EXPOSURE OF FIRST-STAGE LARVAE OF *MUELLERIUS CAPILLARIS* (NEMATODA) TO DESICCATION: SMALLER LARVAE AND REDUCED INFECTIVITY IN THE LAND-SNAIL HOST *CANDIDULA INTERSECTA*

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Summary :

First-stage larvae (L1) of the nematode *Muellerius capillaris* are resistant to dehydratation in desiccated faeces of definitive host. The L1 were exposed to increasing desiccation with different durations (non desiccated; 70% dry-matter (DM), 0 h; 70 % DM, 240 h; 90 % DM, 240 h; 95% DM, 48 h). The larger L3 in snails were recovered from infection using the less desiccated L3 and the infection rates were also higher. The size of L3 could be an indicator of the stress that L1 underwent before infection of the intermediate hosts.

KEY WORDS : nematode, lungworm, land-snail, *Muellerius*, desiccation, larval size.

Résumé : LA DESSICATION DES LARVES DU PREMIER STADE DE *MUELLERIUS CAPILLARIS* (NEMATODA): DES LARVES DE PETITE TAILLE ET UNE INFESTIVITÉ RÉDUITE POUR LE MOLLUSQUE TERRESTRE *CANDIDULA INTERSECTA*

Les larves du premier stade (L1) du nématode Muellerius capillaris sont résistantes à la déshydratation au sein des fèces. Des L1 dans des fèces ont été soumises à des conditions de déshydratation croissantes (non-deshydraté; 70% de matière sèche (MS), 0 h; 70 % MS, 240 h; 90 % MS, 240 h; 95 % MS, 48 h) avant l'infestation. L'infestation des mollusques hôtes intermédiaires est moins importante et les larves L3 sont moins grandes dans les lots infestés par des L1 soumises au préalable à la déshydratation. La taille des L3 pourrait être un indicateur des stress subis par les larves L1 avant l'infestation des hôtes intermédiaires.

MOTS CLÉS : *nématode, protostrongle, mollusque terrestre, Muellerius, déshydratation, taille des larves.*

INTRODUCTION

The *Muellerius capillaris* protostrongylid nematode is a very frequent parasite of small ruminants. It has a two-host life-cycle: small ruminants excrete first-stage larvae (L1) in their faeces, which infect terrestrial molluscs. The L1 develop into second-stage larvae (L2) and finally third-stage larvae (L3). The L3 are ingested by small ruminants, and develop in the lungs into fourth-stage larvae and finally adults; the eggs shed evoluate into L1 in the lungs and are passed in the digestive-tract after regurgitation, and then excreted in faeces.

Desiccation of faeces is an important factor in survival of first-stage larvae (L1) in faecal pellets (Cabaret *et al.*, 1991; Morrondo-Pelayo *et al.*, 1992). The infectivity of the survivors originating from the desiccated faeces is significantly reduced (Morrondo-Pelayo *et al.*, 1992): they penetrated less and developped in smaller numbers into second-stage and third-stage larvae. Morphometric variations are often good indicators of fitness traits in free-living organisms (Jones, 1987; McKenzie & Clarke, 1988). Desiccation during larval development induces reduction of size of infective larvae of several species of trichostrongylid nematodes (Rossanigo & Gruner, 1992), possibly resulting from a reduced feeding activity in desiccated faeces.

We will consider morphological changes in the larval stages of the protostrongylid nematode *M. capillaris* in snails after the L1 had been submitted to varied environments, i.e. several desiccation conditions. The snail *Candidula intersecta* is a particularly convenient host as it is usually found in dry sites, where rapid desiccation of faeces is likely to occur. We will try to answer two questions: *i)* does desiccation of L1 result in smaller L3 in snails?, *ii)* and does the size of L3 reflect infectivity of the L1 for snails?

MATERIALS AND METHODS

PARASITE MATERIAL

The L1 were obtained from a goat infected with *M. capillaris*. The excretion at the period of experiment was approximately 1,200 L1/g of faeces. This goat was reared indoors and fed on a diet

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of hay and commercial pellets: the range of faecal dry-matter (DM) was 42-36 %. Freshly collected faeces were dehydrated to obtain 70, 90 an 95 % of DM. This was performed in a ventilated chamber at 31 ± 1 °C on faecal pellets and lasted approximately 3 h to 21 h to obtain 70 and 95 % DM respectively. After desiccation process, the faeces were left from 0 to 240 h according to groups at 20 °C before extraction of L1, in order to reveal the influence of interaction between desiccation and duration of stay in desiccated faeces. The L1 were then extracted (Cabaret *et al.*, 1980) from control (non-desiccated faeces) and from desiccated faeces.

