

MIGRATION OF INFECTIVE LARVAE OF SHEEP GASTROINTESTINAL NEMATODES

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Abstract

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The migration of infective larvae of nematodes of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Chabertia* on herbage in the soil was studied under laboratory conditions on three artificially seeded grass plots with different methods of irrigation.

The capacity for vertical migration was found to be the highest, i. e. up to 27 cm, in infective larvae of *Trichostrongylus* spp. Most of the infective larvae of this nematode were found in the herbage 6-15 cm above the soil surface. The rate of vertical migration is proportional to the moisture of the herbage. During 3 hours of watering the herbage, infective larvae were found 12-15 cm above the soil surface, whereas within 4 hours of desiccation of the herbage the larvae were concentrated maximally up to 4 cm from the soil surface.

Infective larvae of *H. contortus*, *Trichostrongylus* spp. and *Chabertia ovina* migrated horizontally to a maximal distance of 21 cm from the middle of the faeces sample established on grass irrigated twice a day.

Infective larvae of the above nematode species occurred only sporadically in soil in the depth of 2 cm; only in one case were infective larvae of *Ostertagia* spp. found in a depth of 7 cm.

Infective larvae, herbage, soil, rate of migration

From the epizootiological point of view, the infective larvae occurring in the herbage of pastures are one of the most important sources of distribution of helminth invasions.

Several authors have studied the annual occurrence of infective larvae in the external environment, especially on herbage. B ü r g e r et al. (1983) reported that larvae began to migrate from the faeces to the herbage at the time when the average daily air temperature had exceeded 18 °C for more than a week and after at least 4 days of rainfall when average precipitation was minimally 20 mm. Callinan and Westcott (1986) stated that the occurrence of larvae on herbage was significantly dependent on the temperature and humidity of the environment.

In the herbage of the studied pastures (in November) in South Australia, Overend et al. (1984) found as much as 4800 infective larvae per 1 kg of dry grass. Prosl (1986) studied the quantity of larvae on vegetation during the day and found that the counts of infective *O. ostertagi* larvae were maximum at 6.00 a. m. (i. e. 2470/kg of fresh grass) and minimum in the course of the day (i. e. 390/kg of fresh grass).

In general, in experiments conducted directly on pastureland, infective larvae migrate horizontally to a distance of 10-12 cm, and vertically 17 cm. Fincher and Stewart (1979) reported that *O. ostertagi* larvae in the field migrated vertically at least 12.5 cm, and in glass tubes in the laboratory 15 cm (from the soil). On the contrary, Callinan and Westcott (1986) found 71% of the larvae recovered from the vegetation concentrated within 0-2 cm of the herbage. Prosl (1986) stated that larvae migrated vertically on the herbage only a few cm and that during day they descended downwards to the lower grass layer above the soil. Delgado (1983) recovered infective *Trichostrongylus* and *Haemonchus* larvae on grass 50-130 cm above the ground. Rose (1961) found the infective larvae to migrate more vertically than horizontally. He found the counts of larvae to be the highest in tufts of grass near the faeces. Goldberg (1970) collected 80% of *O. ostertagi*, *C. punctata* and *O. radiatum* larvae on grass

within 13 cm of the faeces. Prosl (1986) found infective *Ostertagia* and *Cooperia* larvae on herbage as much as 40–50 cm from the faeces, the majority of larvae being collected within 10 cm of the faeces.

In addition to the migration of larvae in herbage, many authors also investigated the migration of larvae into the soil in connection with their survival during the cold periods and their higher occurrence on herbage during spring and in the dry period. Callonan and Westcott (1986) recovered 8 times more larvae in the soil than on the herbage down to a depth of 4–6 cm. Al Saquar et al. (1982) collected soil samples from October till September and found that infective *Ostertagia* spp. larvae were present in each sample to a depth of at least 15 cm. Analysis of the vertical distribution suggested a possible migration into the soil during winter followed by an upward trend during spring and summer. Gruner et al. (1982) observed that towards the end of winter, during two weeks when soil temperature was lower than 6 °C, the larvae migrated to a depth of 40 cm, and more. This migration through the soil was supported by the content of soil water, but the larvae were capable of active migration upwards even under relatively dry conditions. Rose and Smill (1985) stated that the ability of larvae to migrate into the soil was not even affected by the period of heavy rainfall. The results of studies of these and other authors have proved that infective larvae of many nematodes migrate horizontally over the herbage much more quickly than in the opposite direction – into the soil. These authors concluded that the soil does not serve as a reservoir where large numbers of infective larvae might survive during the winter.

