



Original Contribution

SEASONAL CHANGES IN MORPHOLOGY AMONG POPULATIONS OF LAND SNAIL *HELICELLA OBVIA* AND EFFECTS OF THESE CHANGES ON CIRCULATION OF PROTOSTRONGYLIDS IN PASTURES OF STARA ZAGORA, SOUTH BULGARIA

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ABSTRACT

The aim of the present study was to examine the role of the size groups in the populations of the terrestrial gastropods *Helicella obvia* on the circulation of the protostrongylid nematodes in pastures of sheep and goats. Two pastures in the region of Stara Zagora were studied. We examined *H. obvia* infection in 796 specimens. Snails were grouped in six size classes on the basis of the diameter of the shell (2-4, 4-6, 6-8, 8-10, 10-12 and 12-15 mm). The size structure of populations was characterized by the relative abundance of the size classes. We observed that *H. obvia* of the same age (that is same generation) were prevalent among the isolated nematode population. The prevalence, the mean abundance and mean intensity showed a gradual increase from snails with diameter of shell 2-4 mm to those with diameter of shell 10-12 mm. Therefore, adult snails had more important role in the circulation of protostrongylids than juvenile snails.

Key words: Protostrongylidae, *Helicella obvia*, population structure, infection

INTRODUCTION

The nematodes of the family Protostrongylidae Leiper, 1926 are parasites on a wide range of various domestic and wild herbivorous mammals (Schultz and Gvozdev, 1970, 1972; Boev, 1975, 1976; Anderson, 1978, 2000; Schmidt and Roberts, 1986; Vasilev et al., 1986; Kamburov et al., 1994) In Bulgaria, 5 species of this group are known to infect goats and sheep. The life cycle of protostrongylids involve land snails as intermediate hosts. In snails, nematodes develop to the third larval stage, which is infective for final hosts.

Two basic approaches are used in the study of intermediate hosts of protostrongylids. The first one is directed at examining the natural infections in snails. The second one involves experimental characterisation of the susceptibility of snail species to infection with protostrongylid larvae.

Some general patterns can be outlined

relative to the participation of various gastropod species in the life cycles of protostrongylids from sheep and goats. Among biological peculiarities of snails, their activity is essential for the protostrongylid infections (Trushin, 1973a; Cabaret, 1981). The involvement of various snail species in the transmission of protostrongylids is also believed to depend on the duration of life cycle of molluscs (Cabaret, 1984).

According to Cabaret (1984), the age structure is probably the most important among the population characteristics of the intermediate host, as the infection of adult snails is usually higher than that in young ones. This suggestion is supported by the observation that the abundance of protostrongylid larvae was 4-10 times lower in juvenile than in adult snails in 3 gastropod species in Tunisia (Lahmar et al., 1990). Similar conclusions were achieved in France on *Cepaea* sp.: adults were considerably more infected than young snails (0.957±0.02 vs 0.053±0.015 larvae / individual, Cabaret et al., 1983).

With this background knowledge of the nematode and its life cycle, we aim to

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go further in the present study, to examine the role of the size groups in populations of *Helicella obvia* in the circulation of protostrongylid nematodes in sheep and goat pastures in Stara Zagora.

MATERIAL AND METHODS

The dynamics of the size structure of *Helicella obvia* and the infection parameters of the size groups were examined in two pastures in South Bulgaria, these at the villages of Oryakhovitsa and Rakitnitsa. The pastures were selected on the basis of several criteria: high density of the intermediate host *H. obvia*, presence of the complex of all protostrongylid species occurring in the region and high parameters of infection recorded in the course of our previous study (Georgiev et al., 2003). At each monthly visit in both experimental areas, 15 primary samples were collected. The monthly sampling of each pasture was performed within a day during the second ten days of the respective month. The day of the visit was chosen to be a rainy one (if possible) or after a rainy one. In warm months (May –

October), the collections were done between 7.00 a.m. and 9.00 a.m.

