressor examination.

Table 2. Infection of snails with larvae of *Elaphostrongylus cervi* in Białowieża National Park in 2000

Snails	Examined	Infected	%	Intensity
<i>Succinea putris</i>	257	12	4.6	1 – 12
<i>Perforatella bidentata</i>	17	1	5.8	5
<i>Bradybaena fruticum</i>	97	3	3.08	4 – 9
<i>Zonitoides nitidus</i>	2	-	-	-
total	373	16	4.29	

Results

All results of infection of particular species of snails in successive years are presented in tables No. 2 – 8.

During the period 2000 – 2004 in Białowieża National Park were collected 3572 terrestrial snails belonging to 13 species. The most numerous species was *S. putris*. Also common species were *B. fruticum*, *P. bidentata* and *Z. nitidus*. Other species occurred incidentally and only some specimens have been collected. Among 13 examined species *E. cervi* was found in 4 of them but the prevalence of invasion varied. The most infected snail in all examined periods was *P. bidentata* – 12.04 %, and in 2001 prevalence of invasion in it was also the highest and reached 20.6 %. Prevalence of infection of the other species with larvae of *E. cervi* was lower and extended in *S. putris* 8.7 %, *Z. nitidus* 6.2 %, and *B. fruticum* 4.2 %. In 2001

Table 3. Infection of snails with larvae of *Elaphostrongylus cervi* in Białowieża National Park in 2001

Snails	Examined	Infected	%	Intensity
<i>Succinea putris</i>	1393	159	11.4	1 – 202
<i>Bradybaena fruticum</i>	433	33	7.6	1 – 242
<i>Perforatella bidentata</i>	87	18	20.6	1 – 23
<i>Zonitoides nitidus</i>	41	6	14.2	1
<i>Arion subfuscus</i>	7	-	-	-
<i>Vitrina pellucida</i>	2	-	-	-
<i>Trichia hispida</i>	2	-	-	-
<i>Limax cinereo-niger</i>	1	-	-	-
total	1966	216	11	

Table 4. Infection of snails with larvae of *Elaphostrongylus cervi* in Białowieża National Park in 2002

Snails	Examined	Infected	%	Intensity
<i>Succinea putris</i>	158	2	1.27	1 – 3
<i>Zonitoides nitidus</i>	46	-	-	-
<i>Bradybaena fruticum</i>	24	-	-	-
<i>Perforatella bidentata</i>	21	-	-	-
<i>Laciniaria sp</i>	3	-	-	-
<i>Limax cinereo-niger</i>	1	-	-	-
total	253	2	0.8	

prevalence of infection in these species was 11.4 %, 14.2 % and 7.6 % respectively.

The highest intensity of infection – 242 larvae was found in *B. fruticum*, and little lower – 202 in *S. putris*. In two other species intensity varied from 1 to 23 larvae.

In the years 2000, 2002, 2003 and 2004 examined snails were collected in September and October, and because of it samples from separate years were not numerous and not exceeded some hundreds snails. In the autumn months

Table 5. Infection of snails with larvae of *Elaphostrongylus cervi* in Białowieża National Park in 2003

Snails	Examined	Infected	%	Intensity
<i>Succinea putris</i>	327	18	5.7	1 – 21
<i>Bradybaena fruticum</i>	277	6	2.17	1 – 5
<i>Perforatella bidentata</i>	38	1	2.7	2
<i>Zonitoides nitidus</i>	2	-	-	-
<i>Cochlicopa lubrica</i>	6	-	-	-
total	650	25	3.85	

number of collected snails decreased significantly and it caused of their drying the summer heats, and by high mortality of infected specimens. It could influence on found then low prevalence and intensity of infection. Only snails examined in 2001 were numerous (1964 specimens) and were collected also in summer months (from June to October). It allowed us to establish seasonal dynamics of invasion with larvae *E. cervi* in three most important species of snails. It has been determinate that from June to August the percentage of snails *S. putris* infected with *E. cervi* larvae had been on constant level and in September and October decreased. Snails *B. fruticum* the highest prevalence of invasion showed on July and later intensity successively decreased. However in case of *P. bidentata* prevalence of infection of *E. cervi* larvae was the lowest in August, and then raised up to the highest value in October. The last of mentioned species lives in forest litter, goes on plants only some centimeters up and it is very resistant on environmental conditions, in it drying. It makes that these snails can be infected during all vegetation season.

Table 6. Infection of snails with larvae of *Elaphostrongylus cervi* in Białowieża National Park in 2004

Snails	Examined	Infected	%	Intensity
<i>Bradybaena fruticum</i>	162	-	-	
<i>Succinea putris</i>	105	3	2.86	1 – 15
<i>Valonia costata</i>	26	-	-	-
<i>Cochlicopa lubrica</i>	15	-	-	-
<i>Limax sp.</i>	9	-	-	-
<i>Zonitoides nitidus</i>	6	-	-	-
<i>Perforatella bidens</i>	3	-	-	-
<i>Clausilia pumila</i>	3	-	-	-
<i>Arion subfuscus</i>	1	-	-	-
total	330	3	0.9	

