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Effect of simulated rainfall timing on faecal moisture and development of *Haemonchus contortus* and *Trichostrongylus colubriformis* eggs to infective larvae

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ABSTRACT

Three climate-controlled chamber experiments were conducted to determine the effect of 32 mm simulated rainfall applied prior to (days −4 to −1) or after (days 0–7) faecal deposition and as a single (32 mm) or split (2 × 16 mm) application on faecal moisture (FM) and development of *H. contortus* and *T. colubriformis* to third stage infective larvae (L3). The timing of simulated rainfall regulated extra-pellet L3 recovery for *H. contortus* ($P < 0.05$) but not *T. colubriformis*. Recovery of L3 was highest ($P < 0.05$) when simulated rainfall was applied on the day of deposition followed by days −1, 1 and 2, which resulted in similar but lower development success rates. Recovery of intra-pellet *T. colubriformis* L3 was two-fold greater ($P = 0.008$) than for *H. contortus* and was higher ($P = 0.007$) following simulated rainfall on days 0 and 1 than on other days. There was a positive association between FM and total L3 recovery indicating the importance of FM in the period 48–72 h (*H. contortus*) and 72–96 h (*T. colubriformis*) after deposition. Simulated rainfall on the day prior to deposition was as effective in supporting total L3 recovery as application on days 1 or 2 and this effect could be predicted through FM. This highlights the importance of soil in transferring moisture to the faecal pellet. The importance of precedent rainfall and soil moisture in determining the development success of *H. contortus* and *T. colubriformis*, in addition to the general effects of the timing of simulated rainfall, need to be accommodated in grazing management programs to combat these species.

1. Introduction

The sheep parasitic nematodes, *Haemonchus contortus* and *Trichostrongylus colubriformis* have a direct life cycle, spending part of their life inside the host (parasitic stage) and part outside the host (free-living stage). Temperature and moisture are the two most important environmental variables controlling the development and survival of free-living stages. Effects of temperature have been extensively studied and reported (Veglia, 1916; Rogers, 1940; Berberian and Mizelle, 1957; Rose, 1963; Hsu and Levine, 1977; McKenna, 1998) but the regulating role of moisture has received less attention (O'Connor et al., 2006). Rainfall and evaporation are the components that regulate the moisture regimens experienced by third stage infective larvae (L3) on pasture and have been incorporated in the prediction models for the development of *H. contortus* (Barger et al., 1974) and *T. colubriformis* (Barnes et al., 1988). The predictive capacity of these models is yet to be fully validated in the field with grazing animals. More recently, O'Connor et al. (2007a,b, 2008) investigated the effect of simulated rainfall amount and distribution and

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evaporation rates on the development of *H. contortus* to L3. These studies indicated greater development of *H. contortus* with increased rainfall amount but as evaporation rate increased development success in response to a given amount of rainfall decreased.

What remains less clear is the effect of rainfall occurring before faecal deposition on the development of *H. contortus* and *T. colubriformis* to L3 and on faecal moisture (FM). For rainfall which occurs before faecal deposition to influence development success, effects must be mediated by soil moisture and/or microclimatic effect that alter FM. Such effects are important to quantify and, if found to be significant, ultimately incorporated with existing prediction models and tested in the field.

Three controlled-climate chamber experiments were conducted to better elucidate the effects of rainfall on FM and development of *H. contortus* and *T. colubriformis* to L3. We hypothesised that (i) by day 14 post deposition, the majority of *H. contortus* L3 will be found in the soil (i.e. extra-pellet) whereas the majority of *T. colubriformis* L3 will be present in the faecal pellets (i.e. intra-pellet); (ii) total recovery of *H. contortus* and *T. colubriformis* L3 will be greater the closer the time of rainfall to faecal deposition; (iii) rainfall prior to faecal deposition will increase recovery of *H. contortus* and *T. colubriformis* L3 relative to unwatered controls, but to a lesser extent than rainfall following faecal deposition; and (iv) effects of moisture on development success are mediated through changes in FM and detected as a positive association between the recovery of *H. contortus* and *T. colubriformis* L3 and FM.

