EFFECT OF THE INFECTION BY *NEOSTRONGYLUS LINEARIS* ON THE SURVIVAL OF THE INTERMEDIATE HOST *CERNUELLA (CERNUELLA) VIRGATA*

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Summary :

The molluse intermediary host *Cernuella (Cernuella) virgata* was infected both in nature and in the laboratory with *Neostrongylus linearis* larvae. The infection rate was higher in the natural infection than in the experimental one all through the study. Accordingly, the survival of the molluscs was lower among those that had been naturally infected than among uninfected control and experimentally infected ones. The differences between survival curves of the different batches were only significant in the months when the infection rate in naturally infected batches was very high, from November to February. So we can conclude that the mortality rate is higher among the snails which harbour a considerable number of *N. linearis* than among non-infected or moderately infected molluscs.

KEY WORDS : *Neostrongylus linearis*, Protostrongylidae, *Cernuella (Cernuella) virgata*, mollusc, survival, natural infection, experimental infection.

Résumé : EFFET DE L'INFESTATION PAR *NEOSTRONGYLUS LINEARIS* SUR LA SURVIE DE L'HOTE INTERMÉDIAIRE *CERNUELLA (CERNUELLA) VIRGATA*

Le mollusque hôte intermédiaire Cernuella (Cernuella) virgata, *a été infesté avec des larves de* Neostrongylus linearis *en milieu naturel et au laboratoire. Pendant toute la durée de l'étude, le taux d'infestation a été plus élevé dans les infestations naturelles. Les mollusques infestés naturellement ont présenté une survie plus faible que ceux infestés expérimentalement ou ceux du lot témoin non infesté. Les différences entre les lots ont été significatives seulement lorsque le taux d'infestation était élevé – de novembre à février – pour les infestations naturelles. Nous pouvons conclure que les mollusques qui hébergent un plus grand nombre de larves de* N. linearis *présentent une mortalité plus élevée que ceux qui ne sont pas parasités ou faiblement infestés.*

MOTS CLÉS : Neostrongylus linearis, *Protostrongylidae*, Cernuella (Cernuella) virgata, *mollusques, survie, infestation naturelle, infestation expérimentale.*

Protostrongylid nematodes complete their biological cycle in terrestrial molluscs, which act as intermediate hosts. In previous studies (Prieto *et al.*, 1993, Díez *et al.*,1994), the most frequent protostrongylid of small ruminants in Galicia was *Neostrongylus linearis,* which develops properly in the intermediate host *Cernuella (Cernuella) virgata*, a species of Helicidae mollusc frequent in the Northwest of Spain. Most of the experimental protostrongylid infection studies (Cabaret & Dakkak, 1979 ; Skorping, 1985 ; Morrondo *et al.*, 1987, 1988) indicate that there is no statistical difference between mortality of infected and non-infected molluscs, although Ramirez (1967) and Marcos (1975) have observed a high mortality percentages in molluscs infected by Protostrongylidae larvae. Moreover, in other patterns of parasite infection, as *Fasciola hepatica* (Sindou *et al.*, 1991) or *Schistosoma* spp. (Anderson & May, 1979), intermediate host mortality were increased. The aim of present study is to assess the role of the protostrongylid infection on the intermediate host mortality.

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MATERIAL AND METHODS

THE MOLLUSCS

Adult specimens of *Cernuella (Cernuella) virgata* were collected in Lugo (Galicia, Northwest Spain). Previously, we had checked that there were no sheep grazing in the area in which the molluscs were collected. The absence of natural infection in this mollusc by protostrongylid larvae was assessed by examining 10 % of every sample collected.

NEOSTRONGYLUS LINEARIS FIRST-STAGE LARVAE

First-stage larvae (L-1) were collected from an ewe naturally infected with *N. linearis*. The ewe was placed in a metabolic cage one day a month in order to obtain 350-400 g of faeces for the experimental infection of the molluscs and another 350-400 g for the infection in natural conditions. We placed 10 g of these faeces in a Baermann apparatus to determine the number of first-stage larvae per gram of faeces (l.p.g.).

