The dynamics of nematode transmission in the red grouse (*Lagopus lagopus scoticus*): studies on the recovery of *Trichostrongylus tenuis* larvae from vegetation

L.M. Saunders*, D.M. Tompkins and P.J. Hudson

Institute of Biological Sciences, University of Stirling, Stirling, FK9 4LA, UK

Abstract

Attempts to recover the infective third-stage larvae of *Trichostrongylus tenuis* from heather (*Calluna vulgaris*) vegetation in the field are rarely successful because the larvae may be: (i) concealed within heather leaflets; (ii) concentrated in dew droplets which are lost from the plants upon sampling; or (iii) simply highly aggregated in the field. Heather plants were exposed to *T. tenuis* larvae in the laboratory and kept under suitable conditions for larval migration. Few larvae were found in dew droplets or concealed within heather leaflets; most larvae were recovered from the plant surface. This suggests that the low larval recovery from vegetation in the field simply reflects a highly aggregated distribution of few larvae. In a second experiment, the efficiency with which infective larvae migrate up the structurally complex heather plants was compared with migration up two control plant species with simpler structures: a monocotyledon, wheat, and a dicotyledon, hebe. After constant exposure, significantly more larvae were recovered from heather than from either of the control plants. This implies that the structural complexity of heather causes no problems for the infective larvae.

Introduction

Previous workers have assumed that infective larvae (L3) of the gastrointestinal nematode *Trichostrongylus tenuis* achieve transmission to their primary host, the red grouse *(Lagopus lagopus scoticus),* through ingestion with their preferred food, the growing shoots of heather *(Calluna vulgaris)* plants (Hudson, 1986; Hudson *et al*., 1992). Infective larvae are both positively phototactic and negatively geotactic and have been recorded ascending to the growing tips of heather plants (McGladdery, 1984). However, the collection of heather samples from the field rarely reveals the presence of *T. tenuis* larvae, providing a problem in estimating the availability of infective stages and predicting disease outbreaks (Hudson, 1986; Shaw *et al*., 1989).

There are three explanations of why *T. tenuis* larvae are rarely recovered from samples of heather collected in the field. First, electron microscope studies have revealed that L3 enter the curled heather leaflets, where they may be difficult to recover using the traditional extraction techniques (Watson, 1988). Second, larvae may be concentrated in the dew droplets that form on heather tips under the high humidity levels required for larval locomotion (Wilson & Leslie, 1911). If this were the case then larvae would be knocked from vegetation during sampling. Third, the low recovery may simply be due to low numbers of *T. tenuis* larvae being present on vegetation in the field at any one time, perhaps as a consequence of a highly aggregated larval distribution. Since red grouse faeces are highly aggregated on a grouse

*Fax: (44) 1786 464994
E-mail: lms1@stir.ac.uk

https://doi.org/10.1017/S0022149X00700381 Published online by Cambridge University Press

moor (Hudson, 1986) it is likely that *T. tenuis* larvae are concentrated in restricted hot spots around caecal faeces. Thus, successful sampling may require the identification of larval hot spots. To distinguish between these three explanations heather plants, treated with infective *T. tenuis* larvae, were intensively sampled.

To maximize the possibility of transmission to a host, one may expect *T. tenuis* larvae to have developed effective means of migrating up heather, since this is the principal food plant of red grouse. This may be particularly important since heather is a structurally complex plant. Previous studies on other trichostrongylids (Krecek *et al*., 1990) have tended to study larval migration on structurally simple monocotyledons such as grass. To examine whether larval migration was as efficient on heather as on a monocotyledon or on a structurally simple dicotyledon we undertook a second experiment. Larval migration on heather was compared with migration on the monocotyledon, wheat (*Triticum* spp.) and the dicotyledon, hebe (*Hebe* spp.).

Materials and methods

Cultivation of T. tenuis *larvae*

Third stage infective *T. tenuis* larvae (L3) were cultured from red grouse caecal faeces and caecal contents collected from both Gunnerside and Danby Moor in North Yorkshire. Cultures were incubated at 22°C for 7 days to allow nematode eggs to hatch and larvae to develop (Wilson, 1979). Larval cultures were mixed together to give a homogenous suspension, in which the number of larvae per ml was estimated using the modified McMaster technique (MAFF, 1978).