The sampling was done by a modification of the method of Gilyarov (1987). Briefly, a square frame with a side of 0.5 m (area of 0.25 m²) was used for collection of 15 primary samples from each pasture. During collection, the snail specimens were stored in separate labelled plastic boxes with ventilation openings.

The snails from each sample were divided into size groups according to shell diameter at a 1 mm step. The shell of each snail was removed and it was studied between heavy glasses. The larvae of protostrongylids were found in the musculature of the leg. They were isolated mechanically or after a digestion with trypsin solution. The statistical analysis was performed by Kruskal-Wallis Test (Sokal and Rolf, 1995).

RESULTS AND DISCUSSION

The size groups of *H. obvia* were arranged in six size classes according to their shell diameters: Class 1: 2-4 mm, Class 2: 4-6 mm, Class 3: 6-8 mm, Class 4: 8-10 mm, Class 5: 10-12 mm and Class 6: 12-15 mm (**Table 1**).

Table 1. Seasonal dynamics of the size structure of *Helicella obvia* measured by the relative abundance (%) of the size classes on the pastures of the villages Oryakhovitsa and Rakitnitsa.

Size classes		Months							
No	Diameter of shell (mm)	April	May	June	July	August	September	October	November
Oryakhovitsa									
1	2-4	14.1	3.2	-	-	-	-	-	-
2	4-6	49.3	12.8	1.3	4.5	5.1	-	-	-
3	6-8	29.6	16.0	21.8	10.4	13.6	3.6	-	-
4	8-10	7.0	35.0	20.5	34.3	37.3	37.3	-	8.3
5	10-12	-	30.9	44.9	40.3	28.8	33.7	57.1	25.0
6	12-15	-	2.1	11.5	10.5	15.2	25.4	42.9	66.7
Rakitnitsa									
1	2-4	60.0	13.7	3.8	-	-	-	-	-
2	4-6	37.8	47.1	7.8	3.4	-	-	3.6	-
3	6-8	2.2	25.5	32.5	24.1	-	-	-	-
4	8-10	-	13.7	33.8	20.7	-	6.2	14.3	-
5	10-12	-	-	20.8	41.5	75.0	68.8	60.7	50.0
6	12-15	-	-	1.3	10.3	25.0	25.0	21.4	50.0

The study showed that the predominant proportion among populations of *H. obvia* was that of the same age. The snails from the small size groups appeared in spring. They were absent during the late summer and autumn months, which indicated the presence of one generation per year. The small size classes (Classes 1-3, with shell diameter < 8 mm) prevailed in the population at the Oryakhovitsa pasture in April and on that of

Rakitnitsa in April and May only. During the other months to the end of the year, the participation of snails from the small size groups was either insignificant (as was the case in October on the Rakitnitsa pasture) or they were totally absent in the samples examined (**Table 1**). At the same time, snails from the big size groups (shell diameters > 8 mm) did not participate or had insignificant participation in the population structure in

April. They prevailed in the age structure of snail populations from May or June onward in

the Oryakhovitsa and Rakitnitsa pastures, respectively.

Table 2. Parameters of protostrongylids infection of the size classes of *H. obvia* in the pastures of Oryakhovitsa and Rakitnitsa.

Parameters of infection	Size classes						K-W
	1 2-4 mm	2 4-6 mm	3 6-8 mm	4 8-10 mm	5 10-12 mm	6 12-15 mm	
Oryakhovitsa							
Sample size	13	54	71	131	155	68	
Prevalence (%)	15.4	14.8	25.4	34.4	45.8	30.9	
Mean abundance	0.15±0.4	0.37±1.1	0.92±2.4	1.56±4.7	2.50±6.4	2.07±4.6	H=37.32, **
Dispersion/mean ratio	0.92	3.19	6.04	14.13	16.34	10.44	
Mean intensity	1	2.50±1.7	3.61±3.5	4.53±7.2	5.45±8.6	6.71±6.3	H=17.16, *
Rakitnitsa							
Sample size	36	49	46	45	97	31	
Prevalence (%)	22.2	12.2	15.2	26.7	34.0	29.0	
Mean abundance	0.31±0.6	0.31±1.1	0.22±0.6	1.38±6.6	0.84±1.7	0.74±2.2	NS
Dispersion/mean ratio	1.24	4.05	1.58	31.0	3.60	6.60	
Mean intensity	1.38±0.5	2.50±2.4	1.43±0.8	5.17±12.3	2.45±2.2	2.56±3.6	NS

NS – no statistically significant differences, * - $p < 0.05$, ** - $p < 0.001$.

