Effect of climatic influences on the migrations of infective larvae of Cyathostominae

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ABSTRACT: Migration to herbage of Cyathostominae from experimentally deposited fresh or incubated faecal samples containing a known number of cyathostome L3 was studied in the Czech Republic for up to 1 year. It was found out that most larvae remained quite close to the faecal samples. Of all larvae recovered from herbage 89.18% were collected within 10 cm of the faeces. Temporal variation in the presence of Cyathostominae larvae on vegetation may account for poor recovery of Cyathostominae L3 in the field. A few infective larvae (0.05–2.74% of the larvae placed on the plot) were found as far as 30 cm from the faeces after 1 or 2 weeks. The number of larvae was significantly higher in June, with maximum recoveries of 4.97% ($P < 0.05$). Time of day was also significantly related to the number of L3 recovered, larval recovery was greater in the morning than at noontime, the highest number of L3 was observed at 8 am. Moisture and temperature were the most important weather factors associated with lateral larval migrations. There was a closer relation between the larval yields and monthly rainfall ($r = 0.47$) than between the larval recoveries and weekly rainfall ($r = 0.23$, $r = 0.24$). A significant amount of migration occurred during dew. An insignificant amount of migration occurred during dry weather.

Keywords: Cyathostominae; horse; migration; infective larvae

Transmission of cyathostomine nematodes between horses is via the ingestion of infective larvae (L3) that migrate to the grass. The migrations of infective larvae of horse strongyles have been studied by various workers, principally in relation to their behaviour in faeces or in different types of soil (Bruns, 1937; Lucker and Bureau, 1938; Mfitilodze and Hutchinson, 1988). Numerous data have been published since then on the relation between temperature, moisture and biological variables such as the time of development of eggs into infective larvae, the development rate and survival of the eggs and of the infective larvae (Ogbourne, 1972; Polley, 1986; Mfitilodze and Hutchinson, 1988; Baudena *et al.*, 2000). The factors affecting the extent and nature of the migrations of certain infective larvae on the grass have also received attention. Knowledge of the behaviour of larvae both in soil and on grass is necessary if control methods either by chemicals or by controlled pasturing are to be effective. However, only few of these studies generally establish the effects of climatic conditions prevailing during larval migration.

Previous studies examined the migration and survival on herbage of mixed strongyle infective larvae from experimentally deposited horse faeces in the subtropical or tropical climates (English, 1979a,b; Hutchinson *et al.*, 1989). The purpose of the present experiments was to investigate the migration of Cyathostominae out of deposited horse faeces and the effects of day, season and weather conditions on the migration of these nematodes.

MATERIAL AND METHODS

The experimental plots were located on a pasture at the Czech University of Agriculture in Prague farm. Horses had never grazed on the plot. The pasture consisted mainly of bluegrass (*Poa pratensis*) but other species of grasses and forbs were also present (*Poa trivialis*, *Elytrigia repens*, *Dactylis glomerata*, *Deschampsia cespitosa*).

Experiment 1

Faecal samples were obtained from naturally infected horses maintained at the University. Three

500 g aliquots of fresh faeces were deposited on plots at monthly intervals from June to October. After 4 weeks, herbage samples were collected from surrounding pasture and after that at monthly intervals until November. Herbage sampling was conducted between 6 and 7 a.m. Herbage from each plot was cut with scissors to ground level from the edge of the deposited faeces up to a distance of 150 cm from the faeces. Herbage samples were collected in 15 fractions, at 0 to 10 cm, 10 to 20 cm, 20 to 30 cm etc. The cut grass was placed in separate, identified plastic bags. The grass samples were weighed to determine the wet weight and the larvae were recovered using a modified Baermann technique.

Experiment 2

Faeces obtained from naturally infected horses were homogenized and cultured at 23°C for 10 days. Six 250 g samples of faeces were used for each monthly experiment. Three control samples were examined by the Baermann technique to determine the number of larvae present.