The mechanism of larvae migration in the soil has not yet been fully explained. Based on his experiment with *O. ostertagi* larvae, Gronvold (1979) suggested that in this respect the earthworms might play a certain role; after 50 days he isolated living larvae from their digestive tract and faeces.

The aim of the present study was to find the ability and rate of migration of infective larvae of the most frequently occurring sheep gastrointestinal nematodes on artificially established grassland, and to discuss the presence of infective larvae in soil as a possible reservoir of infective larvae under unfavourable conditions.

Materials and Methods

Various ways of migration on infective nematode larvae of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Chabertia* were studied. Positive faeces with eggs of the above helminths were collected from spontaneously infected sheep and placed on the experimental plots (see further) under laboratory conditions.

(A) Characteristics of the experimental plots

The vertical and horizontal migration of infective larvae was investigated in March and April in a greenhouse on three artificially seeded experimental plots (35 × 56 cm). The composition of the herbage was as follows: *Festuca pratensis*, *Trifolium repens*, *Lolium perenne* and *Dactylis glomerata*.

(a) Experimental plot No. 1

Positive faeces were placed on the grass surface into the centre of the plot in the form of a cake 21 cm in diameter. Before placing the faeces, one half of the plot of grassland was kept at a height of 2 cm frequent clipping. The herbage on the other half of the plot was 50 cm high before the beginning of the experiment. The herbage was watered twice a day.

(b) Experimental plot No. 2

The herbage and establishment of faeces were the same as on plot No. 1. Only once a day, the bottom 20 cm of soil were irrigated by immersing into a large pot with water for 15 minutes.

(c) Experimental plot No. 3

On the third experimental plot the faeces were evenly distributed over the whole surface. The herbage was not cut and was irrigated twice a day as was experimental plot No. 1; at the time of collection of larvae it reached a height of 50 cm.

(B) Methods of examinations

Within 35 days of establishment of the faeces samples with the eggs of the above nematode species, the herbage and soil of the experimental plots were divided into squares 7×7 cm. The high herbage was gradually cut into 3 cm parts, from the soil surface up to the top. Soil samples were taken to a depth of 2 and 7 cm. In pilot field samplings, the soil was taken on the experimental plots in the Ecological Station Klec in April and November to a depth of 20 cm from 5×5 cm squares.

All samples were examined for infective nematode larvae of the genera *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Chabertia* using the modified method according to Baermann.

(C) Determination of the rate of migration of the infective larvae

The rate of migration of the infective larvae in the herbage was ascertained in three 13×13 cm containers into which the grass mats were set, and the faeces with the infective larvae were over the whole surface. The herbage was continuously watered.

Samples of the herbage were taken gradually from these 8 plots at 15 and 30 min intervals, then at 1 to 6-hour intervals. The grass was cut in 2 cm parts and samples were examined using the modified method according to Baermann. The ability of reverse migration was investigated after desiccation using warm air (45°C).

Results

I. Vertical and horizontal migration

The average air temperature and relative humidity during the whole experimental period from the establishment of the experiment till its termination was 21.3°C (from 12°C to 28°C) and 74% (from 52% to 85%), respectively.

(1) Migration of infective larvae on the herbage

The results are given in Fig. 1 and 2. Fig. 1 shows that on the regularly watered plot the infective larvae migrated horizontally only in the middle part of the experimental plot, maximally 21 cm from the middle of the faeces sample (part A).

At this distance, infective larvae of *Trichostrongylus* spp., *Chabertia ovina* and *Haemonchus contortus* were recovered. Within 14 cm of the centre of the plot, infective larvae of *Trichostrongylus* spp. and *Ch. ovina* were collected.

As concerns vertical migration of larvae, the figures in the middle of the squares of Fig. 1 give the maximal height (in cm) from the soil surface where infective larvae were recovered on the herbage. The present investigations proved that infective larvae of *Trichostrongylus* spp. had the highest ability of migration, i.e. up to a height of 27 cm. Infective *H. contortus*, *Ostertagia* spp. and *Ch. ovina* larvae were found up to a height of 6 cm, 15 cm and 9 cm, respectively, from the faeces. In cut herbage (part B), infective larvae of *Trichostrongylus* spp., *Ostertagia* spp. and *H. contortus* were recovered.

