The influence of water on the migration of infective trichostrongyloid larvae onto grass

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SUMMARY

Detailed knowledge of the effects of water on the migration of infective larvae of economically important trichostrongyloid species is urgently needed to feed into prediction models of future epidemiology. The influence of water on the migration of the parasitic nematodes *Nematodirus battus*, *Haemonchus contortus* and *Teladorsagia circumcincta* from sheep dung onto grass was examined in a series of laboratory experiments. Turf plots were seeded with larvae, which were recovered from grass clippings by serial sampling. Free water was necessary for larvae to escape from dung, but not for vertical migration onto grass. When temperature and relative humidity were held constant, the proportion of a population of live larvae present on herbage reached a plateau of around 2 (1–10)% after 24 h, and then changed little over time. Larvae in soil and dung formed a reservoir, such that a similar proportion of the larval population was maintained on grass after clipping. These findings suggest continuing random movement of free larvae. Implications for the epidemiology of trichostrongyloid species are discussed in the context of trade-offs faced by the parasites.

Key words: *Haemonchus contortus*, *Teladorsagia circumcincta*, *Nematodirus battus*, epidemiology, transmission, rainfall, drought, climate change, disease, trade-off.

INTRODUCTION

In recent years, the ecology and, consequently, epidemiology of highly pathogenic, economically important, helminth parasites of ruminants appears to be changing. For example, data from UK veterinary surveillance laboratories show up to 5-fold increases in diagnostic rates of helminth-associated disease as well as shifts in the seasonality of disease (van Dijk *et al*. 2008, 2010). Climate change has been identified as a likely driver behind such changes (van Dijk and Morgan, 2010). The effects of temperature and, to a degree, moisture on the development of trichostongyloid parasites has received extensive attention (as reviewed by O'Connor *et al*. 2006) but specifics of the migration of the infective larvae of these parasites onto herbage remains the subject of speculation. In particular, knowledge of the effects of water on the migration of L3 appears core to predicting the degree to which developed larvae will actually pose a risk to hosts. Parasite distribution sometimes seems at odds with existing estimates of this parameter, e.g. in bioclimatographs (Morgan *et al*. 2005). Larval availability could depend on the timing and nature, as well as the amount, of rainfall and other available free water such as condensed fog or dew. Climate change is predicted to increase the frequency of periods of drought, followed by a limited number of days with heavy rainfall (Hennessy *et al*. 1997; Tapiador *et al*. 2007). This adds impetus to the need to incorporate improved understanding of the effects of rainfall on larval dynamics into general predictions of the epidemiology of trichostrongyloid nematodes. Furthermore, it has been proposed that migratory behaviour of these nematodes may vary significantly between species (O'Connor *et al*. 2006) and therefore there is a need to compare multiple species in a standardized laboratory set-up.

Many studies have reported a positive correlation between rainfall and the emergence of trichostrongyloid larvae onto herbage or the increase of worm burdens in tracer animals (e.g. Williams and Bilkovich, 1973; Bryan and Kerr, 1989; Stromberg, 1997; Amaradasa *et al*. 2010). Not surprisingly, such associations are most dramatic in semi-arid regions, where absence of rain may bring parasite transmission to a halt during the driest months and sharp peaks in the abundance of larvae on herbage can be seen after periods of rainfall (Chiejina and Fakae, 1989; Onyali *et al*. 1990; Agyei, 1997; Sissay *et al*. 2007). Water present within faeces is likely to permit development to the infective third larval stage (L3) in most species in all but the most arid conditions (Chiejina and Fakae, 1989; Onyali *et al*. 1990; Garcia Romero *et al*. 1997). With respect to migration away from the dung and onto herbage, several pasture studies (Skinner and Todd, 1980; Krecek *et al*. 1990;

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Niven *et al*. 2002) have suggested that a film of moisture, through which larvae would swim, is needed for the migration onto herbage. This would have important implications for parasite transmission. Continuous films of moisture are rarely witnessed on grass blades, and water may evaporate rapidly on summer days. Thus, larval migration would only be possible either just after rains or when dew is found on grass leaves. Although the continuous film hypothesis has become the established view amongst veterinary parasitologists it remains largely untested as most studies of L3 migration have been conducted in the field, with insufficient separation of different phases of migration, or control of environmental conditions (Skinner and Todd, 1980; Rose and Small, 1985; Krecek *et al*. 1992; Marley *et al*. 2006). Silangwa and Todd (1964) compared migration onto wetted and non-wetted grass in the laboratory and concluded that trichostrongylid larvae climbed wetted grass significantly better. However, the experiments took place in humidity chambers at a relative humidity (RH) of 80–90%. Therefore, the RH around the wetted grass may simply have been higher and this may have supported migration. Indeed, Callinan and Westcott (1986) produced similar results by varying only RH, suggesting that free water is not the driver of larval migration. Crofton (1948) and Rees (1950) suggested that gradients in RH and temperature experienced by the larvae determined their migratory behaviour. However, the work presented by these authors took place outdoors and rainfall and dew were not recorded. Therefore, as RH itself will be correlated to rainfall, the question remains whether it is the presence of free water or a certain RH that is needed for larval migration.

