



The Stara Zagora area in southern Bulgaria is home to pasture vegetation and land snails infected with protostrongylids.

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Abstract. Nine pastures in the Stara Zagora region were studied to determine the association between protostrongylid infections of land snails and the type of pasture vegetation. An abundance of plant life was a distinguishing feature of the pastures. The following species of strongylus larvae were found: Protostrongylus sp., Neostrongylus linearis, Cystocaulus ocreatus, and Muellerius capillaris. Helicella obvia is the primary intermediate host for protostrongylids, and the multiple regression analysis verified connections between vegetation and infection characteristics. For both the overall prevalence of protostrongylids and the prevalence of the most prevalent species, M. capillaris, the only predictor in regression models was the type of the primary vegetation.

Keywords: Protostrongylidae, snails, pastures, Upper Thracian plain, primary vegetation

Introduction

Of the worms parasitizing sheep and goats, protostrongylids are among the most important (Daskalov et al., 1961; Bratanov, 1965; Shultz and Gvozdev, 1970; Boev, 1975; Bankov et al., 1977; Cabaret, 1984; Marinov, 1997; Zurliski and Rusev, 1990; Kamburov et al., 1994; Halacheva et al., 2001). Pneumonic foci formations and pathomorphological changes in the lungs of small ruminants are frequently caused by protostrongylid infections. Ecological methods are used to assess the protostrongylid infection rate in pastures. Several research conducted in Europe and along the African Mediterranean coast have helped to advance effective approaches in this regard. Typically, countries like Russia (Trushin, 1973a), France (Cabaret, 1983, 1984a, 1984b; Cabaret et al., 1983, 1985), Morocco (Cabaret, 1988), and Tunisia (Lahmar et al., 1990) do these kind of investigations. Grasslands in the Stara Zagora region and on the slopes of Sarnena Sredna Gora mountain in Bulgaria have been the subject of comparable research by Georgiev (2003), Georgiev et al. (2003), Georgiev and Georgiev (2002), and Georgiev and Georgiev (2004). They determined the species make-up of land snails and the characteristics of protostrongylid larval infection in connection to certain environmental variables. The current research set out to ascertain the vegetation's significance as a feature that can characterize the characteristics of protostrongylid infection of Helicella obvia, the primary intermediate host.

Material and methods

The area under study is located in the Apennine-Balkan soil subregion of the Mediterranean soil region, according to the Bulgarian geographical zonality of soils (Ninov, 1997). The majority of the meadows were located in the region of Thracian and Middle Tundzha. the province of Sredna Gora, where several of them reside. Gruev and Kuzmanov's (1994) biogeographical zonality pattern for Bulgaria was used in this analysis. Xerothermic forests of European turkey oak (*Quercus cerris* L.), Italian oak (*Quercus*

frainetto Ten.), and pubescent oak (*Quercus pubescens* Willd.) make up the typical primary plant communities for the Upper Thracian plain subregion. However, most of these forests have been cleared for agriculture or pastures (Gruev and Kuzmanov, 1994). According to Bondev (1991), the most prevalent contemporary plant communities in the subregion are as follows: (1) agricultural land that replaced forests of pubescent oak (*Q. pubescens*) and Italian pubescent oak (*Q. virgiliana* Ten.); (2) agricultural land that replaced mixed forests of European turkey oak (*Q. cerris*) and Italian oak (*Q. frainetto*); (3) agricultural land that replaced mixed forests of European turkey oak (*Q. cerris*) and Italian pubescent oak (*Q. virgiliana*), often mixed with pedunculate oak (*Q. pedunculiflora* C. Koch); (4) agricultural lands that replaced forests of field elm (*Ulmus minor* Mill.), raywood ash (*Fraxinus oxycarpa* Willd.), pedunculate oak (*Q. pedunculiflora* C. Koch) etc. We took stock of each pasture's flora in the spring, summer, and fall. At each site, we took samples of the most frequent species and used visual inspection to identify the dominant pen. Using plant identification guides for higher Bulgarian plants (Stoyanov and Stefanov, 1948; Delipavlov et al., 1992; Andreev et al., 1992; Petrova et al., 1999), the plants were identified. We compared the dominant and common species found in each pasture with data from the 1964 treatise "Vegetation of meadows and pastures in Bulgaria" (Ganchev et al.) and phytocenotic traits from the Stara Zagora region (Radanova, 2005; Radanova, 2007; Radanova and Ivanova, 2007). This allowed us to determine the plant formations of the various pastures. Snail infection rates were calculated using specimens of *Heterodonta obvia* collected. Prior research established that this particular type of snail is crucial for the dissemination of parasites within the area under consideration. Between 7 and 9 in the morning, before dew-rise, snails were painstakingly picked by hand. The specimens that were gathered were placed in *Marrubium peregrinum* for storage. Perennial ryegrass predominates in plastic containers with holes for air circulation and a label that provides *Lolietia* with details about the pasture's flora, including the date of collection, species, and the amount of perennis grass that resides in the mesophytic formations. Plains, hollows, and the subtype of mesophytic grass (Ganchev et al., 1964) were the sites of the infections measured quantitatively. were in agreement with the following Bush et al. (1997) definitions: frequency or Field next to Rakitnitsa settlement. Barley infection extent (percentage), mean intensity, and mean abundance (A) in vegetation. the eryngo, strawberry, restharrow, and grasslands were All hypotheses were dominant because all data had aggregate distributions. Using non-parametric approaches, we were unable to refer test the observed plant community. In order to assess the relationship between the factors and the transient plant forms, Ganchev et al. (1964) used Spearman's rank correlation coefficient. The paramount importance of (rs). Two *Hordeum leporinum* did not show a categorical pattern when comparing the distribution of the characteristics that were investigated, however