Fig. 2 shows the migration of infective larvae on herbage on an unwatered plot with bottom watering. No horizontal migration of infective larvae was observed on this experimental plot and vertical migration occurred only on the high herbage in three squares above the faeces. Examinations of grass samples from these squares showed only the occurrence of infective larvae of *Trichostrongylus* spp. up to a height of 18 cm.

On the third experimental plot (watered, with regularly distributed faeces), the maximal ability for vertical migration of infective larvae was examined. Infective larvae of *Trichostrongylus* spp. were found to migrate most frequently up to a maximal height of 27 cm, the majority being collected 6–15 cm above the soil surface. Infective larvae of *Ostertagia* spp. were recovered from grass samples of 3–9 cm in height. Infective larvae of *H. contortus* were found in two samples only, i.e. 27–30 cm and 15–18 cm above the soil.

(2) Migration of infective larvae in the soil

The results are given in Fig. 3 (plot No.1) and Fig. 4 (plot No.2). Infective larvae of *Trichostrongylus* spp., *Ostertagia* spp. and *H. contortus* were recovered from the soil to a depth of 2 cm. Only infective *Ostertagia* spp. larvae were found in one square in the depth of

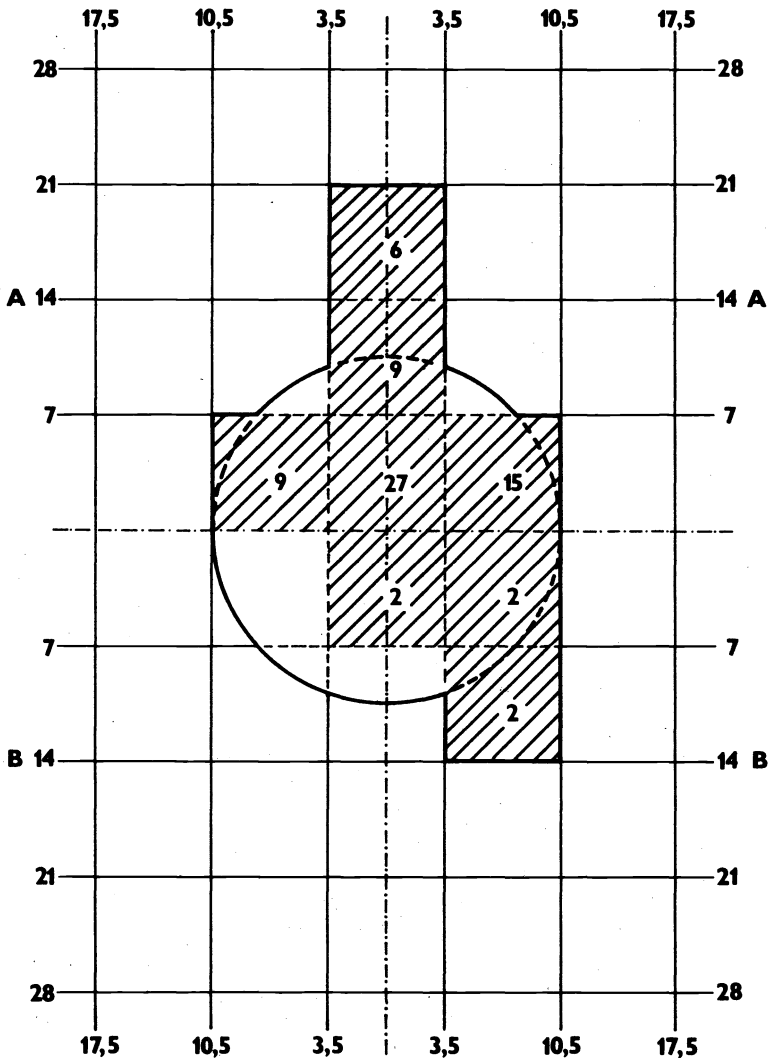


Fig. 1 Results of investigations of infective larvae migration in herbage on experimental plot No. 1

2–7 cm. In pilot field samplings in April, no infective larvae were found in a depth of 20 cm, in November we collected infective *Trichostrongylus* spp., *Ostertagia* spp. and *H. contortus* larvae in a depth of 5–10 cm.