INFECTION OF SNAILS

Snails were deposited in a natural pasture near to the place where the molluscs had been collected. We

used a 0.1-hectare plot completely fenced off in order to prevent cattle from going in. No sheep had ever grazed in this area. The predominant vegetation was made up by *Poa trivialis, Dactylis glomerata, Trifolium repens, T. pratense, Bellis perennis, Taraxacum officinalis and Plantago lanceolatum*. Once a month, from November 1990 to October 1991, pieces of land of of 1 x 1 meter were enclosured and divided with metal fabric, buried into the ground at a depth of 10 to 15 cm to obtain plots of 0.5 × 0.5 meters. Every month, 350 g of faeces with *N. linearis* larvae were placed in one of the plots and 100 uninfected *C. (C.) virgata* adult snails were placed on them for the natural infection. At the same time, a group of 100 molluscs was infected in laboratory conditions using the Kassai method, with 200 *N. linearis* L-1 per snail and after that, they were placed in nature in a plot beside the naturally infected group. A hundred uninfected *C. (C.) virgata* were also placed in another plot as control group. The total number of plots was 36: 12 with naturally infected batches, 12 infected in the laboratory and 12 with control groups. Each of the 36 batches remained in its plot until all snails were dead.

Estimation of mollusc mortality and infection intensity

In order to assess the mortality and infection intensity in *C. (C.) virgata*, all snails were examined every 14 days. Dead molluscs were counted and removed in every batch. The infection intensity was examined by choosing at random three to five molluscs from each infected batch and killing them by immersion in water for 24 hours in a container covered to avoid air bubbles (Manga, 1983). The foot was crushed firmly between glass plates and examined under dissecting microscope. We considered both the average of total larvae (L-1, L-2 and L-3) and the average of L-3 per mollusc to know the infection intensity in the different batches.

Processing of data and statistical analysis

The data were analysed using the Kaplan-Meier survival technique of the computer program SPSS (SPSS Inc., 6.1.3 version, 1995) which derives survival curves. In the infected molluscs, the alive snails that were sampled to know the intensity of infection and the evolution of *N. linearis* larvae were considered censored. The dead specimens in every sampling were considered events. In every case a strata by month was applied in data. Likewise, log-rank test and Breslow test were used to test the significant difference among batches (1. control batch; 2. natural infection batch; 3. experimental infection batch). The log-rank test is based on the summed observed minus expected score for a given group and its variance estimate and gives more weight to mortality in the tail of the survival curve, whereas the Breslow test gives more weight to the earlier part of the survival curve.

RESULTS

The number of total larvae and of L-3 observed in the snails was always higher in the molluscs that had been naturally infected than in those infected in the laboratory. This was particularly so from November 1990 to February 1991 (Table I).

	Natural infection			**Experimental infection**			**Uninfected control**
	Total larvae/mol (mean±S.E.)	L-3/mol	Survival (days)	Total larvae/mol	L-3/mol	Survival	Survival
November	140±15	333±55	152±6	31±3	54±8	212±10	206±11
December	90±16	257±54	129±7	14±2	17±6	108±6	192±11
January	126±15	223±60	115±5	13±1	12±3	137±8	202±12
February	113±24	112±35	90±6	13±2	12±3	207±12	188±12
March	50±6	60±7	154±10	5±1	6±1	218±8	204±11
April	27±4	25±4	188±7	10±1	11±1	198±5	192±10
May	57±9	64±9	180±4	2±0.5	2±0.5	177±4	175±4
June	29±5	33±6	134±5	13±2	14±2	146±4	144±4
July	60±9	67±10	121±4	2±1	2±1	119±5	125±5
August	26±6	31±7	89±3	7±1	8±2	85±3	92±4
September	17±3	10±3	64±2	6±1	7±2	74±3	77±4
October	6±1	—	105±4	3±0.5	—	97±4	106±3

Table I. — Infection intensity and mean survival time in the different *Cernuella (Cernuella) virgata* batches.

The survival curve (Fig. 1) shows that cumulative survival was longer in uninfected control snails (average and standard error 161 ± 3) and in laboratory infected molluscs (158 ± 3) than in naturally infected snails (132 ± 2). The pairwise comparison shows that the survival curve of the naturally infected molluscs is significantly different from that of uninfected control mollusc (χ^2 = 17.6; p < 0.001 with log-rank test and Z = 9.5; p = 0.002 with Breslow test) and from the survival curve of experimentally infected snails (χ^2 = 17.6; p < 0.001 and Z = 12.3; p < 0.001). No difference was found between uninfected control and experimentally infected mollusc survival curves (χ^2 = 0.10; p = 0.7530 and Z = 0.56; p= 0.4562).

