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SURVIVAL OF FIRST-STAGE *NEOSTRONGYLUS LINEARIS* LARVAE IN OVINE FAECES UNDER ENVIRONMENTAL CONDITIONS IN GALICIA (NORTH-WEST SPAIN)

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SUMMARY

A study was made on the survival of first larval stage of *Neostrongylus linearis,* from November 1990 to October 1991, under natural conditions in an inland locality in Galicia (North-West Spain). The faeces were obtained from a sheep naturally infected with this nematode. Once a month, faeces were placed on a 0.5 × 0.5 m plot, in natural conditions, until we had 12 deposits.

Samplings were done weekly until there was no more faecal matter in the plots. Larval survival was determined using the Baermann migration technique, and we calculated the number of larvae per gram after the transformation of the weight of the faecal sample, in order to discard weight variations owing to climatic conditions.

Over the whole period of this study, the percentages of larval survival in the faeces of the 12 deposits changed from the first week onwards. In those samples that were left in the pasture more than three weeks, survival was less than 50 %. After the seventh week only very small numbers of active *N. linearis* larvae were obtained.

We observed a positive influence of relative humidity on survival during the first week's presence of the faeces in the pasture. In the same way, a statistically significant negative correlation was proved between larval survival in faeces and temperatures during the first three weeks post-deposit.

RÉSUMÉ : **Survie des larves du premier stade de *Neostrongylus linearis* dans des fèces d'ovins exposées aux conditions naturelles en Galice (nord-ouest de l'Espagne).**

La survie, en conditions naturelles, du premier stade larvaire de *Neostrongylus linearis* a été étudiée de novembre 1990 à octobre 1991, en Galice (nord-ouest de l'Espagne). Les fèces sont issues d'une brebis infestée naturellement. Une fois par mois, un dépôt de fèces est réalisé dans une parcelle de 0,5 × 0,5 m. Dix grammes de fèces étaient déposées à titre de contrôle dans le dessein de déterminer les changements de poids, et postérieurement de calculer le nombre des larves survivantes par gramme de fèces.

Les pourcentages de survie des larves dans les fèces diminuent dès la première semaine après avoir été déposées. La survie est inférieure à 50 % au bout de trois semaines. A la septième semaine après le dépôt, seul un nombre très faible de larves 1 actives de *N. linearis* est retrouvée.

Une corrélation négative significative existe entre l'humidité relative et le taux de survie des larves 1 au cours de la première semaine de séjour des fèces dans la microparcelle. Il existe une corrélation négative entre la survie des larves dans les fèces et la température au cours des trois premières semaines après le dépôt.

INTRODUCTION

First-stage larvae (L1) of the different species of parasites of the family Protostrongylidae are excreted with the faeces of their final hosts (sheep, goats and small wild ruminants). The L1 must survive until they successfully meet and penetrate an intermediate host (terrestrial gastropod molluscs). Within the latter, larvae continue their development until they reach the third larval stage, infective for the final hosts.

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Some authors (Reguera *et al.,* 1986; Cabaret *et al.,* 1991 and Morrondo *et al.,* 1992*a*) have experimentally demonstrated that temperature and humidity play a role on the survival of first-stage larvae of these nematodes, and, specially, the negative effect of dessication on *Neostrongylus linearis* larvae. In the same way, in a previous paper Díez *et al.* (in press), showed that *N. linearis* had low survival capacity in Autumn in natural conditions in the Touraine region (France).

In dry inland areas in Spain and France, *N. linearis* is the protostrongylid with the smallest prevalence and intensity of parasitism in sheep and goats (Reguera *et al.,* 1983; Cabaret, 1986*a* and Morrondo *et al.,* 1991). Inversely, *N. linearis* is the most common species in sheep in Galicia, a humid region in the North-West of Spain (Díez *et al.,* 1992 and Morrondo *et al.*, 1992*b*). Therefore, in the present paper we intend to study the degree of influence of climatic parameters on larval survival of this lungworm, so that we may know the most suitable periods for infection of intermediate-host molluscs.

MATERIALS AND METHODS

1 — Obtaining of first-stage *Neostrongylus linearis* larvae

First-stage larvae of *Neostrongylus linearis* were collected from a sheep infected naturally with this prostostrongylid, that was reared indoors during the experience and fed on a diet of hay and commercial dry food.