II. Rate of migration

No infective larvae were found on herbage within 15, 30 and 60 minutes at 19°C and relative air humidity 40%. After 2 hours, infective larvae of *Haemonchus contortus*, *Trichostrongylus* spp., *Ostertagia* spp. and *Chabertia ovina* were recovered on the herbage up to 8 cm above the soil; after 3 hours, larvae were found 12–15 cm above the soil.

At 22 °C and 45–50% relative air humidity, infective larvae were found on herbage 2 cm, 6 cm, and 12–15 cm above the soil within 30 minutes, 1 hour, 2 hours and 3 hours, respectively. The infective larvae of all the species, i. e. *Ostertagia* spp., *Trichostrongylus* spp., *Haemonchus contortus* and *Chabertia ovina*, were found to migrate.

Reverse migration was also investigated; the herbage with the infective larvae was dessicated with warm air (the temperature above the herbage was 25 °C and relative air humidity 30%) and within 1 hour, 2 hours and 4 hours of dessication infective larvae were found in a height of 10 cm, 6 cm and 4 cm, respectively.

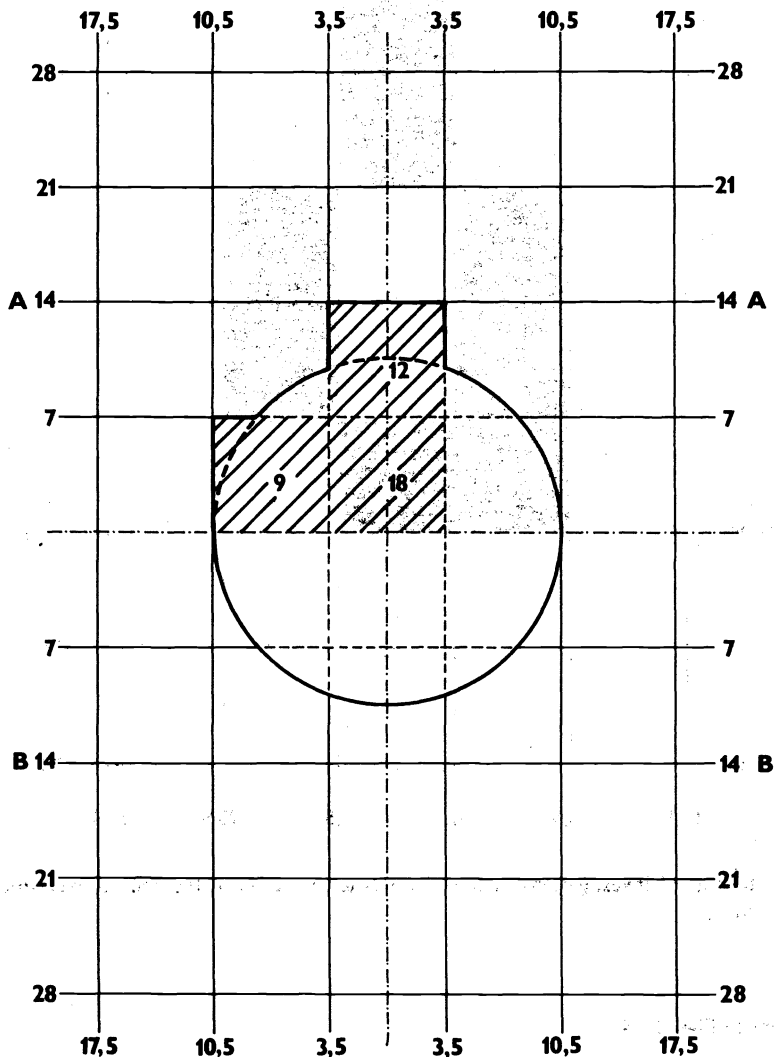


Fig. 2 Results of investigations of infective larvae migration in herbage on experimental plot No. 2