In order to get a more accurate idea of the influence of infection over mollusc survival, we compared survival between the three batches (natural infection, experimental infection and control group) every month. Mean survival time is presented in table I and the significant differences in table II.

Mortality was similar and no difference in survival time was found in the months with low infection intensity in naturally infected batches, from April to October (Table I) and the uninfected control molluscs. However, in batches deposited from November to March, survival time in those infected in nature was lower than the survival time in the control groups and the ones infected in the laboratory. When trying to account for the meaning of these differences, we find statistic differences between time survival in the batches infected in nature and the control groups from November to February (Table II), in which the natural infection was very high (90.5-140.2 total larvae/mollusc and 111.6-333.5 L-3/mollusc). Likewise, there are differences in survival time in the batches deposited in November, February and March between experimentally and naturally infected batches, concordantly with the low infection intensity reached in the experimental infection. The survival in the experimentally infected batches is only lower than in the uninfected control group in December and January, probably because of the low infection intensity of all the experimentally infected batches.

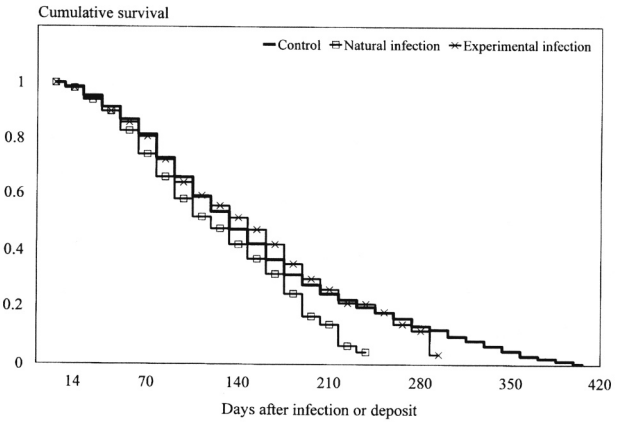

Fig. I. — Survival curve of the control and infected snails

	Log-rank test	Breslow test
Uninfected control versus naturally infected snails	November* December* January*** February***	December* January*** February***
Uninfected control versus experimentally infected snails	November* December*** January*	December*** January*
Naturally versus experimentally infected snails	November*** February*** March***	November* February*** March***

*p < 0.05 ***p < 0.001.

Table II. — Significant differences in survival under pairwise comparison using two statistical tests (log-rank test and Breslow test).

DISCUSSION

Survival of experimentally infected and control molluscs was similar, except the months when the infection intensity was very high. Other authors (Gerichter, 1948; Cabaret & Dakkak, 1979; Morrondo *et al.*, 1987, 1988; Rojo & Cordero, 1974) have already observed that the protostrongylid infection has no effect over mollusc survival in different parasite-host patterns; Skorping (1985) found no difference in survival between infected and uninfected adult snails. On the contrary, Ramírez (1967) pointed out that infection by protostrongylids seemed to cause an increase in mollusc mortality (30 % infected and 10 % control molluscs) and Marcos (1975) found that spontaneous deaths in infected specimens were 4.8 % higher than in non-infected snails, but no statistical analysis was applied in the latter studies to control the significance in mortality differences. Cabaret *et al.* (1990) observed that protostrongylid infection interferes with host survival, causing higher mortality rates in infected *Solatopupa similis* than in control ones and Skorping (1985) observed a consistant decrease in survival rates in juvenile *Arianta arbustorum* with increasing mean number of parasite/snail.

Our results indicate that intermediate host *(C. (C.) virgata)* survival depends on the infection rate of *N. linearis* larvae. Snails which harbour a higher number of *N. linearis* larvae suffer a higher mortality

than non-infected or moderately infected molluscs. Naturally infected batches had always higher infection intensity than those infected in the laboratory, as close contact with faeces permits high infection and induces high death rates (Cabaret & Vendroux, 1986). Mortality can be due to the lesions provoked by the larvae during penetration and development in molluscs, as indicated Marcos, 1975; Cabaret *et al.*, 1990, and also Hourdin *et al.* (1990) in *Lymnaea* sp.
In cases of light infections, protostrongylids do not cause mortality in land snails, whereas heavier infections might result in mortality in the snails.

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