The donor sheep was placed in a metabolic cage one day a month, in order to obtain 350-400 grams of faeces. We placed 10 grams of such faeces in a Baermann apparatus in order to determine the number of larvae per gram of faeces (l.p.g.). The remaining faeces were placed in a natural pasture.

2 — Placement of faeces in natural conditions

Once a month, from November 1990 until October 1991, we placed the faeces infected with L1 in plots of 0.5 × 0.5 m such as they were entirely covered with faeces. The plots were located in a natural pasture. The major vegetal species were *Poa trivialis, Dactylis glomerata, Trifolium repens, T. pratense, Bellis perennis, Taraxacum officinalis* and *Plantago lanceolatum*. The height of grass was always adjusted to 15-20 cm.

The faecal deposits were done monthly, until 12 depostis were completed.

Each month, at the same time that faeces were deposited in each plot, we placed a small basket containing 10 g of control faeces so as to assess weight fluctuations in faeces subjected to environmental conditions. We considered these variations to calculate the number of surviving larvae per gram of faeces.

The initial number of l.p.g. in each of the 12 faecal deposits varied as a consequence of the discontinuous rythm of larvae excretion of the donor sheep *(Table I)*.

3 — Sampling of the faecal deposits and estimation of larval survival

Once a week, from the beginning of the experiment, 5 grams of faeces were sampled in each plot, and faeces from the small control basket were weighed. We determined weight variations of the 10 initial grams of control faeces, in such way that we used the weight of control faeces at the moment of sampling (n), to calculate the number of grams (x) to which correspond our present 5 grams sampled:

$$x = \frac{(10 \text{ g control faeces}) \times (5 \text{ g sampled faeces})}{n}$$

Thus, we discarded variations in the number of l.p.g. owing to weight variations of faeces as a consequence of the environment. The faeces collected in each weekly sampling were placed in a Baermann apparatus, and the number of *N. linearis* living larvae was determined. We calculated the l.p.g. by dividing the number of larvae by (x) grams of faeces.

Sampling were done weekly, until they mixed the ground of the plot and we could not collect any more faecal matter.

Table I. — *Weekly variations of percentage of larvae survival of* N. linearis *throughout the 12 months of study* (p.d.: post-deposit).

Weeks p. d.	Nv	Dc	Ja	Fb	Mr	Ap	My	Jn	Jl	Au	St	Oc
0*	728	335	229	547	36.5	233	25.8	124.3	20	250	140	38.6
1	49.6	37	52	34.9	43.2	5.1	42.3	16.9	40	4	67.9	56.4
2	5.5	46	54.6	3.3	29.7	8.6	7.7	17.7	0	4.6	52.9	2.6
3	14	48.4	34.5	4.9	18.9	2.6	15.4	4	10	4.8	0.7	0
4	11.8	31	9.6	3.7	10.8	0.9	16.3	4	5	6.8		
5	0.6	22.1	6.5	0.7	5.4	0.9	19.2	8.1	5	7.2		
6	2.1	26.5	14.8	2.4	8.1	4.3	3.9	0	5	0		
7	1.4	1.3	34.1	4.6	5.4	2.6	3.9	2.4	5	0		
8	2.2	4.5	17.5	0.6		0.4	3.9	1.6	0	1		
9	3	5.4	22.3	0.4		0.9	3.7	4.8	15	0		
10	0.3	6.9	7.4			0.4	0	3.2	5			
11	0	7.9	9.2				0	0.8	0			
12		9	8.3					2.4	10			
13		4.5						0	0			
14		4.5										
15		0.6										
16		3										

* **Absolute number of larvae per gram of faeces when placed**

4 — Climatic parameters

The climatic data was obtained from a Weather Station located in the same field where this experiment was carried out. Absolute minimum and maximum temperatures, average temperatures, percentage of relative humidity, and rainfall measured in millimeters (mm) were taken into account. We calculated the values of the different climatic parameters in accumulative way, from the moment of deposit of faeces on the plots until the day of sampling.

5 — Statistic analyses

Variance analysis was performed on weekly larval survival after the deposition of faeces, taking into account the 12 monthly deposits.

By means of multiple-range analysis we studied the existence of homogeneous groups in relation to the larvae survival until seven weeks post-deposit of faeces on the plots.

Regression analysis was used to determine the possible relationship between the percentages of larval survival (transformed as a square root) and the different climatic parameters. Data was processed using the « Statgraphics » computer program (Statistical Graphics Corporation EXE (1986) 2.1 version).