Infection of snails and measures of larvae

The adult *C. intersecta* snails originated from an isolated wild colony in Nouzilly, free of any protostrongylid infection. Snails were infected individually according to a method modified from Kassai (1957): each snail was in contact with a larval suspension of 100 living L1 for 3 h. Six batches of 20 snails each were exposed to *M. capillaris* L1 that had survived in desiccated faeces. The influence of desiccation and its duration on L1 survivals has been previously tested: *i)* very high survival (78-86 %) was found in control and in desiccated faeces stored for less than 12 h whereas lower survivals (average 67 %) were recorded in batches stored for 240 h; *ii)* increased desiccation combined with longer storage decreased L1 survival (Morrondo-Pelayo *et al.*, 1992). The following conditions may be considered as a gradient of increasing stress resulting from desiccation: M1 (control), M2 (70 % DM, 0 h), M3 (70 % DM, 240 h), M4 (90 % DM, 240 h), and M5 (95 % DM, 48 h). The coding for batches is as follows: (70 % DM, 0 h) means that faeces were dehydrated up to 70 % of dry-matter, which lasted 3 h and the larvae were then extracted whereas (70 % DM, 240 h) indicated that faeces, dehydrated the same way, were maintained during 240 h at 20 °C before extraction and use for infection of snails. The snails were maintained and fed on lettuce at 20 °C until they were serially killed from day 4 to 30 after infection in order to obtain successively L1, L2 and L3. The extraction of larvae from the foot of snails for the identification of larval stages was performed as previously described (Morrondo-Pelayo *et al.*, 1980). The intensity of infection (number of larvae/infected snail) was assessed in each of the 20 snails per batch. The following measures were performed by means of a camera lucida on each larval stage: length of larvae LL and width of larvae at the end of oesophagus, WL.

Processing of data

The estimation of arithmetic averages, standard-deviation, one-way analysis of variance – Anova (using Newman-Keuhls test of between batches comparison) were classical. The size and shape of larvae were estimated in a simple manner as respectively LL × WL and LL / WL, and the difference between batches were evaluated by means of a discriminant analysis. The relationships between size (LL × WL) or shape of L3 and intensity of infection were established by means of least-square regressions and then validated using resampling procedures (bootstrap with 500 repeats).

RESULTS

The morphological characteristics of larval stages

They are recorded in table I. The L1 were significantly smaller (Anova, $P < 0.05$) in the previously most desiccated L1 group (95 % - 48 h). The L2 and L3 in desiccated groups (M2 to M5) were smaller (Anova, $P < 0.05$) than in the control group (M1).

The size (LL × WL) and shape (LL/WL) of larvae L1 and L2 was not significantly different between control and desiccated group (data not shown). Conversely, differences between size and shape of L3 were found as shown in table II. The size of L3 was significantly larger in the control group compared with the desiccated groups. The shape was modified too: the larvae were less slender (ratio LL/WL being larger) in the control group.

Relationship between size and shape and intensity of infection

Significant differences ($P < 0.05$) in intensities were recorded between the control and the desiccated groups (Anova with Newman-Keuhls test).

Only size (S) could be related to intensity of infection: $S = (665.8 \times \text{intensity}) + 20{,}635$ ($R = 0.96$; $P = 0.01$) Confidence intervals at P = 90 % based on bootstrap procedures were 19,380 to 22,180 for the constant and 541 to 910 for the slope of regression indicating a good fit of the regression to actual data.