Intensive sampling of heather plants for T. tenuis *larvae*

Thirty-six potted heather plants were trimmed to equal size and allocated to one of four groups: ten for Baermann funnel analysis, ten for dissection, ten for water droplet analysis and the remaining six as untreated controls. A volume of larval suspension containing approximately 3500 L3 was taken up by syringe and placed on the soil surface at the base of each of the experimental plants. All 36 plants were kept in a cooled incubator under favourable conditions for *T. tenuis* larval migration: 12°C with a high relative humidity (min. = 75% RH, max. = 92% RH over 24 h). Each plant was kept under plastic covers and an aerial spray of water was administered twice daily to maintain a high relative humidity and allow the formation of dew droplets. Plants were sampled for the recovery of larvae 10 days later since previous studies have shown that larvae take at least 7 days to complete their migration up the plant (McGladdery, 1984).

Three sampling procedures were employed, each on ten of the treated plants and two of the untreated plants. In the first procedure, the plant was divided and cut into four sample types: the growing shoots, the upper 3 cm of vegetation, the lower vegetation and the stem. Larvae were recovered from each sample using Baermann apparatus (MAFF, 1978). Each sample was placed in a muslin bag in a funnel containing warm water (32–35°C) to approximate to the temperature of the host's gut. *Trichostrongylus tenuis* larvae were collected in the stem of the funnel and, after 12 h, were drawn through the apparatus in 2 × 10 ml aliquots. Further tests confirmed that no larvae were recovered after this time. Larvae were concentrated by centrifugation at 1500 rpm for 2 min, with the upper 9 ml of supernatant being discarded. The sediment was re-suspended in the remaining 1 ml of water and transferred to a Petri dish where the number of larvae present were counted under a × 40 binocular microscope. To determine whether the Baermann procedure was failing to recover any *T. tenuis* larvae that had entered the growing tips, leaflets were subsequently dissected under a × 40 binocular microscope.

The second procedure counted the number of larvae that had entered the leaflets by carefully dissecting a subsample of heather leaflets from three parts of the plant (growing shoots, upper vegetation and lower vegetation) and then examining these with the stems under a × 40 binocular microscope.

The third sampling procedure counted the number of larvae in water droplets that had collected on the surface of the plant. Water droplets were removed from plants with a pipette and collected in a Petri dish and the number of larvae counted under a × 40 binocular microscope. Each plant was then separated into the four sample types as before, and processed through the Baermann apparatus.

Migration of T. tenuis *larvae up heather, hebe and wheat stems*

Eighteen plants of the three study species were trimmed so that each had one vertical stem that extended 7 cm above the soil surface. A volume of larval suspension containing approximately 600 L3 was taken up by syringe and placed on the soil surface at the base of 15 plants of each species. The remaining nine plants were left as untreated controls. All 54 plants were kept in an environmental chamber, again under favourable conditions for *T. tenuis* larval migration (12°C and 80% relative humidity). After 5, 10 and 15 days, five treated plants and one untreated plant of each of the three species were removed. Each plant was divided into the upper 4 cm and the lower 3 cm of vegetation. Larvae were then collected using the Baermann apparatus, (as described above).

Statistical analyses

Unless stated otherwise, data were analysed using Generalised Linear Models with either Poisson or negative binomial error distributions. Significance levels were calculated, using chi-squared tests, from the deviance explained by each factor following stepwise deletion (Crawley, 1993). During the examination of samples there were unavoidable time delays (≤ 4 days) between actual sampling of vegetation and the processing of samples. Since this may have influenced numbers of larvae recovered, time delay was included as a covariate in all analyses whenever relevant.

Results

Intensive sampling of heather plants for T. tenuis *larvae*

No *T. tenuis* larvae were recovered from the untreated control heather plants by any of the three sampling protocols. The numbers of larvae recovered from exposed plants using the first and second sampling procedures (Baermann and dissection) followed a Poisson distribution (fig. 1); log-transforming the data produced a normal distribution with normally distributed residuals (Anderson-Darling Normality test, $P = 0.15$).