RESULTS

As is shown in *Table I,* the percentage of survival of *N. linearis* larvae in faeces varied from the first week onwards, differing greatly according to the months of study. In all samples kept for more than three weeks in the pasture, survival was less than 50 %. In the same way, it was observed that after the seventh week post-deposit only the smallest quantity of active *N. linearis* larvae were found, as the percentages of survival were less than 5 % (except in December, with 34.1 %). By means of variance analysis statistically significant differences ($F = 8.633$; $p = 0.000$) were found with regard to the survival of L1 of *N. linearis* during the first seven weeks after the placement of faeces in natural conditions, considering the different months when deposits were done.

Using multiple-range analysis four homogeneous groups were established with regard to the larval survival: first week; second week; third and fourth weeks; and the fifth, sixth and seventh weeks.

With regard to the influence of climatic factors on survival of *N. linearis* (standardized values), we appreciated that during the first week following placement of faeces in the field *(Fig. 1)*, humidity was related positively to first-stage larvae survival. Thus, from November to March, and in May, July, September and October, when relative humidity figures were high, larval survival was correspondingly high. In the same way, during the second week post-deposit *(Fig. 2)* a positive correlation between survival and rainfall was established, thus, the survival was higher in December, January, March and September.

Inversely, at three weeks post-deposit, temperatures (absolute maximum and minimum, and average) are correlated negatively with larval survival *(Fig. 3).* In accordance with

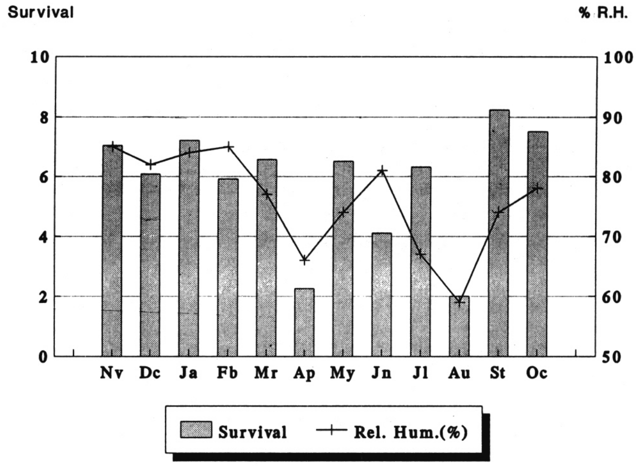

Fig. 1. — Standardized survival (%) of *N. linearis* larvae in relation to percentage of relative humidity (R.H.) during the first week post-deposit).

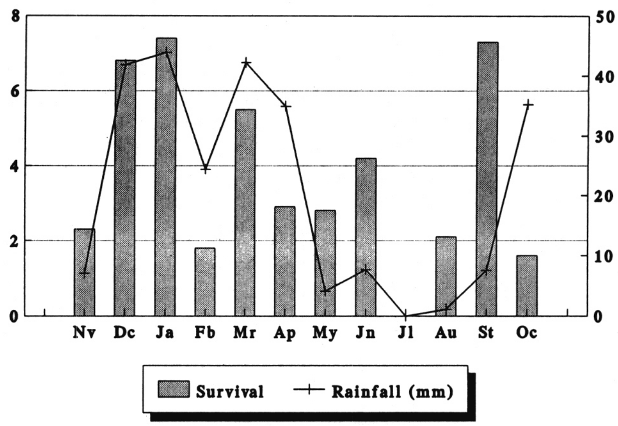

Fig. 2. — Standardized survival (%) of *N. linearis* larvae in relation to rainfall during the second week post-deposit.

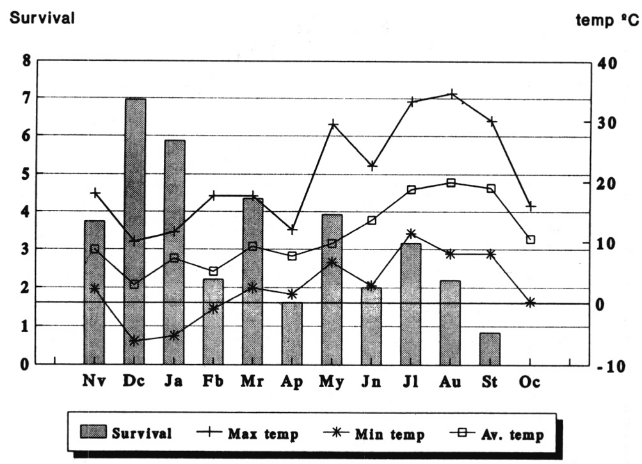

Fig. 3. — Standardized survival (%) of *N. linearis* larvae with regard to temperatures recorded during the third week.

this result, it was observed that from April to October, during which period the highest temperatures were recorded (specially maximum), the survival values of *N. linearis* were the lowest.