Moisture may also influence migration by limiting entrapment in dung. For *Ostertagia, Haemonchus* and *Cooperia* spp. present in the dung of cattle it has been shown that, once development is completed, L3 cannot escape once dung becomes desiccated (Williams and Bilkovich, 1973; Chiejina and Fakae, 1989; Agyei, 1997; Silva *et al*. 2008). On dry, warm, days, cattle faeces, which are normally more liquid than sheep faeces and dropped in pats rather than in pellets, rapidly form a crust on the pat surface. It appears that this crust, although it may protect them from further severe desiccation, cannot be penetrated by L3. In the tropics, the sudden migration of these larvae, made possible by the arrival of the rain season, may result in very sharp and dangerous peaks of larval emergence (Fakae and Chiejina, 1988; Chiejina and Fakae, 1989; Agyei, 1997). In temperate regions it was similarly documented that drought-breaking rains suddenly increased larval density on herbage 10-fold as larvae, which had been trapped for months, migrated *en masse* (Bryan and Kerr, 1989). Cattle faeces can clearly function as a reservoir of larvae during drought. Sheep faeces have a very different shape and consistency but may have a similar function (Silva *et al*. 2008). Whether moisture is essential for the migration of larvae out of sheep faeces is not known. Skinner and Todd (1980) published the only study measuring larval presence in sheep pellets as well as on herbage and suggested that larvae stayed in the pellets when there was no rain. Estimations of larval death rates in desiccated dung have not been published.

Studies on the larval migration of gastrointestinal nematodes (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986) show remarkable consistency in the proportions of larvae recovered from herbage. Unfortunately, no study has quantified the sensitivity of the recovery method and thus true proportions of larvae migrating onto grass cannot be estimated. However, it appears from existing data that the vast majority of larvae may not make it onto the herbage at all. This raises the question of whether larvae form a reservoir in the soil and mat layer from which individuals emerge over a prolonged period of time, or whether larvae that remain in these layers perish without being presented to their herbivore hosts. Longitudinal studies of larval emergence have not been undertaken.

The aims of the present study were to establish, for selected trichostrongyloid species: (i) whether larvae can migrate out of desiccated sheep dung; (ii) whether the presence of free water is essential for the vertical migration of larvae; (iii) the fate of larvae that initially remain in the soil or dead vegetation layer; (iv) the likelihood of differences in migratory behaviour at the species level.

MATERIALS AND METHODS

All experiments were carried out in the laboratory at room temperature (20–24 °C) and relative air humidity between 71 and 83% (as measured with a calibrated TinytagPlus® data logger; Gemini Data Loggers, UK).

Validation of larval recovery method

The modified sucrose-interface larval recovery method, initially developed by Eysker and Kooyman (1993), was used in all of the following experiments. Briefly, watery suspensions, containing larvae and sediment collected on a 38 μm screen, were collected in 50 ml Falcon tubes and spun at 600 ***g*** for 2 min. The supernatant was removed and the sediment resuspended in the remaining 15 ml. Then 30 ml of a 60% sucrose solution was introduced to the tube, injecting underneath the watery suspension with a 50 ml syringe attached to a blunted needle. The tubes were spun at 600 ***g*** for 5 min after which 15 ml of the now sediment-free water located at the sucrose-water interface, containing the larvae, were removed,

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making use of a clean syringe and a blunted needle, and examined in a 1 ml nematode slide (Chalex Corporation, USA).