On one day per month, from January to December, six 250 g samples of horse faeces with known infective larval count, were placed on a plot with 2 metres distance between each faecal sample.

Every month, on day 7 and 15 after the deposition of the faecal samples, the grass was sampled by cutting from the area around the faeces. During the first sampling period the grass was cut, at a distance of 5 to 30 cm from the faeces at two opposite sectors and during the second sampling from the two remaining sectors. The grass was cut with scissors 1 cm above the ground and subsequently placed in a plastic bag. The temperature of the grass was recorded at all times as well as air temperature, dew, rain and weather conditions. This procedure was repeated at 6.00, 7.00, 8.00, 9.00, 10.00, 11.00 and 12.00 a.m., in order to find out the numbers of larvae at and after dew time, respectively. All meteorological details were carefully noted.

The method used to recover larvae was the modified Baermann technique. The results from the migration plots were computed in the form of percentages (larvae per sample/larvae in the faeces).

The weather variables measured each day and at each of the seven sampling times were air temperatures, temperatures at soil surface and the presence of rainfall, snowfall, dew or hoarfrost. Rainfall measurements (total rainfall) for the study period were obtained from a weather station located at 1 km from the pasture study site.

Statistical analysis

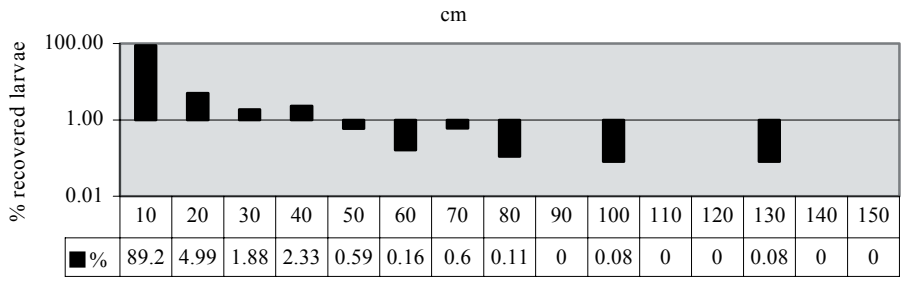
The Kruskal-Wallis test was applied to evaluate the differences in pooled data for pasture larval recovery with respect to number of larvae in the deposited faecal samples. An analysis between larval counts and temperatures, rainfall and dew was carried out using Spearman correlation of SAS Institute Inc. (2000). Levels were considered significant when $P < 0.05$.

RESULTS

The larval counts from all individual plots are shown in Figures 1–4. There were no significant differences between larval yields of collection after 1, 2, 3 or 4 months (experiment 1; $\chi^2 = 5.48$, df = 3, $Pr > \chi^2 = 0.139$) as well as after 1 or 2 weeks (experiment 2; $\chi^2 = 0.81$, df = 1, $Pr > \chi^2 = 0.369$). However, a statistically significant higher larval yield was found after heavy rain in one sample collected 4 months after deposition in April 14. It was found that most larvae remained quite close to the faecal samples. Of all larvae recovered from herbage 89.18% were collected within 10 cm of the faeces (experiment 1).

Temporal variation in the presence of Cyathostominae larvae on vegetation may account for poor recovery of Cyathostominae L3 in the field. In experiment 2, a few infective larvae (0.05–4.97% of the larvae placed on the plot) were found as far as 30 cm from the faeces after 1 or 2 weeks. The number of larvae recovered varied during year. The number of larvae was significantly higher in June, with maximum recoveries of 4.97% ($P < 0.05$). There was no migration of larvae from faecal samples deposited on pasture in February and December. Very small numbers of larvae migrated onto pasture from faecal samples deposited in January, October and in November. The highest yield of larvae was obtained from collection at 8 am, followed by collection at 7 and 6 a.m. The lowest numbers of infective larvae were recovered at 12 a.m. ($P < 0.05$).

