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Soil moisture influences the development of *Haemonchus contortus* and *Trichostrongylus colubriformis* to third stage larvae

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ABSTRACT

Two climate chamber experiments were conducted to determine the effect of varying initial soil moisture (0, 10 and 15%), simulated rainfall amount (0, 12 and 24 mm) and simulated rainfall timing (days −1, 0 and 3 relative to faecal deposition) on development (day 14) of *Haemonchus contortus* and *Trichostrongylus colubriformis* to the third stage larvae (L3) and faecal moisture (FM). Increasing initial soil moisture content from 0 to 10 or 15% led to higher recovery of total L3 ($P < 0.001$). Total L3 recovery increased with each level of simulated rainfall ($P < 0.001$) in the ascending order of 0, 12 and 24 mm. There was an interaction between the effects of initial soil moisture and simulated rainfall amount on the recovery of total L3, showing that the benefit of increased simulated rainfall lessened with increasing soil moisture. Simulated rainfall on the day of deposition resulted in higher recovery of L3 ($P < 0.001$) than simulated rainfall on other days. FM on day 3 relative to faecal deposition was best associated with recovery of total *H. contortus* and *T. colubriformis* L3 ($R^2 = 0.32–0.46$), reinforcing the importance of sufficient moisture soon after faecal deposition. The effects of initial soil moisture, and the amount and timing of simulated rainfall on development to L3 were largely explained by changes to FM and soil moisture values within 4 days relative to faecal deposition. These results highlight the influence of soil moisture and its interaction with rainfall on development of *H. contortus* and *T. colubriformis* to L3. Consequently we recommend that soil moisture be given greater importance and definition in the conduct of ecological studies of parasitic nematodes, in order to improve predictions of development to L3.

1. Introduction

Haemonchus contortus and *Trichostrongylus colubriformis* are the two most important parasitic gastrointestinal nematodes (GIN) of sheep in tropical and temperate summer rainfall areas. The development of the free-living stages of these nematodes depends on environmental variables, mainly temperature and moisture. When the temperature is suitable for development, sufficient moisture is needed to ensure successful development to the third stage larvae (L3). Moisture available in the faeces is more important for the development of eggs to L3 for *H. contortus* than *T. colubriformis* as the egg shell of *H. contortus* is more permeable to water at all temperatures (Waller, 1971), making this species more suceptible to dessication (O'Connor et al., 2006).

Rainfall timing relative to faecal deposition (Barnes et al., 1988; Besier and Dunsmore, 1993; O'Connor et al., 2007a), rainfall amount (Gordon, 1948; Levine and Andersen, 1973) and evaporation rate (O'Connor et al., 2008) are important regulators, and thus predictors, of

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moisture in the faeces following deposition. Availability of moisture in the faeces can also be measured directly to provide faecal moisture (FM) and O'Connor et al. (2006) have suggested that measurement of this variable is likely to integrate all of the other moisture-mediated influences on development to L3.

In a prediction model for *Teladorsagia (Ostertagia)* and *Trichostrongylus* spp. developed by Callinan et al. (1982), soil moisture was one of the most important factors for successful development to L3. This is in agreement with the earlier work of Levine and Todd (1975) who suggested that temperature and soil moisture are the most important factors affecting development and survival of *H. contortus* L3 as soil moisture is influenced by the interaction of rainfall, soil type and evapotranspiration.

While the importance of soil moisture as a moisture regulator for development of eggs to L3 on pasture has been established (Bullick and Andersen, 1978; Levine and Todd, 1975), the effect of varying soil moisture and the interactions between soil moisture, rainfall timing and rainfall amount on FM and subsequent development of *H. contortus* and *T. colubriformis* to L3 have not been investigated. In fact, little consideration has been given to soil moisture in recent ecological studies on the effect of simulated rainfall (O'Connor et al., 2007a, 2007b, 2008; Khadijah et al., 2013) and herbage height (Sakwa et al., 2003) on the development of *H. contortus* to L3.