Larvae of *Muellerius capillaris*, *Neostongylus linearis*, *Cystocaulus ocreatus* and *Protostrongylus* sp. were detected in the snails studied. The prevalence and the mean abundance of protostrongylids varied

between 7.0% and 67.6% and between 0.15 and 3.54 larvae / snail, respectively. The parameters of infection for the various size classes are presented on **Table 2**.

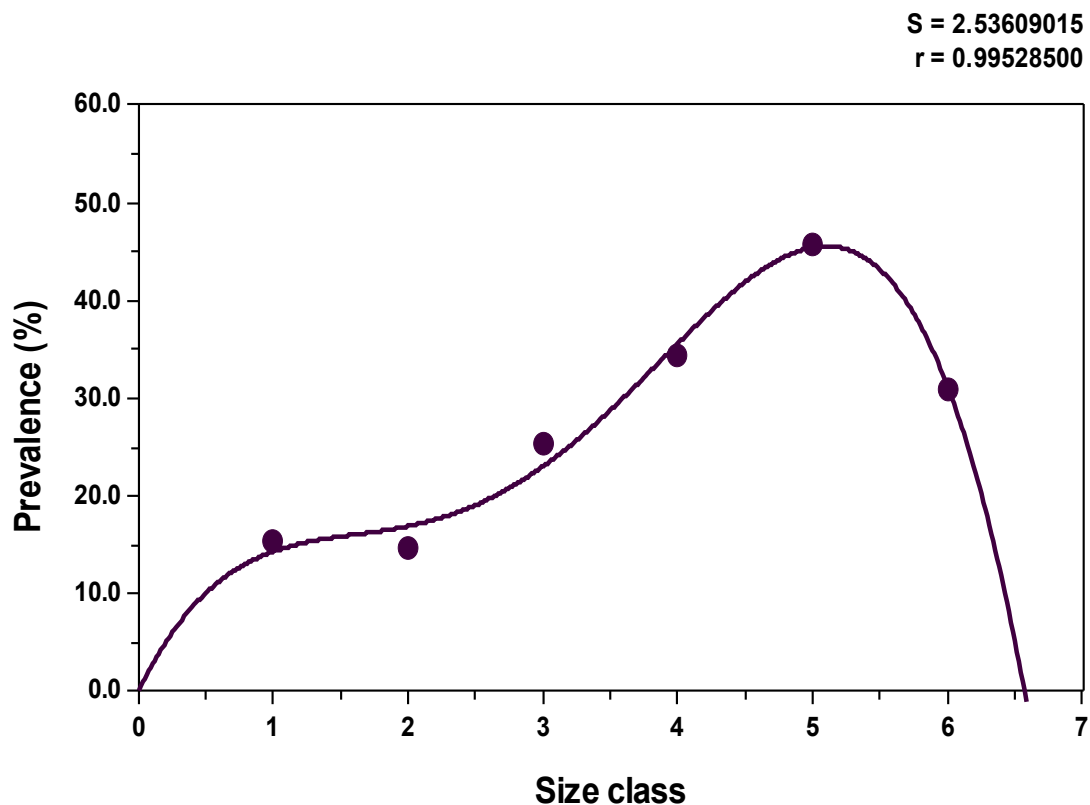


Figure 1: Prevalence of protostrongylid larvae in the size classes of *Helicella obvia*.

On both pastures, snails of *H. obvia* belonging to size classes 4 and 5 were most numerous. For the Oryakhovitsa pasture, the values of the mean abundance and the mean intensity of protostrongylids according to size groups

were statistically significant. The prevalence, mean abundance and mean intensity (**Figure 1, 2**) showed a gradual increase from Class 2 (diameter of shell 4-6 mm) to Class 5 (diameter of shell 10-12 mm).