From these results we can conclude that the standard percentages of larvae survival over three weeks post-deposit on the plots were higher between the months of November and March, and in May, September and October.

Using regression analysis *(Table II),* significant differences were shown between survival in the first week post-deposit and humidity. Furthermore, a negative statistical correlation exists between temperature and L1 survival in faeces during the first three weeks. After the seventh week, a negative correlation between survival and climatic parameters was found.

Humidity and temperature are key factors on the survival of *N. linearis* in natural conditions. The first factor plays a positive role on the contrary to temperature.

TABLE II. — *Correlation coefficient* (r) *between standardized survival percentage and climatic parameters* (* Significant differences ($p \leqslant 0.05$); p.d.: post-deposit).

Week p.d.	temperatures (°C)			r.h. (%)	Rainfall (mm)
	Max.	Min.	Aver.		
1	-0.15	-0.02	-0.09	**0.60***	0.38
2	-0.20	-0.15	-0.13	0.24	0.47
3	**-0.52***	**-0.51***	**-0.51***	-0.42	0.22
4	-0.11	-0.34	-0.44	0.22	-0.21
5	0.10	0.24	0.10	-0.04	-0.21
6	**-0.67***	-0.48	**-0.64***	**-0.52***	**0.55***
7	-0.47	-0.41	-0.40	0.20	0.40

DISCUSSION

In this study, we conformed that the maximum number of weeks *N. linearis* first-stage larvae may survive in natural conditions was 16 weeks in January, which agrees with previous findings by Cordero *et al.* (1982) and Cordero and Castañón (1989). However, we observed that survival rate decreased remarkably after the third week of permanence of faeces in the pasture, with very small percentages at six weeks. On the contrary, Cordero *et al.* (1982) and Cordero and Castañón (1989) estimated the mean survival rate of *N. linearis* to be between five and seven weeks.

Sattlerová (1982) found longer survivals for *Neostrongylus linearis* and *Muellerius* spp. from chamois faeces. Living larvae were recorded after a year under natural conditions. However the survivors were not identified as *Muellerius* spp. or *N. linearis.*

In our experiment, survival was high from November to March and in May, September and October. The latter have been identified by Cabaret (1986*b*) as the most suitable periods for survival of first-stage larvae of *Muellerius capillaris* and *Neostrongylus linearis* in the Rabat area.

We found a positive correlation between survival and relative humidity, but a negative correlation taking temperatures into account. On the contrary, Cabaret (1986*b*) found that survival of *M. capillaris* and *N. linearis* larvae in natural conditions in Rabat only correlated statistically with minimum temperatures.

The percentage of L1 survival in the first three weeks post-deposit in November was higher than data obtained by Díez *et al.* (in press) when we studied survival of *N. linearis* larvae from goat faeces deposited on ryegrass and alfalfa short plots in November 1989 in Touraine (South-West of France). Differences can be attributed to different climatic conditions in both studies, owing to the higher temperatures and rainfall registered in Galicia in November 1990, compared with the same parameters when the experience had been undertaken in the Touraine region.

In dry months, the survival of *N. linearis* after the second week was clearly lower than data found in the humid season, a similar finding to those observed by Morrondo *et al.* (1992*a*) in an experimental study of desiccation on the survival of L1 of *N. linearis,* in which they proved that drier faeces were related to lower larval survival rate. Similarly, Cabaret *et al.* (1991) demonstrated lower survival rates of protostrongylid larvae in desiccated than in dry faeces. Reguera *et al.* (1986) stated that under their own experimental conditions, combining the highest temperatures with the lowest humidity percentages, survival of *N. linearis* was low, which coincides with our results.

The high survival rate of *N. linearis* in cold, humid months casts no doubt on its influence on the different prevalence and geographic distribution of this protostrongylid species. In this way, we may relate to survival capabilities of L1 the abundance of *N. linearis* in sheep in Galicia (Díez *et al.,* 1992; Morrondo *et al.,* 1992*b*) and sparser distribution in dry areas in inland Spain (León province), where relative humidity is lower (Reguera *et al.,* 1983; Morrondo *et al.,* 1991).

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