More larvae were recovered per gram of plant material with the Baermann procedure than by dissection (fig. 2; deviance = 362.69, d.f. = 1, $P < 0.001$). No larvae were recovered from the leaflets dissected after the use of the Baermann apparatus, suggesting that all of the larvae had been recovered. Within procedures, there were different numbers of larvae recovered from the four sample types with more larvae being recovered from heather stems than from upper or lower vegetation (fig. 2; deviance = 1155.28, d.f. = 3, $P < 0.001$). Also, under the Baermann procedure, more larvae were recovered from the growing shoots than from either upper or lower vegetation, whilst, under the dissection procedure, fewer larvae were recovered from the growing shoots than from either upper or lower vegetation (interaction deviance = 2529.65, d.f. = 3, $P < 0.001$).

Under the third sampling protocol, where water droplets were removed from plants prior to processing through the Baermann apparatus, only a small proportion of the total number of *T. tenuis* larvae recovered were present in the water droplets (fig. 2). The Baermann procedure again recovered different numbers of larvae per gram of plant material from the four sample types (deviance = 577.45, d.f = 3, $P < 0.001$): once again, more larvae were recovered from heather stems and growing shoots than from either upper or lower vegetation (fig. 2).

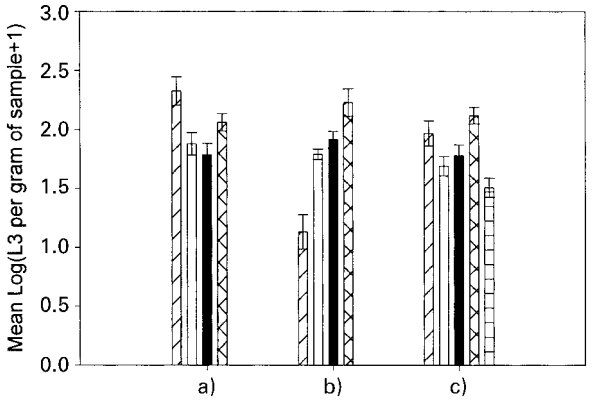

Fig. 2. The numbers of *Trichostrongylus tenius* L3 recovered (± S.E.) from heather plants by a) processing through Baermann apparatus, b) complete dissection and c) removing dew then processing through Baermann apparatus (▨, growing shoots; □, upper vegetation; ■, lower vegetation; ⊠ stem; ▤, dew droplets).

Comparing the migration of T. tenuis *larvae up heather, hebe and wheat stems*

No *T. tenuis* larvae were recovered from untreated heather, hebe or wheat stems. The numbers of larvae recovered from treated stems followed an aggregated pattern that was not significantly different from a negative binomial distribution (chi-squared test; $\chi^2 = 6.75$, d.f. = 7, $P = 0.46$).

There was a significant difference in the number of larvae recovered from the three plant species (deviance = 97.58, d.f. = 2, $P < 0.001$). The highest numbers were recovered from the heather stems; the lowest numbers were recovered from the wheat stems (fig. 3). Both sample type and sampling day also had significant effects on the number of larvae recovered (figs 3 and 4). Overall, more larvae were recovered from lower stems than from upper stems (fig. 4, deviance = 106.52, d.f. = 1, $P < 0.001$),

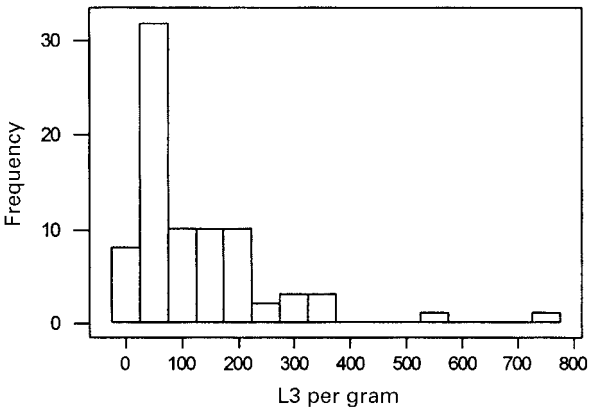

Fig. 1. Frequency distribution showing the Poisson distribution of *Trichostrongylus tenuis* L3 recovered from heather plants with the Baermann and dissection protocols.