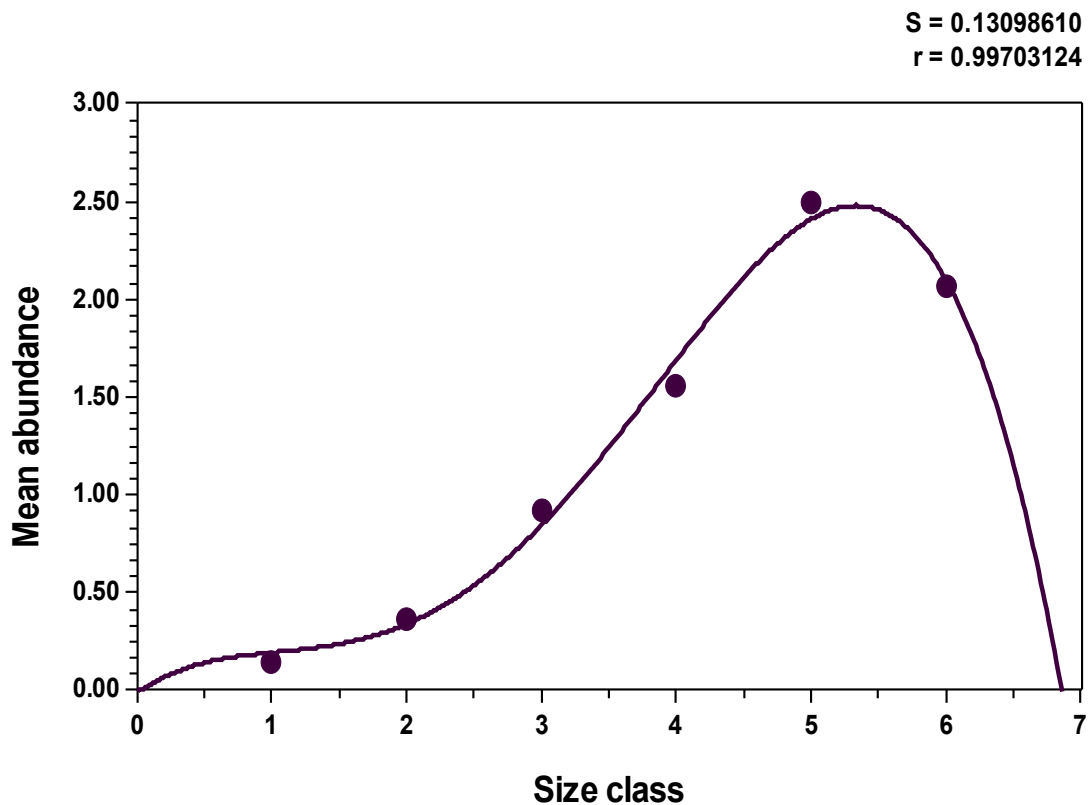


Figure 2: Mean abundance of *protostrongylid* larvae in the size classes of *Helicella obvia*