The proportion of larvae recovered from grass was measured in order to validate the method. Large numbers of pure culture *Teladorsagia circumcincta* and *Haemonchus contortus* L3 were obtained from the Moredun Research Institute, Edinburgh, UK. It was deemed highly unlikely that occasional passages over hosts of these laboratory-maintained strains had influenced their migratory behaviour. For both species separately, suspensions of approximately 2000, 10 000 and 50 000 L3 per 10 ml of water were made by counting the number of larvae per 1 ml in a rostered nematode counting slide (Chalex Corporation, USA) and adding larvae or diluting with water, as appropriate. The number of larvae in 1 ml of each suspension was then counted 5 times to give the mean and 95% confidence interval (CI). Three replicates of 10 ml of each suspension were carefully sprinkled onto approximately 100 g of cut grass in large (40 litre) containers, the grass thoroughly mixed with a spatula and left to stand for 24 h at room temperature and, 24 h later, larvae were recovered from the herbage. Proportions of larvae recovered were calculated, arcsine transformed, and analysed in a two-way ANOVA, with larval density and nematode species as factors. In this and in following tests α was set at 0·05 unless otherwise specified.

Timing of maximum larval recovery

A suspension with mean larval density of 3333 *H. contortus* L3 per ml was created and, under continuous stirring, subdivided into 30 ml portions and then 10 ml portions. Five plastic trays measuring approximately 1 × 0·5 m were filled with grass turf (ryegrass mix, *Lolium* spp.). The turf was purchased at a garden centre and, before experiments commenced, clippings from 1 tray were processed as above to establish freedom of infective nematodes. Grass height did not exceed from 10 cm in any of the experiments and the attached layer of soil and grass roots was approximately 5 cm deep. The trays were subdivided into 3 replicate parts and 10 ml of suspension (containing approximately 33 333 L3) was, after parting of the grass, very carefully spread on the soil midline of the trays, using a 20 ml syringe and a blunted needle. Trays were left for 2 h after which the grass blades were thoroughly wetted with the fine mist of water provided by a plant spray, making sure all parts of the grass were thoroughly wet. The number of spray pumps per tray was equal for each tray. This wetting process was repeated every 24 h thereafter. After 24, 48, 72 and 96 h, grass from each of the 3 replicates of 1 tray was cut with scissors at a height of 1–1·5 cm above soil level and immediately processed. Proportions of larvae recovered were arcsine transformed and compared with time in a one-way ANOVA.

The influence of free water on vertical migration

Six more trays were set up and left to stand until the grass and soil layer were thoroughly dry. Two trays were seeded, as described above, with *T. circumcincta* larvae, 2 with *H. contortus* larvae, and 2 with 2933 *N. battus* L3 per replica. Trays were left for 2 h after which the grass blades and upper soil layer of 1 tray per species was wetted as above. After 5 h, water droplets on herbage had evaporated and the wetting process was repeated. At 24 h after seeding the soil herbage was cut and processed as above. The arcsine transformed proportions of larvae retrieved were analysed in a two-way ANOVA with wetting and worm species as factors, and Tukey's pairwise comparison was applied for the factor worm species.

The influence of water on the emergence of larvae from dung

Dung from mono-infected sheep, containing *T. circumcincta* or *H. contortus* eggs, was incubated at 25 °C for 7 days. No water was added to the cultures during incubation. On day seven, 3 × 10 g of desiccated dung were crushed between 2 fingers and larvae separated from faecal material using the Baermann technique. After 12 h, the numbers of live larvae were estimated by counting those present in 3 × 1 ml of suspension, using a 1 ml nematode counting slide (Chalex Corporation, USA). Another 2 × 10 g were put in an oven at 90 °C for 8 h, in order to estimate the percentage dry matter and thence the number of larvae per gram of dry matter (DM).

Four more grass turf trays were prepared as above (1 wet treatment and 1 dry treatment tray for each worm species) and 71 g of dung were spread evenly over each replica area. Wet treatment trays were thoroughly wetted as above, at the start of the experiment and every 24 h thereafter. After 72 h the grass was clipped from all trays, carefully avoiding picking up any dung particles with the grass, and processed immediately. Approximately 3 × 10 g of dung was picked up from each tray and the crushed pellets put in a Baermann apparatus for 12 h. Examination of dry and wet dung was repeated on day 7 while the dry dung was also examined on days 14 and 21. On each occasion 2 × 10 g of each tray was also put in the oven as above, after which the number of larvae was corrected for dry matter content. Differences in numbers of larvae present on days 0, 3 and 7 were analysed in a one-way ANOVA for each tray, and Bonferroni correction applied, setting the critical P-value at 0·0125. Log transformed numbers of larvae present in dry dung were tested for significant decline with time using Spearman rank

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Table 1. Mean proportions of larvae recovered from herbage

(Ranges in parentheses, $n = 3$.)