the Kruskal-Wallis test (K-W) was used when there were more than two samples, and the Mann-Whitney test (M-W) was used when there were two samples (Sokal & Rohlf, 1981). Prior to using parametric statistical techniques, data about intensity and abundance were transformed using logarithms [$\ln(n+1)$], whereas data regarding prevalence was transformed using arcsines. Quantitative Parasitology 1.0 (Rózsa et al., 2000) software was used to compare prevalence data using the Fisher exact test and mean intensity data using the Bootstrap test in order to verify specific assumptions.

Results

Table 1 displays the data on the vegetation of sheep and goat pastures collected from the Stara Zagora area. Each pasture had the following characteristics: Field close to the Oryahovitsa settlement. The majority of the plant species were identified as *Lolium perenne*. This community might be referred to as the *Hordeeta leporinae* formation; other common species of leguminous plants include *Coronilla varia*, while herbaceous plants include *Taraxacum officinale* and *Carex caryophyllea*.

Meadow next to Malka Vereya settlement. Based on the presence of *Festuca pseudovina*, this grass community was categorized as xerophylic grasses, which is a subtype of the secondary microtherm steppe assemblage group (Ganchev et al., 1964). Meadow close to the towns of Karanovo and Panicherevo. According to Ganchev et al. (1964), these grass communities might be grouped with the xerophylic grasses subtype and the *Dichantieta ischaemi* (= *Andropogoneta ischaemi*) formation, which is a subgroup of secondary mesotherm steppe formations.



Land close to Bogdanovo and Zagortsi. According to Ganchev et al. (1964), this grass community may be classified as *Poaeta sylvicolae*, which is a kind of mesophytic grass vegetation often seen on plains and hollows. Pasture next to the Starozagorski Mineralni bani resort. Species of *Bromus* were most common in the region under investigation. Mesophytic formations, among which *Brometa commutati* is the most homogeneous, are most often found in plains and hollows. Pastures were first shown to have a tentative link with the vegetation subtype ($r_s = 0.5232$, $p < 0.05$) and were then separated into two groups: those placed on plant formations and those at higher altitudes. sides of the mountain Sarnena Sredna Gora (Starozagorski Analysis of *Helicella* (Mineralni Bani, Oryahovitsa, Karanovo, Panicherevo) in one dimension and three dimensions, as well as low-obvia samples from pastures at high altitudes in the Upper Thracian plain (Rakitnitsa, Malka Vereya, Zheleznik, Zagortsi, and Bogdanovo) were the four nematode species that had the most variation. In terms of how common, strong, and plentiful they are. There was a statistically insignificant difference in the distributions of these three infection parameters when comparing the two groups using the Mann-Whitney test ($p > 0.05$) with between species. Among the nematode species, *M. capillaris* had the highest prevalence (17.2%), mean intensity (17.2%), and number of larvae (43), followed by protostrongylid larvae (32 larvae total), *Protostrongylus* sp. (24.4%), and *N. linearis*. Stepwise multiple regression analysis revealed that *C. ocreatus* showed a correlation between the primary vegetation ($p = 0.0000$) and variations in species diversity at the lowest prevalence and infection intensity (up to 9.2% and 14 larvae, respectively) ($R^2 = 0.8388$).

the development of plants ($p = 0.0004$). Nevertheless, these factors account for only In pastures, where nematode species variety accounts for 94% of variability, we compare infection parameters. area of the plains under investigation as well as those around the Sarnena Sredna Several Gora mountain sides exhibited no significant variations in the parameters of nematode infestation. The presence of *Protostrongylus* sp. in pastures was the only variable that showed a statistically significant connection with individual-level vegetation. Regarding the average *M. capillaris* intensity, pastures at higher elevations had a greater value (M-W, $U = 332.1$, $p = 0.0223$).