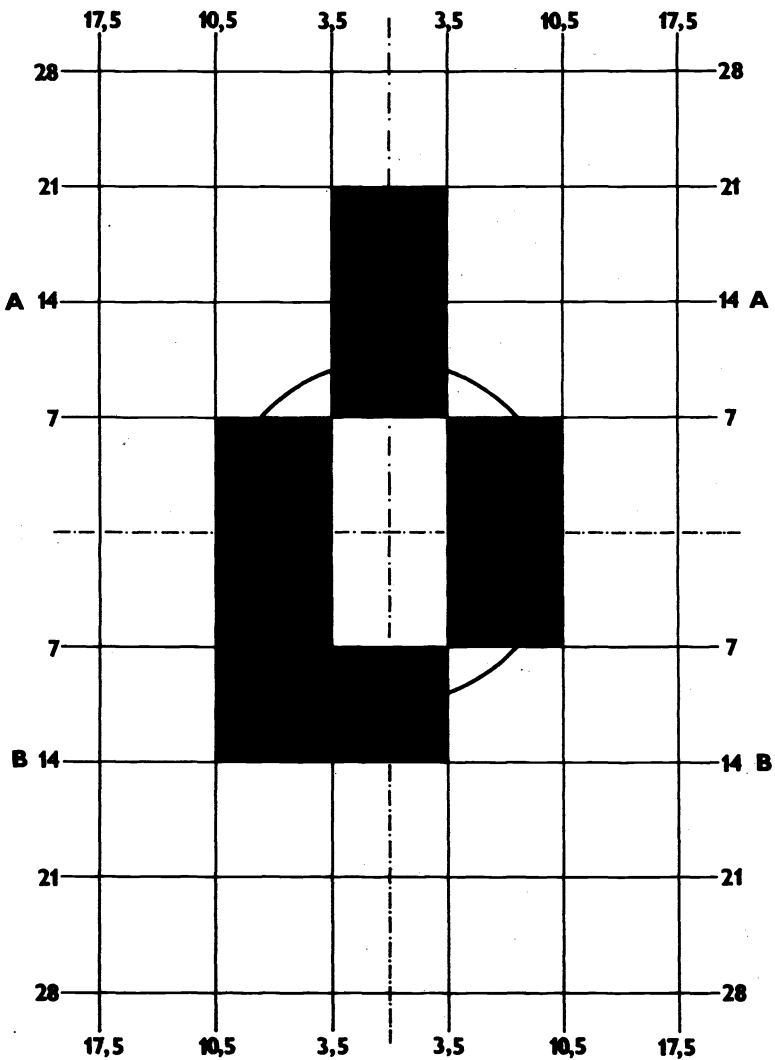


Fig. 3 Results of investigations of infective larvae migration in the soil on experimental plot No. 1

Explanations to Figs. 1-4

Horizontal axis: distance of herbage and soil samplings (in cm) from the centre of the experimental plots

Vertical axis: distance of herbage and soil samplings (in cm) from the centre of the experimental plots

