

Larval development of ovine *Neoststrongylus linearis* in four experimentally infected mollusc species*

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Abstract

Development of *Neoststrongylus linearis* larvae was studied in the snail intermediate hosts *Oestophora* (*Oestophora*) *barbula*, *Oestophorella buvinieri*, *Cepaea nemoralis* and *Helix* (*Cryptomphalus*) *aspersa*. The molluscs of each species, all adults, were divided into groups of 40 for infection purposes. The infection doses for the first two snail species were 90 and 50 first stage larvae (L1) of *N. linearis*, respectively. For *C. nemoralis* two batches were tested: one with 200 L1 kept at 19°C and the other with 250 L1 at a temperature of 21°C. The same was done with *Helix* (*C.*) *aspersa* with 200 L1 at a temperature of 21°C, on the one hand and 300 L1 at 24°C on the other. One or two molluscs of each species were killed in series from the 6th day post-infection (p.i.) until the 44th. Percentage values for total larvae (1, 2 and 3) and L3 were higher with the lower dose for *C. nemoralis*, whilst the same was true with the higher dose in *Helix* (*C.*) *aspersa*. In both cases, the higher temperature appeared to contribute to cycle acceleration. Using one way analysis of variance, statistically significant differences were detected between the species of molluscs tested concerning percentages of L1 which penetrated, total larvae and L3. According to our results, the decreasing order of susceptibility of these species of molluscs as experimental intermediate hosts of *N. linearis* is: *O. buvinieri*, *Oestophora* (*O.*) *barbula*, *C. nemoralis* and *Helix* (*C.*) *aspersa*. It is the first time that *Oestophora* (*O.*) *barbula* and *Oestophorella buvinieri* have been named as experimental intermediate hosts of *N. linearis*.

Introduction

Neoststrongylus linearis (Marotel, 1913) Gebauer, 1932 (Protostrongylidae) is one of the bronchopulmonary nematodes which produce ovine verminous bronchopneumonia. This species often infects flocks in León province (Rojo-Vázquez, 1973; Morrondo-Pelayo *et al.*, 1978; Reguera-Feo, 1983) as well as other countries (Müller, 1934; Cabaret, 1981).

Müller (1934) showed experimentally that *N. linearis* needs to partially develop in molluscs which act as an

intermediate host, in order to complete its biological cycle. Since then various species of molluscs have been quoted as intermediate hosts (Manga-González *et al.*, 1986). However, the two Helicodontinae species *Oestophora* (*Oestophora*) *barbula* (Rossmässler, 1838) and *Oestophorella buvinieri* (Michaud, 1841) had not been tested for experimental susceptibility to this parasite. Both species are endemic to the Iberian Peninsula and mainly found in the western regions (Manga-González, 1983). Consequently we considered it interesting to carry out research which could extend the range of intermediate hosts for this nematode. Moreover, due to the important role that such widespread and abundant mollusc species as *Cepaea nemoralis* (Linnaeus, 1758) and *Helix* (*Cryptomphalus*) *aspersa* Müller, 1774 can

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play in verminous bronchopneumonia epidemiology, we decided to carry out a seriated methodical study of the larval development of *N. linearis* in the molluscs named. This study would allow us, on the one hand, to investigate thoroughly the aspects of *C. nemoralis*, already studied by Müller (1934), and of both species studied by Rojo-Vázquez & Cordero-Del-Campillo (1974) and, on the other hand, to tackle aspects not studied by them. The results obtained should be of great use in understanding the bionomics of this parasite and in taking suitable steps to control the illness it produces.

Materials and methods

The larvae (L1) of *N. linearis* were obtained from the faeces of a ewe experimentally infected with this nematode, using the Baermann-Wetzel method. Manga-González (1983) can be consulted for a description of the four mollusc species used in this work and their distribution in León Province and the rest of Spain. All the molluscs checked with L1 of *N. linearis* were adults. *Oestophora* (*O.*) *barbula* were collected in the Peñarrubia Reservoir (U.T.M. coordinate: 29TPH8004) at 380 m above sea level and phytosociology *Asplenium (rutae-murariae)* O. Bolos, 1968. *Oestophorella buwinieri* were collected in Ribota (30TUN3378) at 520 m above sea level and Quercu-Fagea (Rivas Goday, 1964) Jakucs, 1967 phytosociology. The species of *C. nemoralis* were collected in Crémenes (30TUN2552) and those of *Helix (Cryptomphalus) aspersa* in Sabero (30TUN2445). Both of these places, with *Chenopodio-Sclerantha* Hadac (1956), 1967 phytosociology, are at a height of 997 and 1023 m above sea level, respectively. The first two species of molluscs belong to subfamily Helicodontinae and the other two to Helicinae.