	No. larvae spiked into grass	2000	10 000	*50 000*
Proportion of larvae recovered	*T. circumcinta*	0·079 (0·063–0·092)	0·102 (0·080–0·117)	0·111 (0·091–0·125)
	H. contortus	0·079 (0·060–0·101)	0·100 (0·089–0·110)	0·084 (0·077–0·092)

correlation, with linear regression conducted if the correlation was significant. The slopes of the log-linear regression lines were compared and it was assumed that they were significantly different if their 95% confidence intervals did not overlap.

The fate of larvae initially not moving onto herbage

Vertical migration over time. The wet trays described above were, after initial clipping at 24 h, kept at room temperature and sprayed daily with the same amount of water. On days 7 and 14 the newly grown grass was clipped and processed as above. On day 21 limited grass re-growth prevented clipping. It was assumed that the proportion of larvae recovered from herbage was a function of the number of larvae that had died since their application onto soil, and the number of larvae removed in previous clippings of the same tray/replica area. The proportion recovered on day t was estimated as follows:

$$P(t) = \frac{\frac{1}{Mp} * Nr(t)}{N(0) - N\mu(t) - \left[\frac{1}{Mp}(Nr(t-6) + Nr(t-7) + Nr(t-13))\right]} \quad (1)$$

where Mp is the mean proportion of larvae recovered by the method used, as estimated above, Nr the absolute number of larvae recovered, $N_{(0)}$ the number of larvae applied on day zero and $N\mu$ the number of larvae predicted to have died, with $N_{\mu(t)} = \sum_{t=1}^{t=14} N_{(t-1)}.\mu$. Proportions of *H. contortus* and *T. circumcincta* larvae surviving in soil over time were measured (see below) and the instantaneous larval death rate μ estimated as the slope of the log-linear regression lines. For *N. battus*, μ was estimated from van Dijk and Morgan (2008). As the experiments were not run simultaneously for the three species and the cumulative effect of small differences in temperature, RH, and amount of water applied over 14 days could not be estimated, inter-species comparison was not appropriate for this experiment. The proportions of larvae recovered, corrected according to Equation 1, were analysed in a one-way ANOVA for each tray.

On day 28 the trays were soaked in water and left to stand for 5 h. The turf was rolled up and the water squeezed out into a bucket. The muddy water was passed first over a 2 mm and then over a 1 mm sieve and the mud remaining on the screens discarded. The water was then passed over a 38 μm screen and the material remaining on the screen divided over 2–4 Falcon tubes, depending on the amount of material. This fine sand was mixed with approximately 15 ml of tap water. Larvae were then recovered from the sandy water in the same manner as described for the herbage plucks above. The remaining watery suspension of larvae was thoroughly mixed and then 3×200 larvae were examined in a 1 ml nematode slide (Chalex Corporation, USA) and the number of live larvae recorded. Larvae were considered dead when observed to be immotile and in a characteristic stretched-out position. The number of larvae applied to the *N. battus* tray proved insufficient to recover significant numbers of larvae in the above way.

Survival in desiccated soil. On days 7, 14, 21 and 28 one quarter of the turf of the dry trays was removed, soaked in water and processed as above. For each replica 200 larvae were counted and the number of larvae alive counted as already described.

RESULTS

Validation of larval recovery method

The proportions of *T. circumcincta* and *H. contortus* larvae recovered from spiked grass are given in Table 1. The proportion of larvae recovered did not differ between species ($F_{1,17} = 1{\cdot}883$, $P = 0{\cdot}195$) or with the initial density of larvae ($F_{2,17} = 3{\cdot}815$, $P = 0{\cdot}052$). The mean proportion of larvae recovered was 0·092 (bootstrapped 95% CI 0·062–0·122).

Timing of maximum larval recovery

The percentages of *H. contortus* L3 recovered after 24, 48, 72 and 96 h are illustrated in Fig. 1. Percentages did not increase or decrease significantly after 24 h ($F_{3,11} = 0{\cdot}971$, $P = 0{\cdot}489$).

