Ecology of the free-living stages of *Haemonchus contortus* in a cool temperate environment.

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Summary

Introduction

Current understanding of the ecology of *Haemonchus contortus* is incomplete and seen as an impediment to effective control of the worm in summer rainfall regions, where outbreaks are common and can result in significant stock mortality. Although there has been a wealth of published research on the effects of temperature on free-living development of *H. contortus*, our level of understanding of how moisture in the micro- and macro-environments influences development to infective larvae on herbage is considerably lower.

The current state of knowledge of the free-living ecology of *H. contortus* is reviewed in Chapter 2, with a particular focus on research findings since the 1970s. Knowledge gaps are highlighted, and a proposal made for a framework on which future investigations of *H. contortus* can be based in order to improve prediction of free-living development. The key hypotheses under investigation in the experimental studies concerned the quantitative effects of moisture on free-living development of *H. contortus*, and are summarised in Chapter 1 and in further detail in each of the experimental chapters (Chapter 4-6).

Plot experiments were conducted in the Northern Tablelands of NSW, where *H. contortus* is the most important parasite of sheep. Subsequent laboratory experiments, designed in order to extend and explain the findings of the plot experiments, were conducted in programmable incubators in which temperature was regulated to mimic conditions typical to the Northern Tablelands summer. All experiments focused on testing the effects of a range of rainfall and moisture-related variables on *H. contortus* development from egg through to infective larvae. Details of the experimental sites and the general materials and methods used are described in Chapter 3.

Chapter 4 describes three plot experiments undertaken to determine the effects of amount, timing and distribution of simulated rainfall on the developmental success of *H. contortus*.

Faeces containing *H. contortus* eggs were deposited onto pasture plots under a rainfall-activated retractable roof which eliminated incident rainfall. In October (spring) 2004 and January (summer) 2005, one of four amounts of simulated rainfall (6, 12, 18 or 24 mm) was manually applied to plots at one of four times after faecal deposition (day 1, 4, 8 or 15). In February (summer) 2006, one of three simulated rainfall amounts (12, 24 or 32 mm) was applied by sprinkler in either of two distributions (single event or three smaller but equal split events over 32 h). More *H. contortus* pre-infective larvae (L1 and L2) developed under the d 1 simulated rainfall treatment than later treatments in spring 2004 and summer 2005 ($p<0.001$). There was no effect of rainfall amount on development in either experiment, and negligible recovery of infective larvae (L3). In summer 2006 L3 were recovered from the herbage in one-third of the plots harvested, however recovery was low (0.08% of eggs deposited) and there were no treatment effects. Recovery of L1 and L2 from faeces increased with simulated rainfall amount at d 4 ($p=0.008$), and more L1 and L2 were recovered from the split distribution treatment at d 4. The results indicate that moisture conditions soon after faecal deposition are key determinants of *H. contortus* development success, with significant penalties on development when simulated rainfall was applied 7 days or more post-deposition, and when the duration of simulated rainfall was short. High rates of evaporation during both summer experiments resulted in rapid drying of the micro-environment and this appears to have limited development to L3.

Chapter 5 describes a factorial experiment (3x2x2) conducted in programmable incubators to determine the effects of amount and distribution of simulated rainfall on *H. contortus* development using the daily temperature profile from the summer 2005 plot experiment in Chapter 4. The experiment was conducted under conditions of low evaporation to test the hypothesis that high evaporation rates prevailing in the plot experiments were prohibitive to development of L3, regardless of simulated rainfall treatments applied. Sheep faeces containing *H. contortus* eggs were placed in experimental units containing sterile soil and had one of three amounts (12, 24, 32 mm) of simulated rainfall applied, in either a single event or three split events over 6 days. Treatments were applied either in week 1 only, or in week 1 and 2. Recovery of L3 at 4, 7 and 14 days post-contamination increased with amount of rainfall and was significantly higher under the single simulated rainfall event, compared with the three smaller events. The effect of the week 2 rainfall events on development success was non-significant but regression of recovery with cumulative precipitation and evaporation ratio (P/E) suggested a small additive effect on L3 recovery of the repeated rainfall treatment. Both faecal moisture content and cumulative P/E were strongly correlated with recovery of L3.