Migration of infective larvae occurred in the presence of dew (87.89% recovered larvae), although



cm	10	20	30	40	50	60	70	80	90	100	110	120	130	140	150
%	89.2	4.99	1.88	2.33	0.59	0.16	0.6	0.11	0	0.08	0	0	0.08	0	0

Figure 1. Procentual recovery of L3 larvae (log) from grass in function of the distance from deposited faeces (experiment 1)

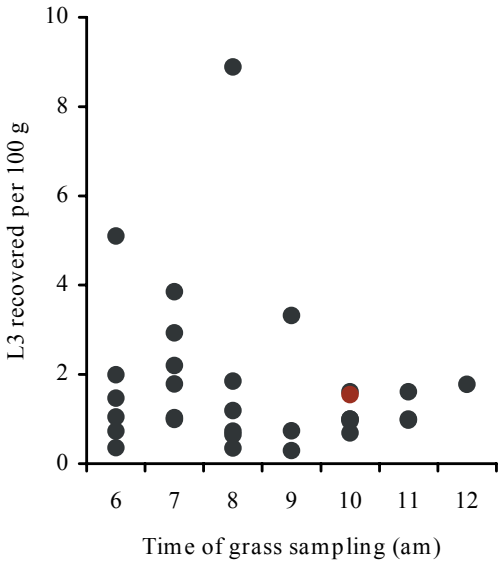


Figure 2. The effect of sampling time on the recovery of Cyathostominae L3 from grass in the field (mean numbers of larvae recovered from grass plots – experiment 2)

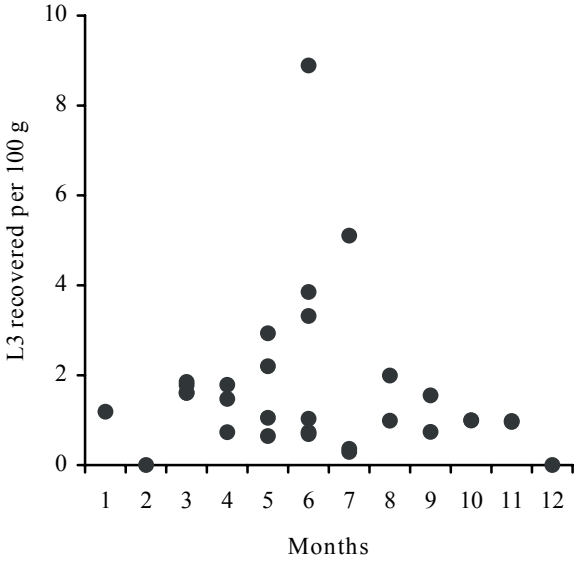


Figure 3. The effect of season on the recovery of Cyathostominae L3 from grass in the field (mean numbers of larvae recovered from grass plots – experiment 2)

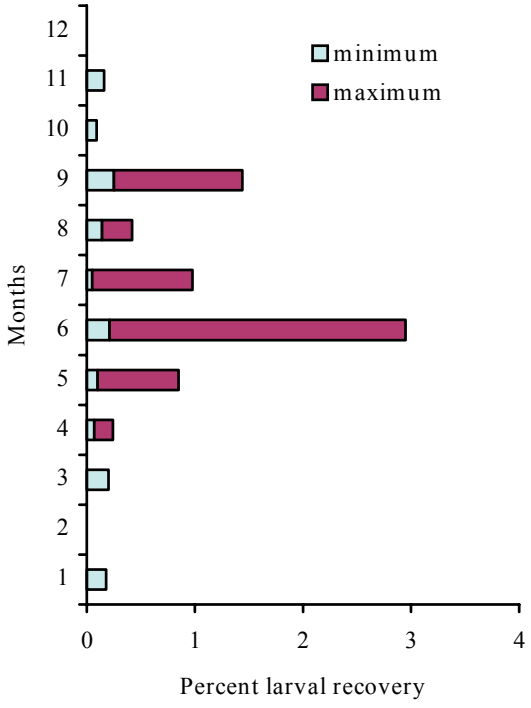


Figure 4. Minimum and maximum of recovered L3 as percent of L3 present in deposited faeces