The influence of free water on vertical migration

The percentages of larvae recovered from grass after 24 h in the wet and dry treatments, and results of the t-tests, are given in Table 2. Wetting the grass leaves did not have a significant effect on the proportions

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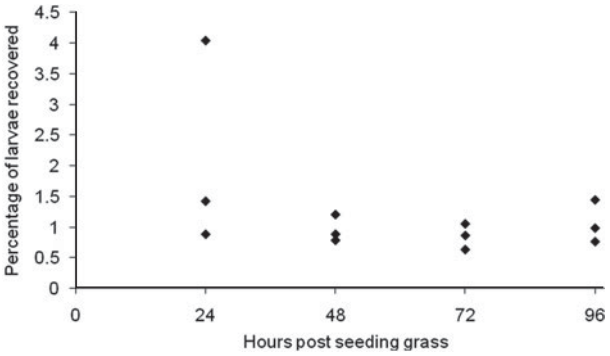

Fig. 1. Percentages of *Haemonchus* larvae recovered at various times (h) after seeding the grass turf with larvae (percentages not corrected for recovery method).

recovered ($F_{1,17}=0{\cdot}933$, $P=0{\cdot}353$), and neither did nematode species ($F_{2,17}=1{\cdot}621$, $P=0{\cdot}238$).

The influence of water on the emergence from dung

The percentages of L3 recovered from the grass in dry and wetted dung trays are given in Table 3. Significantly more larvae were recovered from the water-treated than from the dry trays ($F_{1,11}=51{\cdot}119$, $P<0{\cdot}001$). Significantly fewer *T. circumcincta* than *H. contortus* larvae were recovered ($F_{1,11}=35{\cdot}315$, $P<0{\cdot}001$).

The number of larvae present in the dung (larvae per gram DM) over the 21 days of the experiment is illustrated in Fig. 2. In the wet treatments the number of larvae of both *T. circumcincta* and *H. contortus* that were recovered declined significantly with time ($F_{2,8}=13{\cdot}526$, $P=0{\cdot}006$ and $F_{2,8}=70{\cdot}200$, $P<0{\cdot}001$, respectively). For both worm species, larvae in dung declined markedly in the first 3 days ($P\leqslant 0{\cdot}009$), then reached a plateau between days 3 and 7 ($P\geqslant 0{\cdot}120$). Numbers of larvae did not decline significantly in the dry treatments over the first 7 days (*T. circumcincta*: $F_{2,8}=2{\cdot}146$, $P=0{\cdot}170$; *H. contortus*: $F_{2,8}=1{\cdot}612$, $P=0{\cdot}275$).

Over the full 21 days, numbers of living larvae recovered from dry dung declined significantly for both *T. circumcincta* ($S_p=-0{\cdot}824$, $P<0{\cdot}001$) and *H. contortus* ($S_p=-0{\cdot}847$, $P<0{\cdot}001$). The slopes of the log-linear regression lines for survival in desiccated dung (*T. circ.*: 0·034, 95% CI +/−0·007; *H. cont.*: 0·048, 95% CI +/−0·008) did not differ significantly.

The fate of larvae initially not moving onto herbage

Vertical migration over time. On day 28, a proportion of 0·60 (range 0·55–0·63) of *T. circumcincta* larvae present in the soil of the wet trays was found to be alive. A proportion of only 0·01 (0·00–0·05) of *Haemonchus* larvae had survived. Both values were very close to those predicted by the instantaneous daily dry tray soil survival rates (predicted 0·58 and 0·00, respectively; see below). Therefore, wet-tray larval death rates were approximated as dry-tray rates. The instantaneous daily death rate of *N. battus* larvae was estimated to be 0·020. The proportions (corrected for larvae removed from trays during previous samplings and larvae predicted to have died) and the results of the ANOVAs are given in Table 4. The corrected proportion of larvae recovered did not significantly increase or decrease over time for any of the three species. At constant temperature and relative humidity, the proportion of the total living larval population present on herbage appears stable.

Survival in desiccated soil. The proportion of larvae surviving in dry soil is illustrated in Fig. 3. Numbers of living larvae recovered declined significantly over time for both *T. circumcincta* ($S_p=-0{\cdot}950$, $P<0{\cdot}001$) and *H. contortus* ($S_p=-0{\cdot}926$, $P<0{\cdot}001$). Mortality rates were significantly higher for *H. contortus* than for *T. circumcincta* larvae (slopes of the log-linear regression lines *T. circ.*: 0·015, 95% CI +/−0·002; *H. cont.*: 0·041, 95% CI +/−0·011).

DISCUSSION

Very few infective larvae are able to escape desiccated dung. Therefore, the environment in which the developmental phase is completed (dung for most trichostrongylids; soil for *Nematodirus* spp.) is likely to underpin important inter-species differences in epidemiology on pasture. For species such as *H. contortus* and *T. circumcincta*, if larvae develop during a hot, dry, summer week, a rain event, or heavy dew, has to occur before they are able to migrate onto herbage. Such an event would result in the mass release of larvae from dung and this may explain why clinical haemonchosis in summer is often witnessed when rain follows a period of drought. At times of the year when desiccation of dung is less severe, dew may suffice and larval release may be more gradual.