Places not frequented by small ruminants were chosen to collect the snails. But, in order to confirm the absence of natural infection by Protostrongylidae, 10% of the specimens from each mollusc species were checked.

The Kassai (1957) method was used for infection. The snails were divided into batches of 40 specimens of each species and remained in contact with the L1 for three and a half hours. At the end of this period they were kept in covered vivaria. The snails were handled using the method indicated by Morrondo-Pelayo *et al.* (1980).

Oestophora (*O.*) *barbula* and *Oestophorella buwinieri* were tested with 90 and 50 L1 of *N. linearis*, respectively. Both batches were then kept at 20°C. A low dose was used because in previous experiments with 200 L1 almost all the molluscs had died before day 6 p.i. A batch of *Cepaea nemoralis* and of *Helix* (*C.*) *aspersa* were each infected with different doses of L1 and kept at different temperatures. The first species was infected with 200 L1 (19°C) and 250 L1 (21°C) and the second with 200 L1 (21°C) and 300 L1 (24°C). The number of L1 which penetrated the foot of each mollusc was calculated as the difference between the number put into each Petri dish at the beginning of the infection and that collected from it after infection.

In order to study the development of the larvae in the molluscs, 1 or 2 specimens of each species were killed from day 6 p.i. until the end of the experiment. The methods used for the extraction and later identification of the larvae were the same as those used by Morrondo-Pelayo *et al.* (1980) and Manga-González & Morrondo-Pelayo (1988). In order to determine the level of susceptibility of these mollusc species as intermediate hosts of *N. linearis*, the following

parameters were considered for each of the batches and each snail: percentage of L1 (this is expressed as a percentage in order to compare the infections carried out with different doses), total number of larvae detected in the three stages (L1, L2 and L3) and number of L3, as well as their respective percentages. The last two percentages were calculated based on the total number of L1 that had penetrated the foot. The days when the first L2 and L3 were observed, as well as those when the last L2 were detected and all had become L3 were also taken into account.

One-way analyses of variance were carried out to detect possible interspecific differences among the molluscs with regard to the percentages of: L1 that had penetrated the foot, total number of larvae and L3. The correlation coefficients were calculated in order to discover the degree of dependence between each of the mentioned percentages in the different species of molluscs. The 't' test was used to determine the possible differences between the batches of each species of snail infected with different doses of L1 and kept at different temperatures.

Results

Figure 1 shows that the highest percentage value (94.4%) of L1 of *N. linearis* that penetrated the molluscs corresponded to *Cepaea nemoralis* (infected with 250 L1), whilst the lowest (56.7%) was obtained for *Oestophorella buwinieri*. However, the highest percentage values for total larvae (1, 2 and 3) (8.7%) and L3 per mollusc (9.4%) were recorded for the latter species of mollusc.

By means of one-way analysis of variance, highly significant interspecific differences were found to exist (for 5 d.f. and $P \leq 0.001$) among the species of mollusc tested, when taking the percentages of L1 that penetrated ($F=61.24$), total number of larvae ($F=13.05$) and L3 per mollusc ($F=9.05$) into consideration. When the corresponding correlation coefficients between those percentages were considered, no relation was observed either between the percentage of L1 which penetrated and that of the total of larvae which developed ($r=0.40$), or between that of L1 which penetrated and that of L3 ($r=0.25$). But there was a correlation between the percentages of total larvae and those of L3 ($r=0.08$).