Table 1. Spearman's coefficients of correlation between the indices of larval counts and the indices of temperatures, rainfall and dew

Experiment 1	1 month before collection	
Total rainfall	0.47	
Experiment 2	Collection after	
	1 week	2 weeks
Air temperature	0.16	0.17
Grass temperature	0.20	0.19
Total rainfall 1 week before collection	0.23	0.24
Dew presence	0.30	0.35

larvae were recovered from herbage in the absence of dew. There was a moderate correlation between dew presence and number of L3 per 100 g of herbage during the study period. A significant difference was found between larval yields of experiment 1 and experiment 2. The highest number of L3 was found on grass around faeces of experiment 1.

The values in Table 1 indicate that larval yields and rainfall had the greatest, and temperature had a less significant effect on the numbers of L3 of Cyathostominae. According to the results of Spearman test the closest correlation was found between larval counts and grass temperatures, r = 0.20 or r = 0.19 ($P < 0.05$). A weaker correlation was found between L3/100g and air temperatures (r = 0.16 and r = 0.17).

Meteorological data recorded throughout the experimental period are shown in Figure 5.

DISCUSSION

The purpose of the present experiments was to investigate the migration of infective larvae of equine Cyathostominae from the faeces to the surrounding herbage. An understanding of the relations between the environment and the parasite is necessary for control, environment determines the number of parasites available to a host. By using results from various studies, predictions can be made about the number of larvae that may be available to the host on a given instance. The effect of environment on the ecology of free-living stages of equine strongyles is not well understood, particularly on pasture in Central Europe and as a result the level of infectivity for horses is also hard to determine. From the data presented in this study, it can be seen that only 0.05–2.74% of the total number of larvae deposited on the plots were found on the herbage 1 cm above