Discussion

Earlier investigations of other authors in Białowieża Forest showed that from 6 examined snail's species infection of *E. cervi* larvae have been found only in 5. The highest infection was observed was in *S. putris* – 27 %. Prevalence of infection in remaining species was lower and reached in *B. fruticum* 8.3 %, *P. bidentata* 17.3 %, *A. subfuscus* 12.5 % and *Z. nitidus* 9 %. The highest intensity extending 557 larvae was found also in *S. putris* (Demiaszkiewicz, 1987a). Observed prevalence and intensity of invasion in *S. putris* and *B. fruticum* was much higher than in presented investigation. It was caused by collecting of snails in closed yard for deer in the Show Reserve. Afterwards Misiewicz and Demiaszkiewicz (1994) in experimental hunting grounds of Forest Research Institute in Warmińsko-Mazurskie (Hawa) and Śląskie (Pszczyna) voivodeships have been examined 5 species of snails: *A. subfuscus*, *B. fruticum*, *Cepaea nemoralis*, *S. putris* and *Z. nitidus*. The highest prevalence of infection of larvae *E. cervi* in Hawa forest inspectorate was recorded in *S. putris* – 6.3 % and in Pszczyna forest inspectorate 7.4 % in this

Table 7. Spontaneous infection of snails with larvae of *E. cervi* in Białowieża National Park in 2000 – 2004

Snails	Examined	Infected	%	Intensity
<i>Succinea putris</i>	2240	194	8.7	1 – 202
<i>Bradybaena fruticum</i>	993	42	4.2	1 – 242
<i>Perforatella bidens</i>	166	20	12.04	1 – 23
<i>Zonitoides nitidus</i>	97	6	6.2	1
<i>Valonia costata</i>	26	-	-	-
<i>Cochlicopa lubrica</i>	21	-	-	-
<i>Limax sp.</i>	9	-	-	-
<i>Arion subfuscus</i>	8	-	-	-
<i>Clausilia pumila</i>	3	-	-	-
<i>Laciniaria sp.</i>	3	-	-	-
<i>Limax cinereo-niger</i>	2	-	-	-
<i>Trichia hispida</i>	2	-	-	-
<i>Vitrina pellucida</i>	2	-	-	-
total	3572	262	7.3	

same species. The highest intensity extending 150 larvae has been noted also in *S. putris*. In remaining species of snails infection with the larvae of *E. cervi* was found only in one case in Pszczyna forest inspectorate in *Z. nitidus* – one larva. Another research conducted by Demiaszkiewicz and Drózdź (1997) showed prevalence of invasion of Protostrongylidae in deer farm in Kosewo Górne. Snails collected in fenced yards belonged to 4 species: *S. putris*, *Z. nitidus*, *Zenobiella rubiginosa*, *Arion*

Table 8. Dynamic of infection of most common snails with *Elaphostrongylus cervi* larvae in 2001

Month	<i>Succinea putris</i>				<i>Bradybaena fruticum</i>				<i>Perforatella bidens</i>			
	examined	infected	%	range	examined	infected	%	range	examined	infected	%	range
VI	350	45	12.8	1 – 202	143	7	4.9	1 – 73	12	3	25.0	1 – 23
VII	371	37	10.0	1 – 87	119	16	13.4	1 – 242	29	6	20.7	1 – 23
VIII	288	40	13.8	1 – 83	80	7	8.8	1 – 35	26	3	11.5	1 – 4
IX	245	29	11.8	1 – 35	60	2	3.3	1 – 3	16	3	18.7	1
X	139	9	6.5	1 – 14	31	1	3.2	6	4	3	75.0	1 – 5

circumscriptus. From them only *S. putris* was infected with larvae of Protostrongylidae. In first year prevalence of invasion of larvae *E. cervi* was 41 % and next very dry year only 23 %. The highest intensity of infection reached 105 larvae. High prevalence of invasion in presented examinations was caused by high concentration of animals in the yards. We found more species of snails than cited authors, similar to Feliksiak (1935), who noted in Białowieża Forest from 16 to 33 species according to different types of crops. Big part of noted by this author species are the snails living in litter.

Present examinations showed that except *P. bidens* snails living in litter don't play any important role in distribution of invasion of *E. cervi*.

Found in our investigations prevalence of invasion *S. putris* with larvae *E. cervi* was much lower than presented by foreign authors (Ljubimow, 1959; Panin, 1964; Hale, 1980), and in Poland from Kosewo (Demiaszkiewicz & Drózdź, 1997) and Białowieża (Demiaszkiewicz, 1987a). According to Panin (1964) in Altaj the highest prevalence of invasion *E. cervi* occurred in *Zenobiella nordenskioldi* was 23.7 %. However Hale (1980) in Austria observed that the most infected species was *B. fruticum* – 21.7 %.

Noted in present examinations, infection of *S. putris* was similar to that presented by Misiewicz and Demiaszkiewicz (1994) conducted also in open hunting area. In farm conditions, there is higher concentration of larvae on small area.

In presented research were defined species of terrestrial snails which are the hosts of *E. cervi* in spontaneous invasion. There are: *S. putris*, *B. fruticum*, *P. bidentata* and *Z. nitidus*. Our work has showed that these species play the most important role in spreading of invasion of *E. cervi* in Białowieża Forest. For the first time the highest prevalence

of spontaneous infection has been found in *P. bidentata*. Till now as the most infected species of snail always has been noted *S. putris*.

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