In contrast to widely established opinion, it appears that parasitic nematodes do not need free water to climb onto herbage. This conclusion is strongly supported by agreement in results across the three species. A study comparing the influence of rainfall on the abundance of larval species at pasture, in Australia, reported a strong correlation between rainfall and the abundance of *T. circumcincta* and *T. vitrinus*, whereas the presence of *Nematodirus* spp. L3 was not affected by rainfall (Niven *et al.* 2002). This contrast between species cannot be explained by general dependence of larval migration on free water. However, it could result from water-dependent release from dung, since *Nematodirus* spp. eggs develop in soil and larval migration is likely to take place whenever hatching occurs. For dung-developing species, rapid desiccation of sheep dung may not

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Table 2. Percentages of larvae recovered from the dry and wet treatments after 24 h

(Ranges are given in parentheses. The *t*-statistic refers to dry and wet treatments of the individual species; the Bonferroni-adjusted alpha level is 0·017.)

	H. contortus	*T. circumcincta*	*N. battus*
Wet treatment	0·45 (0·16–0·81)	0·89 (0·51–1·11)	0·24 (0·17–0·31)
Dry treatment	1·11 (0·75–1·44)	0·19 (0·12–0·30)	0·11 (0–0·23)
t statistic (2 D.F.); *P*	−2·41; 0·095	3·54; 0·075	1·29; 0·323

Table 3. Average percentages of larvae recovered from grass in the dry and wet dung treatments after 72 h

(Ranges in parentheses; $n=3$ for each treatment. Differences between treatments and between species are significant (see text).)

	H. contortus	*T. circumcincta*
Wet treatment	3·43 (2·96–3·94)	0·47 (0·40–0·55)
Dry treatment	0·18 (0·11–0·30)	0·04 (0·00–0·11)

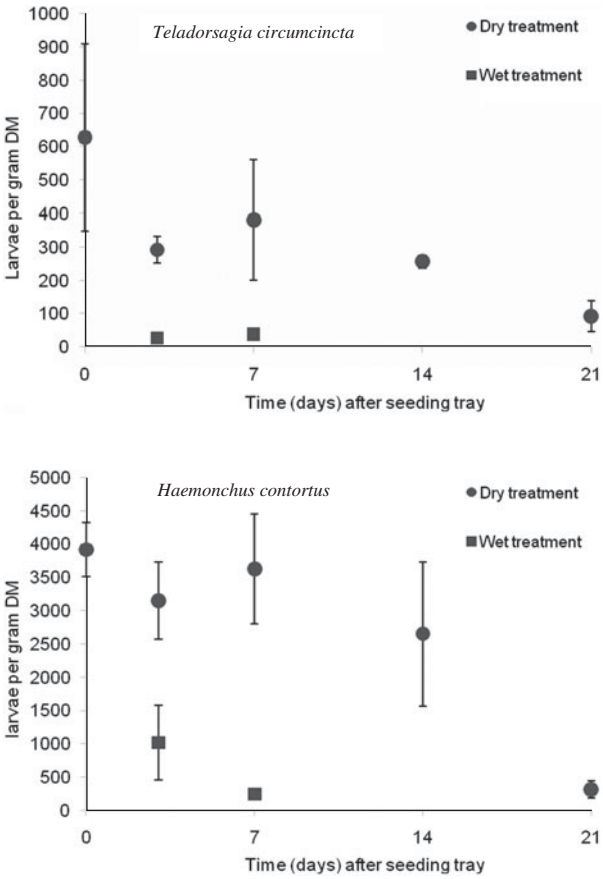

Fig. 2. *Teladorsagia circumcincta* and *Haemonchus contortus* larvae recovered per gram DM of dung during the first 7 (dry and wet dung) and 21 (dry dung only) days of the experiment (error bars represent standard deviations). On day zero, both wet and dry treatments start with the same number of larvae per gram DM.

only trap larvae but also limit their incorporation into soil by Diptera and other organisms (Barth *et al*. 1995; van Dijk, unpublished data).

A reservoir function of sheep dung, with the potential for a sudden, dangerous mass release of larvae, may play an increasingly important role under predicted climate change rainfall scenarios. Studies researching into when pasture, previously grazed by infected animals, can safely be grazed again will have to include examinations of dung as well as herbage larval counts. The length of time for which dung can provide a depot of viable infective larvae is highly relevant to the epidemiological importance of this reservoir. For both *H. contortus* and *T. circumcincta*, at a constant temperature around 20 °C, the estimated instantaneous daily death rates in dung suggest that negligible numbers of larvae will be present in dung after 3–4 weeks. On pasture, day-night fluctuations in temperature may increase, or decrease, these death rates.

Translation of larvae from soil onto herbage initially occurred quickly, with maximum larval recovery from grass recorded at 24 h, and thereafter the proportion of the larval population present on grass remained fairly constant. The soil (and, presumably, also the vegetation mat) therefore represents a second reservoir of larvae from which emergence onto herbage may occur over time. Crofton (1948), Silangwa and Todd (1964) and Callinan and Westcott (1986) all reported maximum larval recovery rates 24 h after larval release. Also, in agreement with non-adjusted recovery rates in the work presented here, the proportion of larvae recovered from grass was consistently estimated at up to 2–3% (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986; O'Connor *et al*. 2007, 2008; Silva *et al*. 2008). The present study is the only one estimating recovery method efficiency and, if the larvae recovery rates in Tables 2 and 3 are corrected accordingly (Table 1), it indicates that the recovered larvae may represent anything up to 20–30% of total available larvae. It appears that larvae distribute themselves over soil and herbage within 24 h. After this, continued emergence onto grass results in a similar proportion of larvae on herbage over time. The most likely explanation for this is continuing random movement.

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Table 4. Mean proportions of larvae recovered (range in parentheses), corrected for previously removed larvae and larval death

(The ANOVA test statistic indicates significance in difference between longitudinally measured proportions.)

Day	1	7	14	ANOVA test statistic
T. circumcincta	0·098 (0·056–0·122)	0·074 (0·024–0·136)	0·045 (0·015–0·068)	$F_{2,8}=1·808$, $P=0·243$
H. contortus	0·051 (0·020–0·091)	0·011 (0·00–0·020)	0·012 (0·00–0·018)	$F_{2,8}=3·132$, $P=0·117$
N. battus	0·072 (0·039–0·098)	0·023 (0·00–0·047)	0·070 (0·035–0·105)	$F_{2,8}=2·553$, $P=0·158$

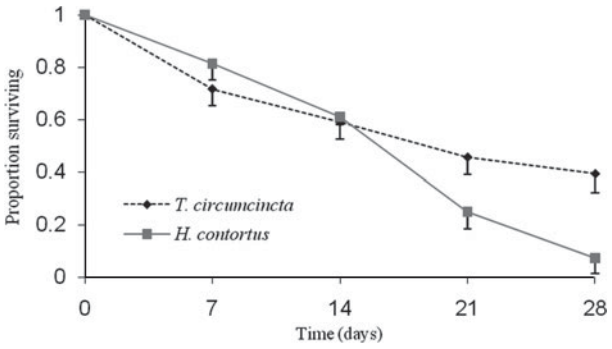

Fig. 3. The proportion of larvae recovered alive from desiccated soil at time (t) (lines drawn for clarity; error bars represent standard deviations).

Fenton and Rands (2004) developed a model framework describing trade-offs between resource depletion of the infective stages of parasites and host encounter rate, and showed that the optimal migration strategy strongly depends on the probability of host contact. They gave gastrointestinal nematodes as an example of parasites showing a switch in strategy: once on the grass blades, further 'cruising' (moving towards a host) would not be advantageous and thus a sit-and wait strategy would be adopted to preserve finite energy reserves.

Once out of dung, since their hosts are unlikely to graze close to faecal deposits unless forced to do so by grass shortage (Hutchings *et al*. 2006), the probability of ingestion of L3 is likely to increase with the horizontal distance travelled. However, further migration is traded off with decreased survival on herbage. Previous work on the gastrointestinal nematodes *H. contortus* (Skinner and Todd, 1980) and *Ostertagia ostertagi* (Williams and Bilkovich, 1973) showed that larvae were able to migrate as far as 90 cm in 24 h yet over 90% were found within 10 cm of the faeces. These findings seem to support a cruise-then-sit-and-wait strategy. The distance travelled may also correlate with the probability of rapid disintegration of dung, and rainfall as well as other abiotic and biotic disrupters of dung may therefore indirectly influence larval migration.

As larvae climb grass blades they are increasingly exposed to lowered relative humidity and other noxes, such as ultaviolet radiation (van Dijk *et al*. 2009). Thus, 'cruising' is not only beneficial but also directly harmful and this is likely to present a second trade-off similar to the first. The consistent finding of 90% of recovered larvae on the lower 2·5 cm of grass stems (Crofton, 1948; Rees, 1950; Silangwa and Todd, 1964; Callinan and Westcott, 1986), not an ideal position for ingestion, is likely to be the result of this trade-off. If larvae were to adopt a sit-and-wait strategy once on grass, then larval numbers on grass would increase over time until death rates matched the rate of migration. In laboratory conditions, with low mortality, this point would not be reached and larvae would accumulate on grass, with numbers increasing until the soil and dung reservoirs were exhausted. This did not occur in our study, suggesting that larvae remain in a cruising mode. This supports the hypothesis by Crofton (1948) of continuous, random movement of larvae within certain temperature and humidity thresholds. Grenfell *et al*. (1986), modelling migration and mortality of *O. ostertagi* and *Cooperia oncophora*, even showed that models in which the migration rate was held constant showed as good an agreement between observed and predicted larval counts as models in which the rate was a function of microclimate. Obviously, whether this truly reflects reality or not depends on the time-steps used in the model and, quite likely, the season of the year.

Crofton (1948), measuring RH, temperature and light intensities at different heights along herbage stems, gave a further insight into vertical movements of larvae. Microclimatic measurements were predictors of the height at which the larvae of *Trichostrongylus retortaeformis* were found on different types of herbage. He proposed that larvae moved at random as long as RH and temperature were favourable, while halting or re-directing their movement when travelling along a higher to lower RH gradient. Rees (1950), who measured *H. contortus* larval recovery from herbage, also found numbers of larvae, differing substantially even between 2-hourly measurements, to be a function of RH providing

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temperature was not limiting their movement. RH-driven migratory behaviour was also demonstrated for foliar nematodes (Jagdale and Grewal, 2006). Yamada and Oshima (2003), studying cues for the migration behaviour of *Caenorhabditis elegans*, described how this species actively avoids higher temperatures, moving along a higher to lower temperature gradient. It appears that the migration of trichostrongylid larvae is adequately modelled by either a continuous cruising strategy or a cruise, sit-and-wait, cruise strategy. Although the distance vertically climbed appears to be influenced by a trade-off between the probability of ingestion by a host and increased death rates higher on the stem, movement from soil onto herbage stems seems to occur at random. Thus, the top few centimetres of soil, and the mat of dead vegetation on it, remain reservoirs for larval emergence onto herbage and consistent percentages of the larval population will appear on herbage until all have died. Larval migration rates, estimated over the first 14 days were, again, shown to be consistent between species. Alongside previous studies, our results suggest that, even during dry summer weeks, living larvae may remain present in the soil for up to 1 (*H. contortus*) or 2 (*T. circumcincta*) months. On pasture, in Texas (USA), significant proportions of *H. contortus* larvae were shown to survive more than 3 weeks in soil (Amaradasa *et al*. 2010). A longer period of 3 months has been suggested for the Netherlands (Eysker *et al*. 2005). Longer soil survival at pasture may result from decreased larval activity during the night as the result of cyclic temperatures.

In theory, larval loss from herbage and the soil surface could be accelerated by heavy rainfall. Sturrock (1965) investigated the influence of heavy rains on the wash-out of *T. axei* larvae from the herbage and top layer of the soil, into deeper soil layers. It was found that, after heavy rains followed by artificial watering of herbage, approximately 85% of larvae were still recovered in the top 5 cm of the soil while only 0·8% reached a depth of 25–30 cm. Rose and Small (1985) confirmed the ineffectiveness of rainfall in washing *T. vitrinus* larvae into the soil. Therefore, losses of larvae as the result of heavy rainfall appear to be very limited, at least in temperate regions.

In conclusion, currently available methods for the recovery of larvae from herbage are sensitive enough to investigate and quantify migration behaviour. The findings were very consistent between the larval species, and also with the existing literature, indicating that results of migration studies are widely transferable between trichostrongyloid nematode species, at least between those infecting sheep. Since free water is not necessary for movement of larvae from the soil onto grass, the well-documented positive correlation between rainfall and the emergence of larvae on herbage is most likely the result of the release of larvae from a dung reservoir. The migration behaviour of trichostrongyloids appears to be the result of 2 trade-offs. Travel in a horizontal plane is determined by a trade-off between energy reserves and the probability of ingestion by a host. In a vertical plane, optimum travel has to balance exposure to damaging environmental influences and the probability of ingestion. As continuous larval movement (cruising) is spatially random, the proportion of a larval population present on herbage is fairly constant in time and declines with the rate of larval death in the soil reservoir.

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