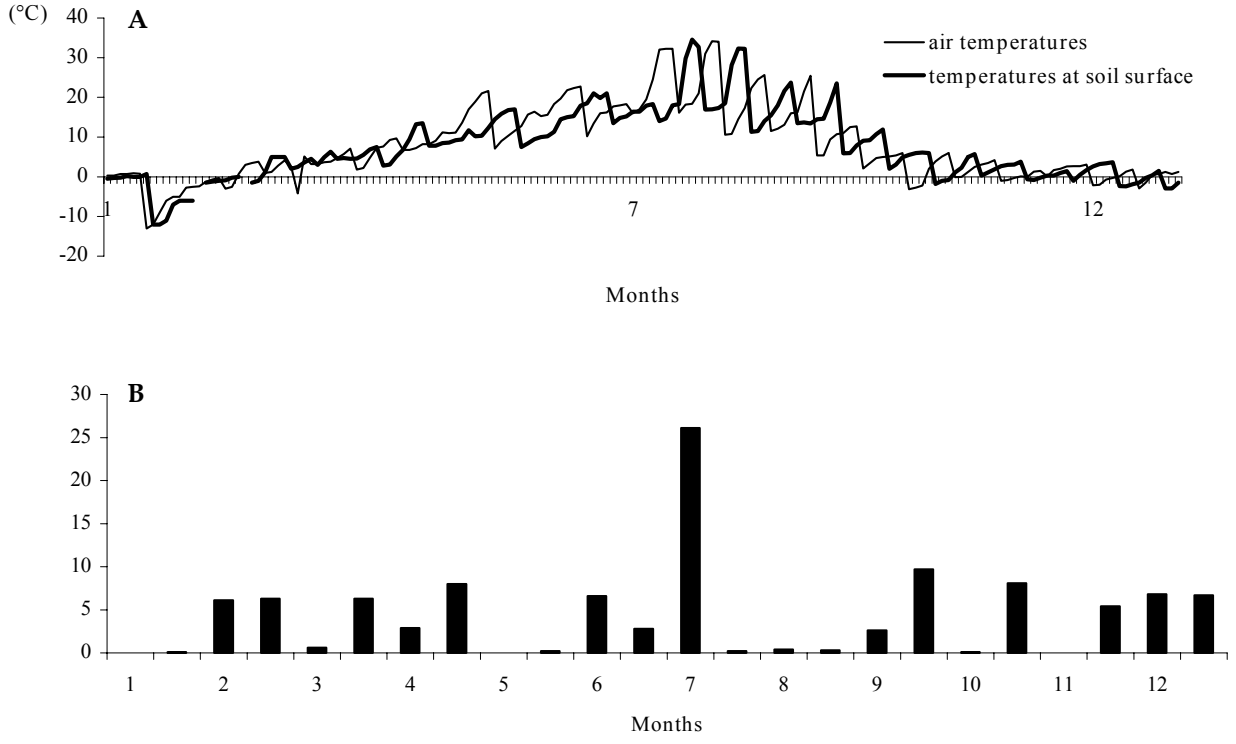


Figure 5. Meteorological data for the period of the study. A – Air temperatures and temperatures at soil surface; B – Total rainfall 1 week before collection (mm per week per square meter)

the top of the soil. It is clear that the active migration of Cyathostominae does not endanger the grazing horses to a great extend. On the other hand, our study also indicates the long-term importance of the faeces as a reservoir for infective larvae. There were no significant differences between larval yields recovered around the same deposited faeces after 1 or 2 weeks as well as after 1, 2, 3 or 4 months. Because of the essentially random movement of infective larvae in moisture films, it is certain that this faeces reservoir constantly contributes to the number of larvae available on pasture during the normal grazing season when adequate rainfall occurs.

The horizontal distribution patterns found in the first experiment confirm that most larvae remain at the base of the faeces, 89.18% larvae were found within 10 cm. This finding is in close agreement with that of English (1979a) who reported that most larvae were found within 15 cm.

In order for infective larvae to migrate, a moist liquid medium is needed. Only a damp herbage on a pasture represents a source of infection for horses. Saueressing (1980) demonstrated in North Queensland that 0.175 mm of dew was capable of inducing translocation of trichostrongylid larvae from cattle pats. The results from the present experiments show that 87.89% of the larvae were found on grass covered with dew.

The number of L3 collected from surrounding pasture in dry condition was small (10.82% of the larvae) in comparison with the number of L3 collected in dew. In the series of experiments of Crofton (1954) on the effects of drying on the movement of larvae, it was shown that on a smooth a number of larvae retreated with the reduction in extent of the water film. In the field, with irregular surface of grass blades, the number of larvae able to retreat would be limited but in some cases this may occur. Larvae are not able to break through the surface tension film, but those which are left in an isolated film are not able to move out of it, and the film can evaporate, leaving the larvae.

Rainfall is limiting for the larval spread of horse strongyles on to pastures. English (1979a,b), Ogbourne (1972, 1973), Hutchinson *et al.* (1989) found infective larvae on herbage as a result of progressive migration of larvae from faecal reservoirs in response to rain. Laboratory experiments of Gronvold and Hogh-Schmidt (1989) documented that more than 90% of the translocated larvae of *Ostertagia ostertagi* were transported passively by splash droplets and only a minor part of the larvae migrated actively in water films or were transported passively by water run-off to the soil surrounding the pats. The experiments conducted here suggest that the infective larvae respond to rain fall by spreading on (or moving up) vegetation, since higher numbers of nematodes were recovered after rainfall. There was a closer relation between the larval yields and monthly rainfall ($r = 0.47$) than between the larval recoveries and weekly rainfall ($r = 0.23$, $r = 0.24$). The importance of rain for Cyathostominae larval migration was clearly demonstrated by the significantly higher larval yield found after heavy rain. However, Hutchinson *et al.* (1989) found no significant correlations between numbers of larvae recovered from herbage in the dry tropical region of North Queensland and the amount of rain, although there was a good correspondence between rainfall and larval translation. The authors considered that this may be because of the amount of rainfall is important only in as much as it provides the minimum amount of moisture required for larval translation.