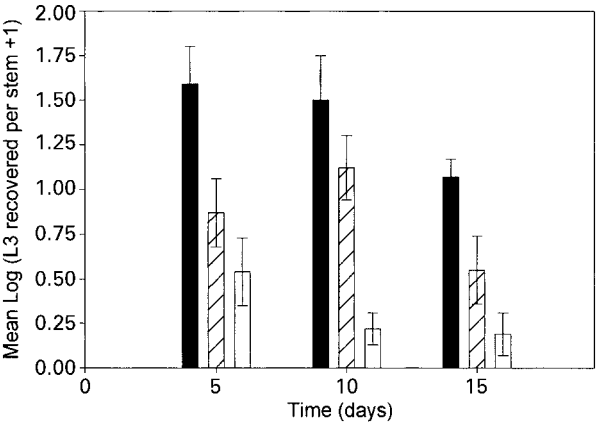

Fig. 3. Number of *Trichostrongylus tenuis* L3 recovered (± S.E.) from heather (■), hebe (▨) and wheat (□) stems after 5, 10 and 15 days.

https://doi.org/10.1017/S0022149X00700381 Published online by Cambridge University Press

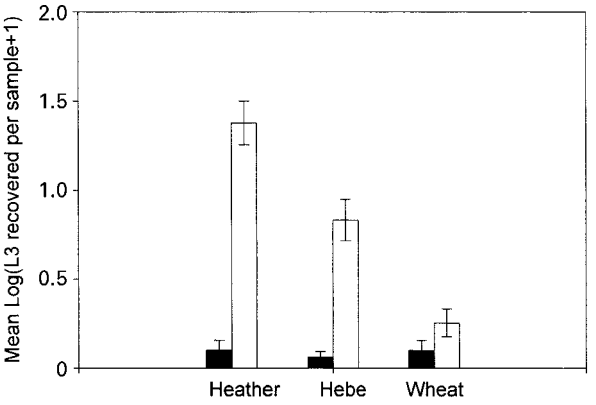

Fig. 4. Number of *Trichostrongylus tenuis* larvae recovered (± S.E.) from upper (■) and lower (□) heather, hebe and wheat stems.

and the number recovered generally decreased with increasing time since exposure (fig. 3, deviance = 7.60, d.f. = 2, $P = 0.006$).

Discussion

Samples of heather taken from grouse moors rarely contain more than a small number of infective *T. tenuis* larvae (L3), although red grouse frequently carry heavy worm burdens (Hudson, 1986). The research conducted here suggests that this is simply due to low numbers of *T. tenuis* larvae being present on vegetation in the field at any one time. The hypothesis that low larval recovery may be due to large numbers of L3 encapsulated within heather leaflets was refuted. Less, rather than more, larvae were recovered per gram of heather growing shoots by complete dissection than by simply processing through Baermann funnels (fig. 2). Moreover, no further larvae were recovered from dissection of heather leaflets following the Baermann procedure. The hypothesis that low larval recovery may be because the L3 are concentrated in the dew droplets on heather tips, which are knocked off during sample collection, was also refuted. Only a small proportion of the total number of *T. tenuis* larvae recovered were present in the water droplets which were removed from the surfaces of heather plants prior to processing through Baermann funnels (fig. 2).

The maximum mean percentage recovery of *T. tenuis* larvae from heather over the course of the second experiment was 10.2%. This figure should be considered an under-estimate since the efficiency of the Baermann apparatus in recovering *T. tenuis* larvae from vegetation is approximately 61% (Shaw *et al.*, 1989). Furthermore, there will have been some mortality in the larvae as indicated by the fall in larval recovery with time of exposure (fig. 3). Regardless of these limitations, the success rate of larval migration up heather is most likely a major limiting factor in the parasite life-cycle since it would drastically reduce the number of infective larvae ingested by grouse feeding on heather plants.

