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Microclimate has a greater influence than macroclimate on the availability of infective *Haemonchus contortus* larvae on herbage in a warmed temperate environment

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ABSTRACT

Global climate change is altering epidemiological patterns of gastrointestinal nematode infections in grazing livestock, including through effects of temperature and moisture on the availability of infective third-stage larvae (L3). While considerable experimental effort has been devoted to the influences of climate on L3 development and survival in major nematode species, knowledge of effects on L3 migration out of faeces and onto herbage is more limited. In this study, we examined elements of this process for *Haemonchus contortus* in controlled and natural climates. The effect of temperature on migration rate from faeces was quantified and found to peak at 15 °C. In glasshouses, a 3 °C difference in mean temperature failed to produce a statistically significant difference in the number of L3 reaching herbage after a single rainfall event, and faecal moisture content (FMC) did not decline significantly more rapidly at the higher temperatures. Most larvae left the faeces and reached the grass within 3 h after simulated rainfall. On natural pasture in temperate summer, FMC was strongly affected by microclimate, with shade and long grass both significantly slowing drying. Results suggest that microclimate is important in determining FMC and larval migration, and that its effects can be greater than those of macroclimate, e.g. moderate differences in average ambient temperature. More work is needed to develop a full predictive understanding of larval availability in natural settings, which is the product of interacting factors acting on overlapping parasite cohorts.

1. Introduction

The abomasal nematode parasite *Haemonchus contortus* causes major problems to the health and productivity of sheep and goats, especially in tropical and sub-tropical areas with adequate rainfall (Waller, 1997; Perry and Randolph, 1999; O'Connor et al., 2006; Van Dijk et al., 2010; Besier et al., 2016). Improved conditions for transmission are further predicted in temperate areas under climate change scenarios (Rose et al., 2016), where resistance to anthelmintic drugs threatens to undermine control (Rose et al., 2015a). Temperature and moisture have long been known to be important for the development, survival and translation of infective third stage *H. contortus* larvae (L3) onto herbage (Veglia, 1915; Rossanigo and Gruner, 1995; O'Connor et al., 2007, 2008). Previous experiments have attempted to characterize the effects of individual climatic factors on components of the *H. contortus* life cycle under controlled conditions (e.g. Hsu and Levine, 1977; Coyne and Smith, 1992), and relationships between climate and infection pressure have been widely observed in the field (e.g. Rose, 1963; Onyali et al., 1990; Troell et al., 2005; Silva et al., 2008).

How temperature and rainfall interact to influence L3 migration out of faeces has received less attention (Todd et al., 1976; Hsu and Levine, 1977, Pandey et al., 1989; Van Dijk and Morgan, 2011; Wang et al., 2014). Under climate change scenarios, variability in temperature and rainfall is predicted to increase (Mason et al., 1999; Pinault, 2012), so the effect of short-term climatic conditions on larval availability, e.g. following brief rainfall events, and the modifying influence of pasture microclimate, might significantly alter transmission patterns. Increased temperatures could hypothetically accelerate larval movement out of

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faeces, while simultaneously leading to faster drying of faeces and thus a shorter period of migration. The net effect of warmer temperatures on migration is therefore hard to predict.

This study aims to determine how temperature and rainfall interact to influence migration of infective *H. contortus* larvae from faeces onto grass, and how this is modified by variation in microclimate. Hence, to assess the likely net impact of realistic gradual climate warming on L3 availability in temperate environments. The overall objective is to improve ability to predict infection patterns under climate change, in order to inform farm adaptation strategies (Morgan and Wall, 2009), as part of wider attempts to understand ecological responses of parasites to global change (Cable et al., 2017).

2. Materials and methods

2.1. General methods

2.1.1. Mono-infected faecal samples

Faecal samples from mono-cultures of MHco3(ISE) isolate *H. contortus* in sheep (Vineer et al., 2016) were provided by the Moredun Research Institute (Edinburgh, U.K.). Briefly, lambs were born and reared indoors to ensure that they were free of pre-existing nematode infections. Donor lambs were drenched orally by syringe with 5000 L3 in a suspension of tap water. Faecal egg counts (FEC) were checked 21 days later, and individual sheep with sufficient egg output were harnessed and a bag attached to collect faeces over a 24-h period. Faecal samples were placed in a sealed plastic bag and sent by post to the laboratory in Bristol, to arrive within 24 h. A modified McMaster method (MAFF, 1986) was used to verify estimated egg density on arrival and ensure that development of eggs was not discernible before starting the experiments.

2.1.2. Larval culture and recovery

For Experiments 1 and 2 below, L3 were prepared as follows. Faeces containing *H. contortus* eggs were placed in six-well plates (Sterilin, U.K.) with a loose lid and incubated at 20 °C for seven days (Versatile Environmental Test Chamber, Sanyo, Japan). Lids were removed briefly each day to ensure enough air exchange for development, without the danger of excessive drying. Faeces containing fully developed L3 were used for the experiments, which aimed to test larval emergence from faeces. Following the various treatments, L3 were harvested from cultured faeces using a modified Baermann technique (Gruner, 1986), and species identity confirmed using the identification keys described in Van Wyk and Mayhew (2013).