Chapter 6 describes a factorial experiment (3x4x2x3) conducted in programmable incubators to investigate the effects of evaporation rate, and amount and distribution of simulated rainfall on the free-living development of *H. contortus*. The experiment aimed to confirm the fundamental effects of rainfall amount and distribution on *H. contortus* development, along with quantifying the effect of interaction between evaporation rate and rainfall variables alluded to by the results from previous sets of experiments. Sheep faeces containing *H. contortus* eggs were incubated on sterilised soil under the same temperature profile used for experiments in Chapter 5. Simulated rainfall was applied in 1 of 3 amounts (12, 24 or 32 mm) and 4 distributions (a single event on the day after deposition, or in 2, 3 or 4 equal but smaller, split events over 2, 3 or 4 days, respectively). Samples were incubated at either a Low or High rate of evaporation (Low: 2.1-3-4 mm/day and High: 3.8-6.1 mm/day), and faeces and soil were destructively sampled at 4, 7 and 14 days post-deposition. Recovery of L3 from the soil (extra-pellet L3) increased over time ($p<0.001$) and with each increment of rainfall ($p<0.001$) but was reduced under the High evaporation rate ($p=0.008$). All rainfall amounts yielded significantly different recoveries of L3 under Low evaporation rates but there was no difference between the 12 and 24 mm treatments under the High evaporation rate. The distribution of simulated rainfall did not significantly affect recovery of infective larvae ($p=0.126$). Faecal moisture content was positively associated with L3 recovery ($p<0.001$), as was the ratio of cumulative precipitation and cumulative evaporation (P/E), particularly when measured in the first 4 days post-deposition. The latter may provide a practical means of predicting the moisture requirements for *H. contortus* development from readily available meteorological data if subsequent field trials indicate the relationship between P/E and *H. contortus* L3 development is applicable under paddock conditions. The results show that evaporation rate plays a significant role in regulating the influence of rainfall amount on the success of L3 development and assists in the interpretation of some earlier studies. Sheep producers may ultimately be able to use the P/E approach to improve decision-making about *H. contortus* control.

Chapter 7 summarises the main findings of the studies and their implications for worm control. The progression from the initial plot studies through to the final set of incubator experiments demonstrated fundamental relationships between *H. contortus* free-living development and simulated rainfall variables (timing, amount and distribution) that were previously unpublished. Amount of rainfall was positively correlated with development to the L3 stage, while distribution treatments applied appeared to act on development through its effects on moisture availability during the first few crucial days after faecal deposition. Similarly, there was a significant difference in pre-infective development depending on whether rain fell in the first few days post-deposition or not until after the first 7 days, and little additive effect on development to L3 when rainfall events occurred more than 7 days post-deposition. However, inclusion of evaporation rate as an effect in the final experiment indicated that rate of drying modulated the effects of rainfall variables on development, particularly the relationship between rainfall amount and *H. contortus* development. The findings suggested that high evaporation rates were the key limiting factor to development in the plot experiments, and highlighted the relevance of the cumulative ratio of precipitation and evaporation (P/E) for predicting *H. contortus* development under the controlled laboratory conditions. If this relationship remains robust under a field setting, it is likely that

P/E can be used as a practical means of predicting pasture infectivity on-farm. However the findings await validation in the field.

Certification

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

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O'Connor, L.J., Kahn, L.P. and Walkden-Brown, S.W. (2007). Moisture requirements for the free-living development of *Haemonchus contortus*: quantitative and temporal effects conditions of low evaporation. *Veterinary Parasitology,* **(Submitted)**.

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