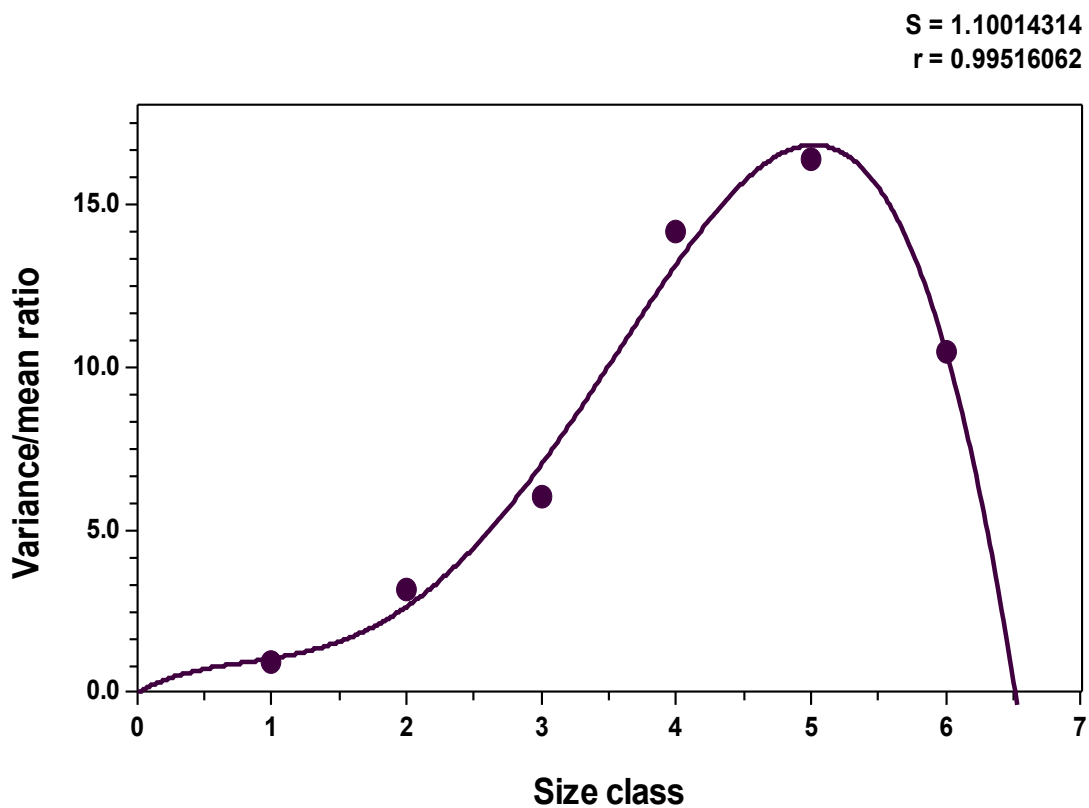


Figure 3. Variance / mean ratio for the abundance of *protostrongylid* larvae in the size classes of *Helicella obvia*

These parameters were with lower values in size class 6 compared to the preceding one. A similar change was observed in the variance/mean ratio of the abundance as a

measure of the aggregation of the distribution (**Figure 3.**). These data confirm the general rule suggested by Cabaret (1984) that the adult snails are more infected than young ones.

While the parameters of infection of the various size classes showed significant differences for the Oryakhovitsa pasture, such variations were not observed for the Rakitnitsa pasture. This is the reason for interpreting the data about the latter pasture as rather relative. As the parameters of host populations on both pastures were very similar, the probable cause for observed differences should be sought in the lower parameters of infection on the Rakitnitsa pasture.

CONCLUSION

On both pastures studied during the period April – November, nematodes belonging to the same generation represent the populations of *Helicella obvia*. In general, the infection increases in direction from small size classes to large size classes of intermediate hosts. The increase of the parameters of infection in large size classes is probably due to the accumulation of larvae in the hosts parallel with the process of the increase of their size and biomass. These results confirm the opinion of Cabaret (1984b) that the higher infection of adult vs juvenile gastropod intermediate hosts could be assumed as a general rule of the infections of land snails with protostrongylids.