2.1.3. Larval extraction from herbage

The method used in Experiment 2 to recover L3 from herbage was derived from several different studies (Martin et al., 1990; Couvillion, 1993; Van Bezooijen, 2006). Grass was cut using scissors at the base of the plant, as close to the soil as possible without including soil or roots, and immediately wrapped in a square of woven cotton muslin cloth, forming a loose ball, and the muslin secured with a clip. A 250 ml inverse conical flask was filled with water until it reached about 1 cm below the rim, a few drops of detergent were added, and herbage samples were submerged in the water. After standing for 12 h, samples were removed and set aside, and the water decanted, leaving about 10 ml of water containing the sediment, including larvae and debris. This residue was agitated and poured into a soft-walled flexible plastic test tube and centrifuged for 2 min at 1500 rpm (c. 400 g). Supernatant was then carefully removed by siphoning from the top of the tube, leaving 1 ml of sediment. Sucrose solution of specific gravity 1.17 was added to fill two thirds of the tube, which was then centrifuged again at 400 *g* to float the larvae. In order to capture all the larvae, the test tube was clamped with haemostatic forceps about 5 mm below the surface, and inverted to pour the larval suspension into a beaker. The top portion of the clamped tube was then rinsed with water to recover any remaining larvae, and the rinsing water combined with the larval suspension. The whole suspension was then transferred to a standard test tube, topped up to 10 ml with water, and inverted several times to mix the larval suspension. A 1 ml aliquot of the suspension was transferred into a Sedgewick Rafter nematode counting chamber together with one drop of Lugol's iodine, and the larvae counted under ×40 total magnification. The number of larvae obtained was multiplied by 10 to estimate total number present in the herbage sample.

The efficiency of the above method was evaluated as follows. Herbage from un-grazed pasture was harvested as above, and 10 equal 5 g aliquots cut into 1 cm pieces and each wetted with 1 ml larval suspension that contained 399 ± 25 (mean ± standard deviation) *H. contortus* L3, evenly distributed by pipette throughout the herbage. After settling for 30 min, larvae were recovered from herbage using the above method and counted to estimate the recovery rate.

2.1.4. Rainfall simulation

A portable rainfall simulator was applied in Experiment 2 below to standardize rainfall delivery. This consisted of a pressurized nozzle with a rubber hose connected to a tap, from which water was released at uniform intensity. For the purpose of calibration, the simulator was run for 1 min, 1 m above an array of cylindrical beakers, and the vertical height and volume of water recovered from each beaker was recorded. The average value showed that rainfall was delivered at a rate of 1.4 mm/min, and this was applied to estimates of rainfall requirements for larval migration.

2.2. Experimental design

This study aimed to quantify the rate of L3 migration from faeces at a range of constant temperatures without moisture limitation (experiment 1). Secondly, to quantify L3 migration following temporary rewetting of faeces in controlled environments (experiment 2) and, finally, the rate of drying of faeces on natural pasture (experiment 3).

2.2.1. Experiment 1: effect of temperature on the migration of H. contortus L3 from faeces

Migration of L3 was assessed using a mesh sieve apparatus based on that in Wang et al. (2014). Five faecal pellets containing *H. contortus* L3 were placed on a sieve 10 cm in diameter and of aperture 0.9 mm, which was inserted into a plastic cup. In order to create ideal moisture for migration, a fine water mist was applied to the pellets using a pressurized garden sprayer (Spraymist 1.25l, Hozelock, UK) for 20 s from a distance of around 1 m. The experimental units were then transferred immediately to, and maintained in, covered plastic boxes at relative humidity (RH) of 95%, which were kept stored at 7, 10, 15, 20, 25 and 33 °C for one hour, with four units in each box. Faecal pellets were then transferred to a series of new sieves, each of which was inserted into a new plastic cup, and the same spraying treatment was once more applied to keep the faeces moist, before again placing them into the constant temperature boxes. Larvae that had migrated from the faeces onto the first sieve during incubation were washed into the respective cups and allowed to sediment for 12 h, after which the supernatant was siphoned off with a pipette. The bottom 10 ml of water containing larvae was mixed and a 1 ml aliquot was transferred into a nematode counting chamber. The process of larval recovery was repeated eight times at intervals of one hour. At the end of the experiment, the number of larvae remaining in the pellets was estimated by Baermann extraction, enabling calculation of the proportion of total recoverable L3 present that had migrated out of the faeces at each time point, and at each temperature. Owing to the ideal moisture conditions and short experimental time, mortality of L3 was ignored.

For each temperature treatment, the mean cumulative proportion of L3 that had left the faeces was calculated for each time point (h) and then logistically transformed as follows: