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Environmental factors influencing the transmission of *Haemonchus contortus*

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ABSTRACT

Infection with the gastrointestinal nematode *Haemonchus contortus* causes considerable losses in the sheep industry. In this study, we evaluated the effect that climate has on third-stage larvae (L3) of *H. contortus* in terms of their migration from sheep feces to *Brachiaria decumbens* grass, as well as their distribution among the forage plants. Fecal samples containing *H. contortus* L3 was deposited on the soil among the herbage at an initial height of 30 cm. Sample collection began 24 h after contamination and was performed on alternate days over 13 days. The L3 were recovered and quantified in three strata (heights) of grass (0–10 cm, 10–20 cm and >20 cm) as well as in the remaining feces and a superficial layer of soil, collected from beneath the feces. In order to obtain results under different environmental conditions, fecal samples containing *H. contortus* L3 were deposited on pasture in January (summer), in April (autumn), and July (winter). In all of the periods, the L3 were able to migrate from the feces to the herbage. However, rains, accompanied by high relative humidity and high temperatures, apparently favored migration. The highest L3 recovery rate in the pasture was in the summer observation period, which had the highest number of days with measurable precipitation, high relative humidity (>68.2%), and the highest temperatures at the soil level (minimum and maximum means of 19 °C and 42 °C, respectively). Under those conditions, larvae began to reach the upper stratum of the grass (>20 cm) by 24 h after the deposition of fecal matter, the number of larvae having reached that stratum peaking at seven days after deposition. In the autumn observation period, there was no rainfall in the first five days post-contamination. During that period, high numbers of larvae were found in the fecal samples demonstrating that feces can act as a reservoir of larvae in the absence of rain. Except for two days in the summer observation period, when most of the L3 were recovered from the tops of blades of grass, L3 where located predominantly at the base of the herbage. In conclusion, rainfall favors the migration of L3 from feces to herbage. In addition, larval migration up and along blades of grass can occur relatively rapidly when the temperature is high.

1. Introduction

Infection with gastrointestinal nematodes causes considerable losses in the sheep industry. In small ruminants raised in tropical and subtropical regions, the main parasite species is the gastrointestinal nematode *Haemonchus contortus*. Anthelmintics have been used routinely in the prophylaxis of parasitic gastroenteritis. However, the frequent use of such drugs has resulted in the selection of parasites with multiple anthelmintic resistance, which jeopardizes the treatment and control of gastrointestinal nematode infections (Almeida et al., 2010).

There is a need to develop new approaches to control parasitic gastrointestinal infection, such as

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grazing management. Detailed knowledge regarding the biology of the free-living stages of nematodes is essential for understanding the epidemiology of parasitic gastroenteritis and can facilitate the development of husbandry strategies that seek to limit contact between the ruminants and their parasites in the infective stage.

Development from the egg stage through infective third-stage larvae (L3) comprises a series of individual pre-infective stages and processes. The migration of L3 from feces to the herbage is influenced by climatic conditions and the microclimate of the environment provided by the forage (reviewed by O'Connor et al., 2006). Parasites behave in a way to maximize infection success. In the case of *H. contortus*, the probability of being ingested by the host is increased if the L3 migrate from the feces, where the initial development took place to the vegetation for ruminant consumption. Fenton and Rands (2004) described a model that predicted three types of infection behavior for macroparasites: always actively seek a host (a pure cruising strategy); always passively await host contact (a pure ambushing strategy); and an initial cruising phase followed by ambushing (a mixed strategy). According to those authors, *H. contortus* is an example of a parasite that adopts the mixed strategy. In this case, in order to maximize their chances of host contact, infective stages must exit the dung and position themselves where they are more likely to be ingested by a host (e.g., at the tips of vegetation). Therefore, an initial cruising phase greatly increases their chances of encountering a host. However, once at the tip of a blade of grass or a leaf, any subsequent activity is unlikely to further increase their chance of contacting a host, at which point they adopt an ambush-only strategy, awaiting host contact on the vegetation, also allowing the L3 to save energy.

In the environment, the initial cruising appears to be greatly influenced by climatic conditions. For fecal matter containing L3 during a dry season, greater numbers of L3 tend to remain in the dung and those able to migrate to the vegetation are most often found at the base of the herbage. Conversely, during times of high humidity and high temperatures, greater numbers of L3 tend to be found higher up on vegetation (Silva et al., 2008).

This study was motivated by the need for more detailed information about the influence of climate on the dynamics of *H. contortus* L3 migration from sheep dung to the herbage. In previous studies carried out in the state of São Paulo, the collection of fecal samples from pasture was initiated at seven days after contamination (Oliveira et al., 2008; Carneiro and Amarante, 2008; Silva et al., 2008). In all of those experiments, L3 numbers were markedly lower than were those recorded in control samples maintained in the laboratory. In the present study, we intended to determine when this loss begins to occur and whether climatic variables, such as temperature, rainfall and luminosity, affect the numbers or behavior of *H. contortus* L3.

2. Materials and methods

2.1. Study site and climatic measurements

The experiments were carried out in a paddock at São Paulo State University, in the city of Botucatu, Brazil. The daily rainfall was measured at the study site, using a rain gauge (Multitec, São Leopoldo, Brazil) as was the maximum and minimum daily temperatures at the soil level, with a thermometer (J Prolab, Curitiba, Brazil). The relative humidity and solar radiation data were obtained from the Meteorological Station of the São Paulo State University School of Agricultural Sciences, Department of Environmental Science, located 8 km from the experimental site.

2.2. Collection and maintenance of H. contortus L3

Details about the isolation of *H. contortus* were previously described by Silva et al. (2008). In the present study, the *H. contortus* isolate was maintained in lamb donors until July 2008. Subsequently, feces were collected and cultured to recover L3 that were used to infect lambs for a test of anthelmintic efficacy, which demonstrated resistance of the *H. contortus* isolate to levamisole, albendazole, ivermectin, moxidectin, closantel and trichlorfon (Almeida et al., 2010). Feces collected from a lamb treated with moxidectin (0.2 mg/kg BW; Cydectin®, Fort Dodge) in October 2008, were cultured to recover L3 which were stored and used to infect two worm-free male lambs, on a single occasion in December 2008. These monospecifically infected animals were kept housed and provided fecal samples for this study.

2.3. Experimental procedures

In order to obtain results under different environmental conditions, fecal samples containing *H. contortus* L3 was deposited on pasture in January (summer), in April (autumn), and July (winter) of 2009. In each of those observation periods, fecal samples were deposited at 70 points, approximately 1.5 m apart.

The experimental area (504 m^2) was located inside a 0.6-ha paddock cultivated with *Brachiaria decumbens* grass, which was maintained ungrazed during the study period. For each observation period, 70 sites in the pasture were chosen and marked with stakes. The criterion for site selection was a minimum herbage height of 30 cm. In order to standardize the height of the pasture, the upper part of the forage was cut down to 30 cm just before the deposition of fecal matter containing the L3. This was done by hand, with scissors, in order to prevent the cuttings from falling onto the soil.

The sheep having the highest fecal egg count was chosen as the donor animal, and a collection bag was attached to its hindquarters. The collection bag was changed twice daily and after each bag was removed it was dated and stored in a refrigerator at 7 °C. Feces were collected in this manner for 5 days and then 10 fecal samples were taken at random to estimate the number of eggs per gram of feces (EPG) by a modified McMaster technique (Ueno and Gonçalves, 1998).

For each deposition, 80 fecal samples were prepared, each with a total of 30,000 eggs. The amount of feces used in each observation period varied depending on the EPG value presented by the donor animal. The methodology for producing the L3 and processing of samples was that devised by Silva et al. (2008). In brief, feces were gently pooled and the samples, previously weighed, placed with intact fecal pellets in 80 Petri dishes that were kept in an incubator (at 25 °C) where they remained for 7 days to allow development to L3. Of the 80 fecal samples, 70 were deposited on the soil, among the herbage, while the 10 remaining samples served as control cultures and were immediately processed in the laboratory according to the technique of Baermann (Ueno and Gonçalves, 1998), which allowed recovery and enumeration of L3 in each sample.

In the field, all L3-containing fecal samples were deposited in the morning (between 8:00 and 9:00 am). The day of deposition was considered day 0. The measurements were taken on alternate days, beginning on day 1 (24 h after deposition). Therefore, samples were collected, between 8:00 and 10:00 am, on days 1, 3, 5, 7, 9, 11, and 13, resulting in seven days of sampling. For each day, there were 10 replicates selected randomly among the 70 samples. To delineate the collection area and herbage height, a 10-cm diameter metal ring was attached to a rod graduated according to stratified grass heights (> 20, 10–20, and 0–10 cm). The ring was placed over the fecal matter deposit, and blades of grass that were inside the circle were cut, stratum by stratum. Each collected stratum was stored separately in a pre-labeled plastic bag for subsequent processing in the laboratory. The remaining feces were collected, taking care to recover all fecal pellets. The superficial layer of soil, to a depth of approximately 1 cm, from beneath the dung was also collected. The samples were packed separately in pre-labeled plastic bags for subsequent processing in the laboratory. For L3 recovery, material was processed in accordance with the method described by Silva et al. (2008).

2.4. Statistical analysis

Data were analyzed by general linear model with the program Statistical Analysis System, version 9.2. (SAS Institute, Inc., Cary, NC, USA). The model included three grass height strata (0–10, 10–20, and >20 cm) and seven collection time points (days), with evaluation of the interaction between stratum and collection day. Means were compared by Tukey's test at a 5% significance level. Results were analyzed following logarithmic transformation ($\log(x+1)$) and are presented as arithmetic means (±standard error).

3. Results

3.1. Climatic conditions and L3 recovery

The *H. contortus* L3 containing fecal pellets deposited on the soil among herbage remained exposed to climatic variations for 13 days. As expected, the maximum and minimum daily temperatures measured at ground level were different for each time of the year (Tables 1–3). The heaviest rainfall occurred in January/February (rain on 7 of the 13 days, a total of 96.0 mm) followed by April/May (rain on 3 of the 13 days, a total of 70.5 mm) and July/August (rain on 3 of the 13 days, a total of 28.0 mm). The mean relative humidity was also higher in January/February than in April/May and July/August (80.9% vs. 71.9% and 73.0%, respectively). As a consequence of the heavy precipitation and high temperatures in January/February, there was progressive degradation of the fecal pellets, which did not occur at the other times of the year (Table 4). Herbage presented external moisture (dew or rainwater) at the time of sample collection on all days, except on four days in July/August (Tables 1–3).

At all times of the year, the percentage recovery of L3 from the field samples (of soil, feces, and total herbage) was considerably lower than that achieved from the control fecal samples (Table 4).

3.2. January/February 2009

In the January/February (summer) observation period, larvae were recovered from fecal samples in high numbers only on the first sampling day (i.e., 24 h) after deposition (Fig. 1A). Thereafter, rainfall apparently favored the degradation of fecal pellets and larval dispersal into the environment, resulting in greater recovery of L3 from the total herbage samples than during either of the other two observation periods. On the first day of collection, the lowest grass height stratum (0–10 cm) showed the highest mean number of L3 (16.1 ± 3.7). Over the subsequent sampling days, the mean number of L3 recovered from the uppermost stratum (>20 cm) gradually increased, peaking on day 7 (at 34.3 ± 10.7), whereas that recovered from the lowest stratum gradually decreased (Fig. 2A). However, on day 13, the number of L3 recovered was similar among the three strata (Fig. 2A).

During this observation period, there was significant interaction between collection day and grass height stratum ($p < 0.05$). However, there were no statistical differences among the different grass height strata, in terms of the mean number of L3 recovered.

3.3. April/May 2009

In the April/May (autumn) observation period, the recovery of L3 from fecal samples was higher than in any other observation period: 1.32% of the total L3 deposited (Table 4). In contrast, the recovery of L3 from total herbage samples was low during the first sampling days. On post-deposition day 7, there was measurable rainfall, which coincided with a sharp reduction in the number of L3 recovered from fecal samples and a small increase in the number of L3 in the total herbage samples (Fig. 1B). In the herbage samples, L3 recovery was always greater in the lowest grass height stratum (0–10 cm), the values obtained on days 7 and 9 (7.1 ± 1.4 and 7.7 ± 1.3, respectively) being significantly higher than those obtained for the 10–20 cm and >20 cm strata ($p < 0.05$; Fig. 2B).

Table 1
Maximum and minimum temperatures (measured at the soil level), daily rainfall, relative humidity (RH), and solar radiation (SR), together with the sample conditions, during the summer observation period (January/February 2009).

Date	Temperature (°C)		Rainfall (mm)	RH[a] (%)	SR[a] (cal/cm^2)	Condition of samples		
	Maximum	Minimum				Herbage	Feces	Soil
(0) January 28[b]	32	18	35	93.8	146	–	–	–
(1) January 29[c]	25	22	3	89.8	215	Humid	Humid/intact	Humid
(2) January 30	38	19	20	89.9	273	–	–	–
(3) January 31[c]	34	20	0	78.5	420	Humid	Humid/intact	Humid
(4) February 1	42	23	0	68.2	511	–	–	–
(5) February 2[c]	41	19	4	78.9	249	Humid	Humid/degraded	Humid
(6) February 3	38	20	0.5	80.4	409	–	–	–
(7) February 4[c]	40	21	26.5	88.1	252	Humid	Humid/degraded	Humid
(8) February 5	34	19	0	74.7	481	–	–	–
(9) February 6[c]	40	19	0	70.4	533	Humid	Humid/degraded	Humid
(10) February 7	39	20	7	74.2	0	–	–	–
(11) February 8[c]	42	21	0	78.9	434	Humid	Humid/degraded	Humid
(12) February 9	39	20	0	84.3	271	–	–	–
(13) February 10[c]	38	20	0	82.3	308	Humid	Humid/degraded	Humid

[a] RH and SR recorded at the Meteorological Station located 8 km from the study site.
[b] Day on which fecal samples were deposited on the soil.
[c] Sample collection day.

3.4. July/August 2009

In the July/August (winter) observation period, it rained immediately after the deposition of fecal matter, which possibly favored the presence of larvae in the total herbage samples on the first day of evaluation (Fig. 1C). Similar to what occurred in the April/May observation period, L3 recovery was greatest in the lowest grass height stratum (0–10 cm) on six of the seven sampling days (Fig. 2C). Although in small numbers, the L3 were found in the uppermost grass height stratum (> 20 cm) on the first sampling day (24 h after the deposition of fecal samples). The overall mean number of L3 recovered from the 0–10 cm stratum was significantly higher than was that of L3 recovered from the 10–20 cm and >20 cm strata.

4. Discussion

Two aspects of *H. contortus* L3 ecology were evaluated in the present study: survival and migration. Regarding survival, there was no question that the recovery of larvae from the fecal pellets, vegetation, and soil surface was poor. Even at 24 h after the deposition of fecal pellets on pasture, the number recovered was only a small fraction of the total number present in the control samples. Similar results have been reported in other field studies (Levine et al., 1974; Carneiro and Amarante, 2008; Silva et al., 2008; Amaradasa et al., 2010). Although the fate of the larvae is unknown, it is thought that the great majority die within a day or two after presentation to the environment. Among the many likely causes of L3 death is exposure to the elements

Table 2
Maximum and minimum temperatures (measured at the soil level), daily rainfall, relative humidity (RH), and solar radiation (SR), together with the sample conditions, during the autumn observation period (April/May 2009).

Date	Temperature (°C)		Rainfall (mm)	RH[a] (%)	SR[a] (cal/cm^2)	Condition of samples		
	Maximum	Minimum				Herbage	Feces	Soil
(0) April 27[b]	29	17	0	74.8	373	–	–	–
(1) April 28[c]	29	15	0	71.0	430	Humid	Humid/intact	Dry
(2) April 29	25	12	0	66.8	409	–	–	–
(3) April 30[c]	19	11	0	59.6	418	Humid	Humid/intact	Dry
(4) May 01	30	12	0	71.7	423	–	–	–
(5) May 02[c]	30	15	0	74.6	410	Humid	Humid/intact	Dry
(6) May 03	30	16	46	76.0	372	–	–	–
(7) May 04[c]	27	16	22	85.8	171	Humid	Humid/intact	Humid
(8) May 05	19	11	0	70.9	385	–	–	–
(9) May 06[c]	29	10	0	63.7	368	Humid	Humid/intact	Humid
(10) May 07	35	15	0	68.7	338	–	–	–
(11) May 08[c]	30	15	0	73.5	327	Humid	Humid/intact	Humid
(12) May 09	30	18	2.5	70.3	314	–	–	–
(13) May 10[c]	29	15	0	79.6	301	Humid	Humid/intact	Humid

[a] RH and SR recorded at the Meteorological Station located 8 km from the study site.
[b] Day on which fecal samples were deposited on the soil.
[c] Sample collection day.

Table 3
Maximum and minimum temperatures (measured at the soil level), daily rainfall, relative humidity (RH), and solar radiation (SR), together with the sample conditions, during the winter observation period (July/August 2009).

Date	Temperature (°C)		Rainfall (mm)	RH[a] (%)	SR[a]	Condition of samples		
	Maximum	Minimum				Herbage	Feces	Soil
(0) July 28[b]	21	14	17.5	90.5	205	–	–	–
(1) July 29[c]	29	16	8.5	83.6	206	Humid	Humid/intact	Humid
(2) July 30	28	15	0	83.7	229	–	–	–
(3) July 31[c]	22	12	0	89.8	305	Humid	Humid/intact	Humid
(4) August 01	25	13	0	83.5	287	–	–	–
(5) August 02[c]	25	15	0	81.1	226	Dry	Dry/intact	Humid
(6) August 03	30	14	2	64.8	258	–	–	–
(7) August 04[c]	27	12	0	77.7	325	Dry	Humid/intact	Humid
(8) August 05	28	13	0	83.1	291	–	–	–
(9) August 06[c]	29	15	0	75.9	376	Humid	Humid/intact	Humid
(10) August 07	28	14	0	49.4	406	–	–	–
(11) August 08[c]	31	15	0	67.5	387	Dry	Humid/intact	Humid
(12) August 09	33	12	0	48.8	407	–	–	–
(13) August 10[c]	33	11	0	42.3	349	Dry	Dry/intact	Humid

[a] RH and SR recorded at the Meteorological Station located 8 km from the study site.
[b] Day on which fecal samples were deposited on the soil.
[c] Sample collection day.

(weather). To evaluate this factor, we measured a number of climatic variables. The temperature at the soil level in the shade of herbage >20 cm in height varied widely. For instance, in the January/February (summer) observation period, the maximum and minimum temperatures were 42 °C and 19 °C, respectively. Additional mortality caused by ultraviolet light exposure could also help to explain the high larval mortality rates on pasture (van Dijk et al., 2009). Although we did not measure the variation in relative humidity throughout the day at the ground level, it is likely that there was also considerable fluctuation between moist and dry conditions on the surface of the herbage. It has been reported that, in the laboratory and in the field, infective larvae are able to survive several cycles of desiccation/rehydration in a process called anhydrobiosis, in which the metabolic activity is decreased and the survival of the larvae is prolonged (Lettini and Sukhedeo, 2006). However, according to Siamba et al. (2011), repeated cycles of desiccation-revival cycles, due to diurnal variations in pasture humidity, can reduce the viability of *H. contortus* larvae, which suffer continuous depletion of irreplaceable lipid reserves. Such a reduction in viability might have occurred in the present study.

The degradation of fecal pellets occurring in the summer observation period was attributed to high temperatures and high humidity. The activities of microorganisms might also have had some influence on fecal degradation and in L3 mortality. For example, nematophagous fungi can trap and kill the L3 of *H. contortus* (Campos et al., 2008).

Despite the low survival of the free-living stages, the number of *H. contortus* larvae produced and remaining on pasture is sufficient to cause severe infections in susceptible sheep. This is possible due to massive egg production. Assuming that, in sheep, fecal output corresponds to approximately 5% of body weight (Amarante et al., 2007), an ewe weighing 50 kg and shedding 1,000 EPG can eliminate approximately 2.5 million eggs per day. If only 0.01% of those eggs give rise to L3 that are able to survive and migrate to pasture, there will be 25,000 L3 reaching the pasture every day.

There is evidence that *H. contortus* L3 reach vegetation merely by chance. According to Sciacca et al. (2002), the behavior of *H. contortus* L3 suggests random crawling, with frequent reversals of direction, which is different from that of *Ancylostoma caninum* and *Strongyloides stercoralis*, two skin-penetrating species, whose L3 exhibit negative geotaxis, crawling against the pull of gravity, presumably in attempts to contact a passing host.

In all of the experimental periods analyzed in the present study, L3 were able to migrate from the fecal pellets

Table 4
Numbers of *Haemonchus contortus* third-stage larvae (L3) recovered from field samples (of soil, feces, and herbage), as well as from control samples, by observation period (time of year).

Observation period[a]	Number of *H. contortus* L3 larvae (mean ± standard error)				
	Control	Soil[b]	Feces[b]	Herbage[b]	Not recovered[b]
Summer	18,610 ± 141	2.24 ± 0.30 (0.01%)	9.74 ± 3.55 (0.05%)	8.23 ± 0.91 (0.04%)	18,589.79 (99.90%)
Autumn	12,160 ± 540	5.96 ± 1.77 (0.05%)	160.70 ± 26.33 (1.32%)	2.30 ± 0.28 (0.02%)	11,991.04 (98.61%)
Winter	11,102 ± 677	1.66 ± 0.29 (0.02%)	20.27 ± 2.73 (0.18%)	3.72 ± 0.36 (0.03%)	11,076.35 (99.77%)

[a] Data represent the overall mean values of 13-day observation period.
[b] Percentages in parentheses represent the proportions of larvae recovered (or not recovered) from the field samples in relation to the number of larvae recovered from the control samples maintained in the laboratory.

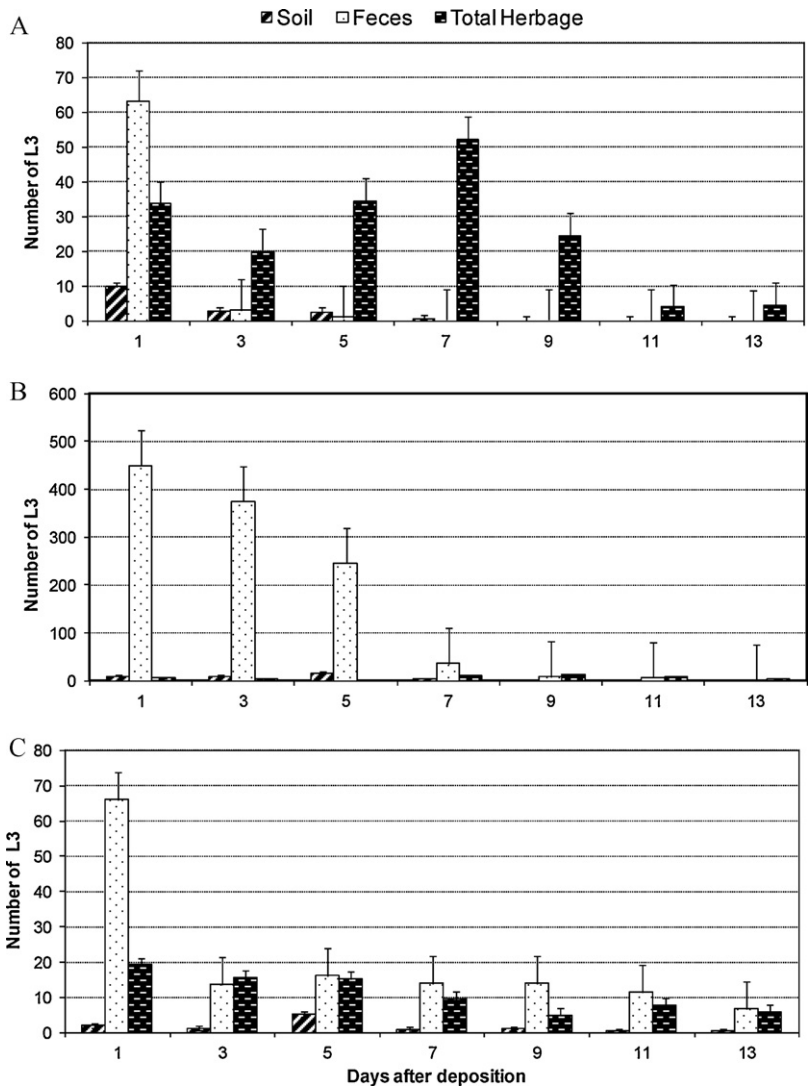

Fig. 1. Average numbers of *Haemonchus contortus* third-stage larvae (L3) recovered from field samples (of soil, feces, and herbage) during each of the three 13-day observation periods: January/February (A); April/May (B); and July/August (C). The mean number of L3 in fecal samples deposited on pasture in those three observation periods was 18,610, 12,160, and 11,102, respectively. Bars represent standard error of the mean.

to the herbage. However, rainfall and high relative humidity accompanied by high temperatures apparently favored migration, with L3 recovery from pasture being greatest in the summer observation period, during which there was measurable rainfall on 7 of the 13 days, high mean relative humidity (>68.2%), and the highest temperatures at the soil level (minimum and maximum means of 19 °C and 42 °C, respectively).

In the April/May (autumn) observation period, there was no rainfall in the first five days after deposition of the fecal matter. The high numbers of larvae found in the fecal samples collected during this observation period suggest that the dung acted as a reservoir of larvae in the absence of rain, as previously described (Silva et al., 2008; Carneiro and Amarante, 2008; van Dijk et al., 2009). In experiments carried out in a laboratory at room temperature (20–24 °C) and relative humidity between 71% and 83%, van Dijk and Morgan (2011) noted that free water appeared to be essential for larval release from dung but was not essential to ascend herbage. Therefore, rain is necessary for making fecal pellets humid and soft, which allows larvae trapped inside those pellets to exit and migrate to herbage that is in close contact with the dung. Therefore, a film of moisture, through which larvae would "swim", appears to be necessary for their migration along the blades of grass. However, even in the absence of rain in the initial 5 days, some larvae were able to migrate to the herbage during the autumn observation period. It is likely that there was a quantity of dew water sufficient to allow this migration.

Field studies have been shown that migration of larvae to pasture increases when rainfall occurs, and the increase appears to be proportional to the intensity and frequency of rainfall (Catto, 1982; Silva et al., 2008). Similar results were reported by Agyei (1997) who observed, in pastures

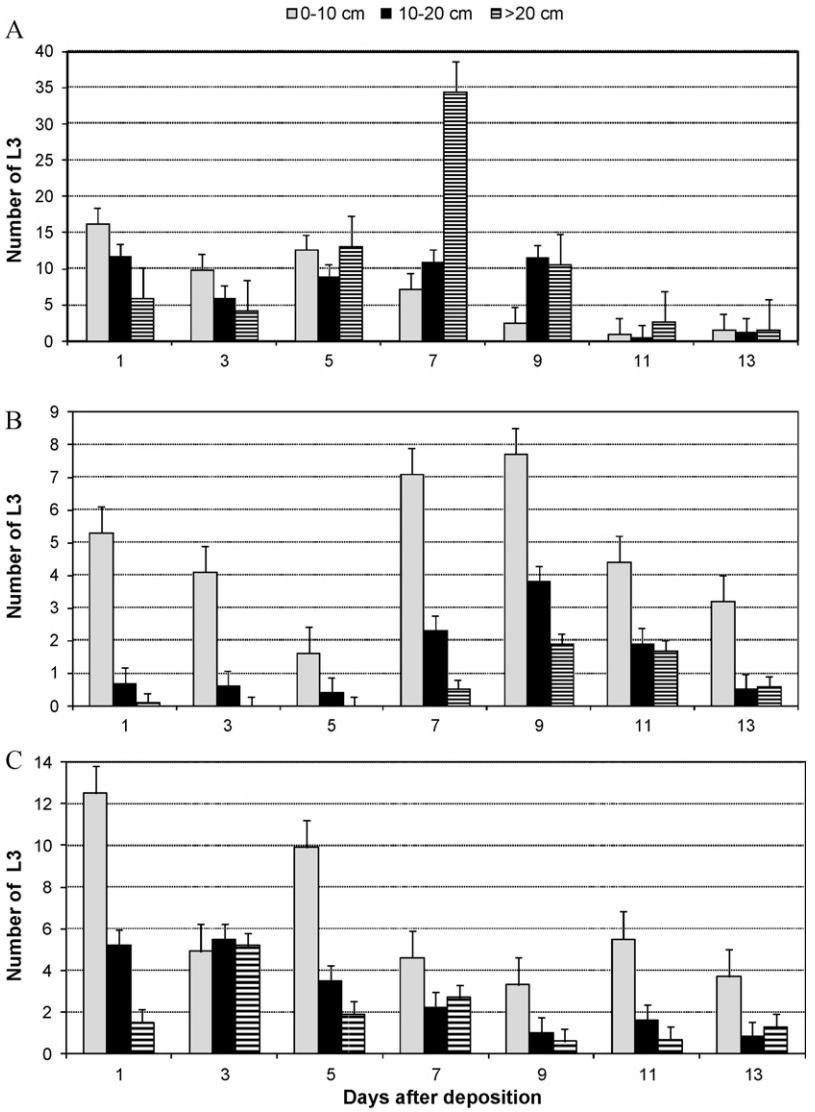

Fig. 2. Average numbers of *Haemonchus contortus* third-stage larvae (L3) recovered from herbage samples, by grass strata (0–10 cm, 10–20 cm, and >20 cm) during each of the three 13-day observation periods: January/February (A); April/May (B); and July/August (C). The mean number of L3 in fecal samples deposited on pasture in those three observation periods was 18,610, 12,160, and 11,102, respectively. Bars represent standard error of the mean.

in Ghana, increased numbers of L3 during the rainy season and decreased numbers or absence of L3 during the dry season. In a study conducted in the state of Texas, Amaradasa et al. (2010) identified a strong positive correlation between rainfall and total average daily *H. contortus* larval counts on pasture. These results also show that rain facilitates the liberation of larvae from sheep feces. However, when heavy rains occur in a short period of time, there is a reduction in larval recovery, as occurred after two consecutive days of rain (total of 68 mm on days 6 and 7) in the autumn observation period of our study. It is likely that the heavy rains washed larvae away from the fecal matter deposition site, resulting in low larval recovery from pasture.

On two days during the summer observation period of our study, L3 recovery was greatest for the uppermost grass height stratum. On all other sampling days in all three observation periods, larvae were predominantly found at the base of the herbage. These results are in agreement with those of other studies (Oliveira et al., 2008; Amaradasa et al., 2010). Silva et al. (2008) also found the largest number of larvae at the base of the herbage, except in the evaluations carried out in summer, when most of the larvae were found at the tops. Coincidently, in the present study, considering the means for each observation period, we found that overall L3 recovery was greatest in the summer observation period. During that period, the larvae began to reach the uppermost grass height stratum soon (within 24 h) after deposition of the fecal matter, L3 recovery peaking on day 7. The high temperatures and abundant humidity during that period probably favored the migration of L3 to all grass height strata.

In the present study, larvae were recovered on all sampling days during each of the 13-day observation periods. These were relatively short observation periods. However, in experiments of longer duration, it has been demonstrated that *H. contortus* larvae can survive on pasture for several weeks, especially when the temperature is mild, around 17 °C (Carneiro and Amarante, 2008). The survival of L3 for extended periods was also observed in a study conducted in Cameroon, where larvae of *H. contortus* migrated to the herbage and survived for approximately 11 weeks during the rainy season (Ndamukong and Ngone, 1996).

In conclusion, rainfall favors the migration of L3 from dung to herbage. In addition, high temperatures can increase the speed of larval migration up and along blades of grass.

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