REFERENCES

1. Anderson R.C. (1978) Keys to genera of the superfamily Metastrongyloidea. In: Anderson R.C., Chabaud A.G., Willmot S. (Eds.) *CIH keys to the nematode parasites of vertebrates*. CAB, 540 p.
2. Anderson R.C. (2000) *Nematode Parasites of Vertebrates. Their development and transmission*. 2nd edition. Wallingford, CABI Publishing, 650 p.
3. Boev S. N. (1975) Protostrongylids. In: *Fundamentals of Nematology*, 25, Nauka, Moscow, p. 268.
4. Boev, S. N. (1976) The family Protostrongylidae Leiper, 1926. *Fundamentals of Nematology*, 26, Nauka, Moscow, p. 82-133.
5. Cabaret J. (1981) *Réceptivité des Mollusques terrestres de la région de Rabat à l'infestation par les protostrongyles dans les conditions expérimentales et naturelles*. Thèse Doctorat es-Sciences, Paris, 214 p.
6. Cabaret J. (1984) Les mollusques hôtes intermédiaires dans la muelleriose caprine en Touraine. *Les maladies de la chèvre*, 9-11, oct., 337-345.
7. Cabaret J., Bouley N., Gruner L. (1983) Caractérisation de zones à risque parasitaire pour des Ovins élevés en liberté sur les parcours des Causses. 2. Protostrongylides. *Annales de Recherches Vétérinaires* 14, 3, 301-310.
8. Egorov Y. G. (1960) On the biology of the lungworm *Muellerius capillaris*. *Proceedings of the Scientific Veterinary Institute*, 1, 160-170.
9. Georgiev D. M., Kostadinova A., Georgiev B. B. (2003) Land snails in the transmission of protostrongylids in pastures in South Bulgaria: variability of infection levels related to environmental factors. *Acta Parasitologica*, 48 (3), 208-217
10. Georgiev D. M. (2003) Identification of periods at maximum risk for infection with protostrongylid nematodes of sheep and goats on pastures in the region of Stara Zagora. *Animal Sciences*, 3-4.
11. Gilyarov M. S. (1987) Quantifying of large invertebrates (mesofauna). In: Gilyarov M.S., Striganova B. R. (eds.) *Quantitative methods in soil zoology*. Nauka, Moscow, 9-26.
12. Golubev N. F. (1963) On the biology of protostrongylids in sheep from Crimea. In: *Problems of parasitology, Proceedings of the IV Scientific Conference of Parasitologists of USSR*, Kiev, 172-175.
13. Kamburov, P., Vasilev I., Georgieva D., Kamenov Y., Koinarski V. (1994) *Veterinary Parasitology, Agropress, Sofia*, 462.
14. Lahmar S., Cabaret J., Cheniti T. (1990) Land snails and periods at high risk for protostrongylid infection on a sheep-grazed pasture of Northeast Tunisia. *Veterinary parasitology*, 36, 105-115.
15. Samnaliev P. (1966) On the infection rate of *Helicella obvia* (Hartman, 1840) and *Zebrina detrita* (Muller, 1774) with *Protostrongylus* larvae in the Sofia region. *Bulletin of the Central Helminthological Laboratory*, 11, 111-117.

16. Samnaliev P. (1968) Studies on the biological cycle of protostrongylids in Bulgaria (PhD thesis). Sofia, Bulgarian Academy of Sciences, Central Helminthological Laboratory, 142.
17. Schmidt G.D., Roberts L.S. (1985) *Foundations of Parasitology*. St. Louis et al.: Times Mirror/Mosby College Publishing, 775 p.
18. Schultz R. S., Gvozdev E. V. (1970) *Fundamentals of General Helminthology, Vol. 1*. Nauka, Moscow, 492.
19. Schultz R. S., Gvozdev E. V. (1972) *Fundamentals of General Helminthology, vol. 2*. Nauka, Moscow, 516.
20. Seinhorst J.M. (1959) A rapid method for the transfer of nematodes from fixative to anhydrous glycerin. *Nematologica*, 4, 67-69.
21. Sokal R.R., Rohlf F.J. (1981). *Biometry*. W.H. Freeman, New York, 887 p.
22. Trushin I. N. (1973b) Role of ecological factors in the natural infection of molluscs with *Muellerius* larvae. In: *Problems of General and Applied Helminthology*, Nauka, Moscow, 344-347.
23. Trushin I. N. (1973a) Evaluation of pastures for mulleriosis. In: *Helminthological Evaluation of Pastures*. Kolos, Moscow, 80-95.
24. Vasilev I., Dzhankov I., Kamburov P. (1986) *Veterinary parasitology and invasive diseases*. Zemizdat, Sofia, p. 479.
25. Žďárská J. (1960) Larvální stadia cizopasných červů z našich suchozemských plžů. *Československá Parasitologie*, 7, 355-379.
26. Zurliiski P. (1994) Study upon intermediate hosts of the family Protostrongylidae Leiper, 1926. *Veterinarna sbirka*, 1, 26-27.