Many authors reported that moisture and temperature were important predictors in the model for strongyles. In the tropics and sub-tropics larval translation is influenced exclusively by moisture (English, 1979a; Hutchinson *et al.*, 1989). In temperate areas, larval translation is also regulated by available moisture but survival on pasture is predominantly influenced by temperature (Ogbourne, 1973; Craig *et al.*, 1983; Eysker *et al.*, 1986). However, the effect of temperature on the migration of third stages of equine strongyles is not well understood. Only the series of experiments of Buckley (1940) show that below 10°C the equine strongyle larvae do not migrate much in a low temperature and their movements are greatly slowed down. At 13–14°C the larvae showed a positive geotropism, at 19 to 24°C the larvae migrated markedly both upward and downward. In the case of trichostrongylids, Saunders *et al.* (2000) found that temperature did not have a significant influence on *Haemonchus contortus* larval availability but greater numbers of *Trichostrongylus tenuis* L3 were recovered at low temperature (10°C) than at higher temperature (20°C). However, Krecek *et al.* (1995) demonstrated that air temperature had the greatest effect on the migration of *H. contortus*, while soil moisture and relative humidity had the least on the numbers of L3 of these nematodes.

In accordance with many research data (English, 1979a,b; Hutchinson *et al.*, 1989; Baudena *et al.*, 2000)

larval translation occurred mainly during the hot wet season. Infective larvae were recovered in greater numbers during May, June and July, when peak numbers of larvae were recovered from herbage samples collected 7 days after faecal June deposition, at 8 am. The present study has clearly demonstrated that herbage contamination is not strictly a seasonal event, some larvae were recovered from herbage in winter time (January).

Time of day was also significantly associated with the number of L3 recovered. Larval recovery was greater in the morning than at noontime, the greatest number of L3 was observed at 7 a.m. These results absolutely bear on the appearance of dew as in order for infective larvae to migrate, a moist liquid medium is needed.

Most authors of epidemiological studies about equine strongyles demonstrated that animals became infected with infective larvae on pasture (e.g. Hasslinger, 1981; Craig and Suderman, 1985; Herd, 1995; Herd and Coles, 1995; Abbott, 1998; Klei and French, 1998). A pasture may have a high level of contamination, but a low level of infectivity. Under suitable climatic conditions for development and migration of infective larvae (e.g. a wet summer) a pasture can rapidly change from a one of a high contamination to a one of a high infectivity and endanger the grazing horses. Only a damp herbage on a pasture represents a source of infection for horses. While the number of larvae on pasture is of great epidemiological importance, equally important are the larval yields in litter of the box stalls. Significant differences ($P < 0.05$) were observed in the larval yield of the two types of technique for collecting herbage samples. Herbage in experiment 1 was cut with scissors to ground level, the grass samples of experiment 2 were clipped 1 cm above the top of soil, because horses do not bite the grass to ground level of soil and the aim of the experiment was to demonstrate the migration of L3 with most danger to horses. It is evident that there exist differences between contamination of pastures and infectivity for horses.

This study loosely followed the epidemiological study (Langrová, 1999). According to the results of Spearman's correlation analysis the closest link was found between EPG by horses and the indices of the risk of infecting a horse in a box stall (L3/kg litter and the time spent on infected litter times the level of infection of that particular litter). A weaker link was found between EPG by horses and the indices of the infectiousness of the pastures ($P = 0.001$). The issue (or the question) can be raised whether epidemiological importance of pastures is not overestimated and whether the importance of (zoo-) hygiene of litter at box stalls is not underestimated.

This study on the active migration of the third stages of nematode parasites of horses on pastures provides new information with important epidemiological consequences in the hope of obtaining results of practical value or of filling up certain gaps in our knowledge of them.

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