2. Materials and methods

2.1. Experimental designs

Three experiments were conducted in controlled climate chambers (SANYO Electric Biomedical Co., Ltd., Japan) to determine the effect of timing and distribution of simulated rainfall application on FM and the development success of *H. contortus* and *T. colubriformis* to L3. Experiment 1 was designed to determine the drying rate of unwatered faecal pellets and changes in FM when simulated rainfall occurred on different days relative to day of faecal deposition. Experiment 2 was designed to determine the effect of varying timing of simulated rainfall relative to faecal deposition on the recovery of *H. contortus* and *T. colubriformis* L3 14 days after faecal deposition. Experiment 3 was designed to determine the effect of the treatments applied in Experiment 2 on FM. Experiment 3 was conducted separately because there was insufficient space in the climate chambers to conduct both experiments at the same time. Simulated rainfall (hereafter referred to as rainfall) was applied to freshly collected faeces which had been placed in experimental containers, on the surface of a uniform mixture of steam sterilised soil and gravel. In Experiment 2, faeces and the top 25 mm soil were collected on day 14 to determine development success of *H. contortus* and *T. colubriformis* to L3. In Experiments 1 and 3, subsampling of faecal pellets within containers occurred daily for 7 and 6 days respectively.

2.1.1. Experiment 1

A single factor experiment was conducted as a completely randomised design with the single application of 32 mm timed to occur on either day 0, 1, 2, 3, 4, 5, 6, or 7 relative to day of faecal deposition. There were 2 replicates per treatment and collection of faecal pellets occurred daily on days 1–7 for determination of FM.

2.1.2. Experiment 2

A 12×2 factorial experiment was conducted as a completely randomised design with rainfall applied on day −4, −3, −2, −1, 0, 1, 2, 3, 4, 5, 6 or 7 relative to faecal deposition. Rainfall was applied as a single application of 32 mm or as two equal but split applications of 16 mm on consecutive days. There were 4 replicates per treatment combination with unwatered controls external to the factorial design. Collection of faecal pellets and the top 25 mm of soil occurred 14 days after faecal deposition for determination of FM and development success of *H. contortus* and *T. colubriformis* to L3.

2.1.3. Experiment 3

A 7×2 factorial experiment was conducted as a completely randomised design with rainfall applied on day −3, −1, 0, 1, 2, 3 or 4 relative to day of faecal deposition. Rainfall was applied as a single application of 32 mm or as two equal but split applications of 16 mm on consecutive days. There were 4 replicates per treatment combination with unwatered controls external to the experimental design. Where rainfall was applied on days −3, or −1, collection of faecal pellets for determination of FM occurred at 24, 48 and 72 h after faecal deposition (because of the precedent rainfall treatments). Where rainfall was applied on days 0, 1, 2, 3 or 4, collection of faecal pellets for determination of FM occurred at 6, 24, 30, 48, 72 and 96 h after rainfall.

2.2. Experimental units

Polycarbonate jars (250 ml, 100 mm height, 60 mm diameter) containing a mixture of sterilised sandy loam soil and gravel (7 mm diameter), to a depth of 60 mm, were used as experimental units. The jars had a single 2 mm drainage hole drilled in the side wall 25 mm from the bottom. The soil moisture was 0% (i.e. oven dried at 80 °C) on day 0 for Experiment 1 and 10% (w/w) on day −4 for Experiment 2 and day −3 for Experiment 3.

2.3. Rainfall simulation

Polycarbonate jars (250 ml volume) with seven holes (5.5 mm diameter) drilled into the base were lined with a single layer of filter paper (No. 42, Whatman® Schleicher & Schuell, England) and used to apply rainfall. These rainfall jars were placed on top of the experimental units (i.e. 40 mm above the soil surface) and filled with the required volume of water. The water was allowed to drip slowly and uniformly over the faecal pellets and soil over a 6 h period. The average rate of application was 32 mm/h. Rainfall of 32 mm required a volume of 94 ml and application of 16 mm required 47 ml.