DISCUSSION

The lengths of L1 were smaller than it has been recorded by Morrondo-Pelayo *et al.* (1980) in Spain: 392 in the control group or even 349 µm in the most desiccated larvae (batch 95 % MS during two days) *versus* 436 µm. This could be due to the fact that larvae L1, prior to infection have different sizes from one site to another (Kulo *et al.*, 1994) or that

	Non-desiccated faeces (M1)	First-stage larvae maintained during 0 to 240 h in desiccated faeces (70 to 95 % DM) before infection of snails			
		70 % – 0 h (M2)	70 % – 240 h (M3)	90 % – 240 h (M4)	95 % – 48 h (M5)
First-stage larvae	6*	2	18	8	16
Length of larvae	392 (21)	396 (2)	385 (24)	396 (10)	349 (27)
Width of larvae	25 (3)	32 (1)	21 (5)	24 (6)	23 (5)
Second-stage larvae	46	27	31	35	23
Length of larvae	541 (38)	541 (32)	501 (42)	512 (56)	521 (45)
Width of larvae	40 (6)	35 (3)	37 (6)	42 (10)	46 (9)
Third-stage larvae	80	58	24	23	38
Length of larvae	633 (37)	610 (38)	608 (26)	604 (32)	603 (32)
Width of larvae	48 (6)	43 (11)	43 (8)	41 (6)	42 (8)

* Number of larvae examined.

Table I. — Averages in µm and standard deviations of the first-, second- and third- larval stage measures of *Muellerius capillaris* in snails *(Candidula intersecta)* in relation to desiccation of infective first-stage larvae before infection (batches M1 to M5).

	Non-desiccated faeces	First-stage larvae maintained during 0 to 240 h in desiccated faeces (70 to 95 % DM) before infection			
		70 % – 0 h	70 % – 240 h	90 % – 240 h	95 % – 48 h
Size (Length × width in µm)	30286 (4824)*	26334 (7943)	26091 (5382)	24689 (3770)	25512 (5719)
Shape (Length/width)	13.2 (1.8)	15 (3.2)	14.7 (2.9)	15 (2.6)	14.9 (3)
Intensity of infection	14.9 (19.4) (20)**	10.6 (15.7) (18)	8.6 (7.7) (15)	6.9 (8.5) (19)	5.8 (8.7) (19)

* Standard-deviation; ** number of infected snails.

Table II. — Size and shape of *M. capillaris* third-stage larvae in relation to desiccation history of first-stage larvae in faeces and actual infection of snails *(Candidula intersecta)*.

development differs according to the species of land-snail. Conversely, the second larval stage in control group (541 µm) was larger than that recorded by Gerichter (1951), Rose (1957: 529 µm) or Morrondo-Pelayo *et al.* (1980: 505 µm). The third larval stage length (633 µm) was similar in size to that found by Morrondo-Pelayo *et al.* (1980: 641 µm) but was much larger than the specimens examined by Rose (1957: 594 µm). The differences recorded in second- and third-stage larvae could be due as above mentioned to origin of L1, species of snail or slug used as intermediate host, or fixation and clearing methods (Fagerholm, 1979).

The sizes of L3 did present a coherent pattern between batches: they were larger in the heavier infections, i.e. in control group. We may hypothesise that larger L1 originating from non-desiccated faeces were more able to infect snails and that they developed better than their counterparts extracted from desiccated faeces. Thus the size of the obtained L3 should reflect the infectivity of L1. These findings are in agreement with those of Rossanigo & Gruner (1992) on trichostrongylid nematodes (size was modified when faeces are desiccated) but they also give indications on infectivity of larvae (this aspect was not evaluated by Rossanigo & Gruner, 1992). Our results could be compared with those obtained on asymmetry in insects submitted to stress (McKenzie & Clarke, 1988). In nematode larvae, larger size would be the positive indicator of infectivity (a putative indicator of fitness) in contrast with asymmetry in insects wich is a negative indicator of fitness.

The size variations of larvae are one case of phenotypic plasticity as an adaptation to variable environ-

ments. It could be an example of allelic sensitivity, in which the expression of individual genes is altered by changes in external conditions (e.g. genes whose products are directly responsive to changes in desiccation) and the phenotypic outcome is continuous and proportional to fluctuations in environment (Schlichting & Pigliucci, 1995).

The results might summarized as follows: desiccation of L1 in faeces was unfavourable for future development within the snail host, fewer larvae become established, and the L1 that completed their development into L3 remained smaller in desiccated larvae groups. The question is open to wether these smaller L3 larvae will be less fit or not to infect small ruminants.

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