In the case of the two infections of *C. nemoralis* (fig. 1) the lowest dose (200 L1) of *N. linearis* produced higher values for the total larvae and L3 per mollusc, as well as for their respective percentages. The percentage of L1 that penetrated was larger in the experiment with higher doses (250 L1). The 't' test was applied and statistically significant differences (for $P \leq 0.001$ and 78 d.f.) were detected between the two batches tested with different doses, as regards the percentage of L1 that penetrated ($t=11.88$) and that of the total larvae which developed ($t=5.52$). In the case of the batches of *Helix* (*C.*) *aspersa* infected with different doses of L1 (fig. 1), it was observed that the opposite to the case of *C. nemoralis* occurred, that is, the absolute values and percentages of the total larvae and L3 per mollusc were greater when infected with the higher dose (300 L1) and the percentage of L1 which penetrated was similar for the two batches. On applying the 't' test, no significant differences were detected as regards the last percentage mentioned ($t=1.05$), but there were (for $P \leq 0.001$ and 78 d.f.) as regards the percentage of the total larvae found ($t=2.17$). It can be deduced from these results

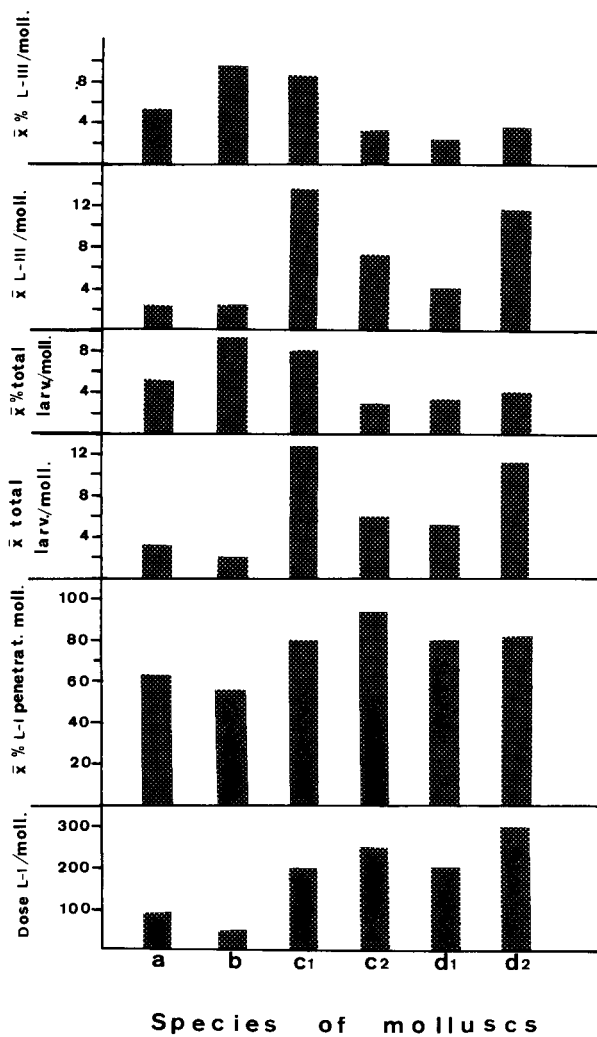


Fig. 1. Larval development results of *N. linearis* in the mollusc species: 1) *Oestophora* (*Oestophora barbula*); 2) *Oestophorella buvinierei*; 3a) *Cepaea nemoralis*, dose of 200 L1 at 19°C; 3b) *C. nemoralis*, dose of 250 L1 at 21°C; 4a) *Helix* (*Crytomphalus*) *aspersa*, dose of 200 L1 at 21°C; 4b) *Helix* (*C.*) *aspersa*, dose of 300 L1 at 24°C.

that the influence of the dose on subsequent larval development depends on the species of mollusc used, as we had already observed in previous studies.

As can be seen in fig. 2, the shortest periods of time before reaching the different larval stages of *N. linearis* were recorded for *Helix* (*C.*) *aspersa*. These periods of time were also shorter for the batches of *C. nemoralis* and *Helix* (*C.*) *aspersa* kept at the higher temperature.