One mechanism by which *T. tenuis* larvae appear to compensate for this low migration success, in attempting to maximize their transmission to grouse, is the increased efficiency of larval migration up heather plants compared with other plant species. Experiments conducted here show that, under constant conditions of larval exposure, more L3 are subsequently recovered from heather stems than from either a monocotyledon or a dicotyledon with simpler structure (fig. 3). This probably reflects a selective advantage of the larvae, which we shall examine in more detail with further experiments. We consider the comparison of plant stems valid since more larvae are normally recovered from the stems and growing shoots of heather plants, than from the rest of the vegetation. This implies that *T. tenuis* larvae simply migrate up the stem and go straight to the growing tips of heather (fig. 2), the preferred food of the red grouse. The recovery of more larvae from lower stems than from upper stems may be because of humidity variations within the chamber, since the presence of a moisture film on vegetation is essential for trichostrongyle larval migration (Callinan, 1979; McGladdery, 1984).

Trichostrongylid larvae from other species exhibit diurnal patterns of migratory behaviour up and down the vegetation (Krecek *et al.*, 1990). If this were to occur in *T. tenuis*, it may mean there is a turnover in larvae at the growing tips of heather plants. However, the relatively large distances the larvae would have to travel on heather would mean the larvae would spend a great deal of time not exposed to the definitive host. We thus expect diurnal migratory behaviour in *T. tenuis* to be unlikely.

We have acquired evidence which suggests that there is no substantial proportion of the infective *T. tenuis* larval population concentrated within heather plants or in water droplets on heather plants. Since the faeces of grouse are highly aggregated on a grouse moor (Hudson, 1986), and this work shows aggregation on the plant, it seems likely that there are a few restricted hot spots containing large numbers of infective *T. tenuis* larvae in the field. Moreover, since grouse are highly territorial, this suggests that a large proportion of the *T. tenuis* infection that a grouse receives is derived through self-infection or from close relatives. Predicting outbreaks of *T. tenuis* from random vegetation sampling would thus be highly inaccurate. Estimates of larval burdens on vegetation would probably be more accurate when obtained from a synthesis of grouse densities, worm burdens and the patterns of worm aggregation within grouse populations (Hudson *et al.*, 1992).

Acknowledgements

We would like to thank Peter Robertson for his guidance during this work and to Dave Newborn for collecting the caecal faeces. A NERC studentship and support from English grouse moor owners through the Game Conservancy Trust funded this study. We would like to thank J.A.C. Briggs for his enthusiasm and suggestions.

References

Callinan, A.P.L. (1979) The ecology of the free-living stages of *Trichostrongylus vitrinus*. *International Journal for Parasitology* **9**, 313–316.

Crawley, M.J. (1993) *GLIM for ecologists*. Oxford, Blackwell Scientific Publications.

Hudson, P.J. (1986) *The red grouse. Biology and management of a wild gamebird*. Game Conservancy Trust.

Hudson, P.J., Newborn, D. & Dobson, A.P. (1992) Regulation and stability of a free-living host–parasite system, *Trichostrongylus tenuis* in red grouse. I: monitoring and parasite reduction experiments. *Journal of Animal Ecology* **61,** 477–486.

Krecek, R.C., Murrell, K.D. & Douglas, L.W. (1990) Effects of microclimatic variables on the availability and movement of third-stage larvae of *O. ostertagi*. *Onderstepoort Journal of Veterinary Research* **57,** 133–135.

MAFF (1978) *Manual of veterinary investigation laboratory techniques*, Part 7 Parasitology. Reference Book No. 368. Ministry of Agriculture, Fisheries and Food.

McGladdery, S.E. (1984) Behavioural responses of *Trichostrongylus tenuis* larvae in relation to their transmission to the red grouse, *Lagopus lagopus scoticus*. *Journal of Helminthology* **58,** 295–299.

Shaw, J.L., Moss, R. & Pike, A.W. (1989) Development and survival of the free-living stages of *Trichostrongylus tenuis*, a caecal parasite of red grouse *Lagopus lagopus scoticus*. *Parasitology* **99,** 105–113.

Watson, H. (1988) The ecology and pathology of *Trichostrongylus tenuis* (Nematoda), a parasite of red grouse *Lagopus lagopus scoticus*. Unpublished PhD thesis, University of Leeds.

Wilson, E. & Leslie, A.S. (1911) Grouse disease. *in* Lord Lovat (*Ed*.) *Grouse in health and in disease*. London, Smith & Elder Co.

Wilson, G.P. (1979) Effects of the caecal threadworm *Trichostrongylus tenuis* on red grouse. Unpublished PhD thesis, University of Aberdeen.

(Accepted 12 November 1998)