To investigate this, two experiments were conducted in climate-controlled chambers to determine the effect of soil moisture, rainfall timing and rainfall amount on FM and development of *H. contortus* and *T. colubriformis* to L3. A number of general hypotheses to confirm established understanding about the development of *H. contortus* and *T. colubriformis* from egg to L3 were tested but the specific hypotheses of particular interest in these experiments were (i) the recovery of L3 will increase with increasing soil moisture at the time of faecal deposition; (ii) increasing initial soil moisture will reduce the beneficial effects of increased rainfall amount on L3 recovery; (iii) FM during the first few days post deposition will be an accurate predictor of development to L3 when temperature is not limiting.

2. Material and methods

2.1. Experimental designs

Two experiments were conducted in climate-controlled chambers to test these hypotheses. Experiment 1 was designed to determine the effects of initial soil moisture, simulated rainfall timing and simulated rainfall amount on the development of *H. contortus* and *T. colubriformis* to L3. Experiment 2 was designed to determine the effects of these factors on FM and soil moisture. In both experiments simulated rainfall (hereafter referred to as rainfall) was applied to freshly collected sheep faeces which had been placed on the surface of experimental containers filled with oven-sterilised soil.

2.1.1. Experiment 1

This was a $3 \times 3 \times 3$ incomplete factorial experiment with a completely randomised design and 4 replicates per treatment combination. The factors and levels of rainfall of either 0 mm, 12 mm or 24 mm, timed to occur on days −1, 0 or 3 relative to faecal deposition, were applied to experimental units containing soil with 0, 10 or 15% (w/w) moisture content. The design was incomplete because of the absence of 0 mm rainfall × rainfall timing. Soil was oven dried at 80 °C prior to the experiment and water was added (w/w) to the respective experimental units on day −1 to attain soil moisture 10 or 15%. Recovery of L3 from faecal pellets and the top 25 mm soil was determined on day 14 after faecal deposition.

2.1.2. Experiment 2

The design was identical to that described for Experiment 1. However, rather than measuring development to L3, faeces and soil were subsampled from experimental units on days 1, 2, 3, 4, 7, and 14 relative to faecal deposition to determine moisture content.

2.2. Experimental units

Development from egg to L3 occurred in experimental units comprising polycarbonate jars (250 ml, 100 mm height, 60 mm diameter) filled with a mixture (ratio of 5:1) of sterilised sandy loam soil and 7 mm (diameter) gravel to a depth of 60 mm. Each jar had a single 2 mm drainage hole drilled in the side wall, 25 mm from the bottom of the jar.

2.3. Rainfall simulation

Rainfall was applied to the experimental units via a second polycarbonate jar (250 ml, 100 mm height, 60 mm diameter) with seven holes (5.5 mm diameter) drilled into the base which was then lined with a single layer of filter paper (No. 42, Whatman® Schleicher & Schuell, England). Water was applied to experimental units by placing the rainfall jar with the required volume of water on the top of them (i.e. 40 mm above the soil surface). The rainfall jars remained in position for 6 h but the average rate of application was 24 mm/h. Rainfall of 12 and 24 mm required a volume of 34 and 68 ml respectively.

2.4. Production of infective faeces

Groups ($n = 8$ animals/group) of Merino wethers (5–6 months old) were used in Experiments 1 and 2 as faecal donors. Following arrival at the animal house, they were weighed and treated with albendazole and levamisole (Rotate®, Novartis Animal Health Australia), naphthalaphos (Rametin®, Bayer Australia) and abamectin (Virbamec LV®, Virbac Australia) at recommended oral dose rates to remove any existing GIN infection. Nematode worm egg counts (WEC) and coproculture were negative for all animals ten days after treatment. The animals were fed daily with chaffed lucerne (*Medicago sativa*; 150 g/d) and oaten (*Avena sativa*; 600 g/d) hay, had free access to water and were kept in individual pens under natural light.