Table 3. Statistical reliability of the differences (comparisons in pairs) to prevalences (Fischer's exact test, above the diagonal) and mean intensity in (Bootstrap test, under diagonal) of protostrongylid larvae in *H. obvia* from nine pastures.

Pasture	1	2	3	4	5	6	7	8	9
1	-	ns	ns	***	ns	ns	*	***	ns
2	***	-	ns	ns	ns	ns	ns	ns	ns
3	**	ns	-	***	**	ns	**	***	ns
4	ns	***	**	-	ns	***	ns	ns	ns
5	*	ns	ns	*	-	**	ns	ns	ns
6	**	*	ns	**	ns	-	***	***	ns
7	*	ns	ns	*	*	**	-	**	ns
8	ns	**	**	ns	*	**	*	-	ns
9	ns	ns	ns	ns	ns	ns	ns	ns	-

Indications of statistical confidence: ns, differences are not statistically reliable; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. Indications for pastures: 1, Starozagorski Mineralni Bani; 2, Oryakhovitsa; 3, Karanovo ; 4, Panicherevo;

5, Rakitnitsa; 6, Malka Vereya; 7, Zheleznik; 8, Zagortsi; 9, Bogdanovo.

The prevalence of protostrongylid larvae in relation to their primary host is shown as a group variable. Only the type of main vegetation was significantly different among the pastures selection processes ($F=11.22$, $p=0.0000$) among all variables given to variable-the area, H. obvia. The overall prevalence of protostrongylid larvae was predicted by the pairwise comparisons of the prevalence predictor. The mean intensity and this found that the mean variable explains a much less percentage (25%) of the overall intensity of protostrongylids (50%) than comparisons did. As opposed to 66.7% in pastures with primary vegetation from type 1 (pubescent oak) and comparisons (Table 3), protostrongylid larvae were most prevalent on statistically significant areas. In terms of the frequency of vegetation of type 3, namely mixed forests from European turkey oak and protostrongylid larvae in H. obvia, two pastures were identified: one near Panicherevo and one with Italian pubescent oak. The lowest incidence was found on pastures with main Zheleznik. The Italian pubescent oak (also called pedunculate oak) is a medium-intensity species. Pastures that were compared had more variation (Table 3). Due to the lesser values (12.4%), there were intermediate Bogdanovo and other pastures in areas with primary vegetation type 2 (mixed woods), which meant that there were no statistically significant differences between the pastures adjacent European turkey oak and Italian oak. The main plants account for the variation in the sample size. On the other hand, this trait is reflective of the actual situation, since the mean abundance of protostrongylids was higher on pastures than in areas with type 2 or 3 primary vegetation, and another snail species, *M. cartusiana*, was more common in areas with type 1 primary vegetation than in pastures (Georgiev et al., 2003). An ANOVA with one way of analysis was used in this research. Differences in the frequency and severity of

For the first assessment of infection parameters with environmental features, *M. capillaris* was associated with the same group of factors. For instance, the model just included the principal vegetation type as a variable. Describing the variation in the prevalence of this snail species became crystal evident using the multivariate regression analysis. Pastures in areas with type 2 primary vegetation were found to have better protostrongylid infection parameters than pastures in regions with type 3 primary vegetation, as well as a higher prevalence of the most common species, *M. capillaris*. This was found to be true across all regions.

There was a slow but steady decline in *M. capillaris* (the medians It was not possible to trace these connections to a direct impact. There were 17.2% and 2.1%. The communities, whose structure is based on a number of climatic mean intensities of *M. capillaris*, were highly impacted (66% of the variation) by the main vegetation type and type of primary vegetation, which comprises climax natural plant subtype. The research found that regions with mesophytic grasses had the lowest infection parameter and edaphic regional characteristics (Iav 1.33-1.47). As a predictor variable, the main vegetation was located in a

The values range from 2.71 to 3.15 in regions with mesophytic and xerophytic grass vegetation, and from 3.54 to 4.62 in pastures with ephemeral and xerophytic grass growth, respectively. Variations in nematode prevalence were also explained by the variety of nematode species. Additionally, the model that accounted for the differences in the mean intensity of *N. linearis* relied only on this component. No evidence of *N. linearis* was found in samples that included either single-species or two-species protostrongylids in our investigations.

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