Figure 1 shows that the percentage of L1 which penetrated each mollusc was high in all the species tested, although the number of larvae that developed was low. That is why the suitability of the mentioned species of molluscs for the larval development of *N. linearis* was based on the absolute values and percentages of the total larvae (1, 2 and 3) and L3, as well as the days when the different larval stages were observed. The order of suitability from greater to lesser was: *O. buvinierei*, *Oestophora* (*O.*) *barbula*, *C. nemoralis* and *Helix* (*C.*) *aspersa*. The first two species of

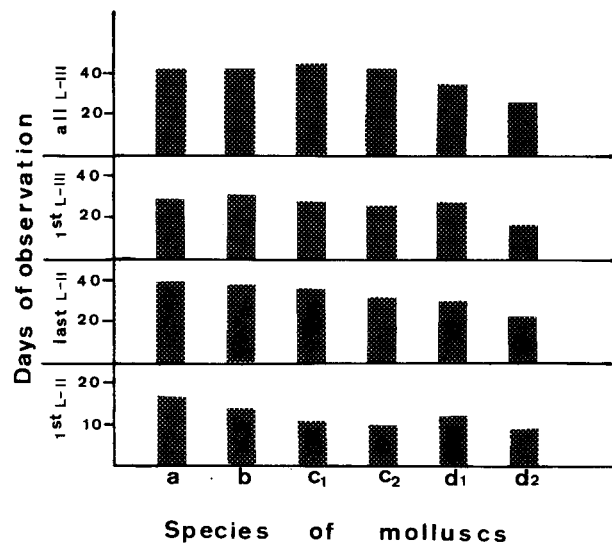


Fig. 2. Observation days of the first L2, last L2, first L3 and all L3 of *N. linearis* in the same species of molluscs as in fig. 1.

mollusc mentioned are quoted as intermediate hosts of *N. linearis* for the first time.

Discussion

On comparing the results obtained for *C. nemoralis* and *Helix* (*C.*) *aspersa* with those of Rojo-Vázquez & Cordero-Del-Campillo (1974), when they infected the same species of molluscs with doses of L1 of *N. linearis* similar to those we used, the percentage of L1 that penetrated the molluscs was higher in our experiments. However, the period of time needed for the observation of the different larval stages of *N. linearis* was longer in our study than in that carried out by Rojo-Vázquez & Cordero-Del-Campillo (1974). Müller (1934) also indicated shorter periods of time than us for detecting the first L2 and L3 of *N. linearis* in *C. nemoralis*.

Cabaret (1981) infected *Helix* (*C.*) *aspersa* experimentally with larvae of *Muellerius capillaris* (Mueller, 1889) Cameron, 1927 and *N. linearis* and concluded that the species was not susceptible to these parasites. This does not agree with the results obtained by us and by Rojo-Vázquez & Cordero-Del-Campillo (1974) as regards the experimental susceptibility of this mollusc as an intermediate host of *N. linearis*. Later, however, Cabaret & Cabaret-Galkin (1985) indicated that *C. nemoralis* and *Helix* (*C.*) *aspersa* are suitable molluscs for the transmission of Protostrongylidae in Touraine (France). We have also found these two mollusc species hosting larvae of *N. linearis* in the wild.

When the results obtained in this study were compared with those recorded for other species of molluscs (Morrondo-Pelayo *et al.*, 1980, 1981; Morrondo-Pelayo & Manga-González, 1982; Manga-González & Morrondo-Pelayo, 1988) it was observed that the values of the percentages of L1 that penetrated, of the total larvae and of L3 of *N. linearis* were similar, except in the case of *Cochlicella barbara* (L., 1758) and *Monacha* (*Ashfordia*) *granulata* (Alder, 1830). Higher values were recorded for the last two percentages for these species.

The number of L3 per mollusc obtained in the species tested for this study is much smaller than that recorded by

Reguera-Feo (1983) and Castañón-Ordóñez *et al.* (1984) when they infected *Cerņuella (Xeromagna) cespitum arigonis* (Schmidt, 1853) with *N. linearis*.

The number of days needed before the observation of the different larval stages in the molluscs was greater than that indicated by Müller (1934), Rojo-Vázquez & Cordero-Del-Campillo (1974) and Marcos-Martínez (1975). It was smaller than that mentioned by Urban (1980) and Manga-González & Morrondo-Pelayo (1988), and similar to that recorded by Morrondo-Pelayo *et al.* (1980, 1981), Morrondo-Pelayo & Manga-González (1982), Reguera-Feo *et al.* (1980) and Reguera-Feo (1983). Nevertheless, it must be taken into account that the molluscs infected by the authors mentioned above belonged to species of Helicidae different to those used for this study.

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