A - the uncut herbage

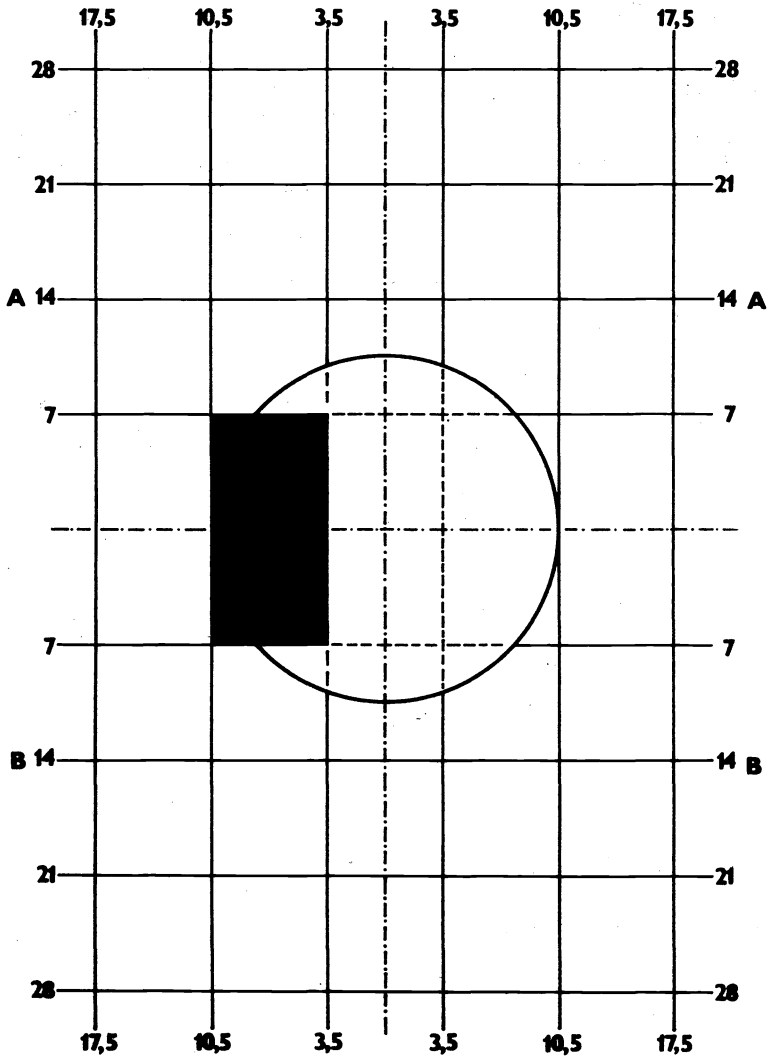


Fig. 4 Results of investigations of infective larvae migration in the soil on experimental plot No. 2

B — herbage regularly cut and kept at a maximum height of 2 cm from the soil surface
 the circle in the middle of the figure indicates the area of established faeces sample
 the crosshatched squares indicate the occurrence of infective larvae in the parts of the grassland
 figures in the middle of the crosshatched sections give the maximum height of migration
 of the infective larvae (in cm)
 black squares indicate the migration of infective larvae in the soil

Discussion

From our observations it is apparent that infective larvae of the genera *Haemonchus*, *Trichostrongylus*, *Ostertagia* and *Chabertia* migrated vertically, a lesser proportion horizontally. Infective larvae were collected on herbage only above the faeces or in the close vicinity. Rose (1961) reported that infective larvae migrated more vertically than horizontally and that the largest counts of larvae were recovered from grass tufts near the faeces in the lower layer of the herbage. In their studies, Goldberg (1970) and Prosl (1986) described horizontal migration.

In our experiment, the infective larvae of *Trichostrongylus* spp., at most, migrated vertically, up to a height of 27 cm; the majority of *Haemonchus*, *Trichostrongylus*, *Ostertagia* and *Chabertia* larvae found at a height of 6–15 cm above the soil with faeces. The results of Fincher and Stewart (1979) and Delgado (1983) were similar. On the contrary, Callinan and Westcott (1986) and Prosl (1986) collected infective larvae in the lower layers of the herbage.

If the infective larvae are to use their ability for migration, they require humidity which is maintained for a longer time in the dense and high herbage. This fact agrees with the present results when infective larvae on the experimental plot regularly watered, migrated up to a height of 27–30 cm, while they remained in the faeces on the plot with bottom watering.

From the present results it further follows that the migration of infective larvae into the soil is probably not markedly affected either by the permeability or moisture of the soil. Infective larvae of *H. contortus*, *Ostertagia* spp. and *Trichostrongylus* spp. were recovered from the soil only sporadically, and only to a depth of 2 cm; only in one case *Ostertagia* spp. larvae were recovered in a depth of 7 cm. It is true that in orientation field samplings carried out in November, infective larvae were found to reach a depth of 10 cm, but this does not conclusively prove the ability of infective larvae to migrate into the soil, because we cannot exclude contamination during sampling or if the larvae were carried down by percolating water during rainfall. The present results agree with results of Borgsted and Boogard (1983), Rose and Small (1985) who recovered very small amounts of infective larvae in the soil. On the other hand, Al Sagur et al. (1982), Gruner et al. (1982) and Callinan and Westcott (1986) reported that infective larvae migrated deeply into the soil. The mechanism of larvae migration in the soil has not yet been fully explained; as mentioned above, some authors attach importance to concrete local conditions, others suggest the role of earthworms in the transport of infective larvae in the different layers and depths of the soil.

Observations on the rate of migration have revealed that, under sufficient air humidity and adequate temperature, the infective *Haemonchus*, *Ostertagia*, *Trichostrongylus* and *Chabertia* larvae are capable of reaching a height of 15 cm on the herbage within 3 hours. During desiccation, the infective larvae migrate downwards again and remain at a height of up to 4 cm where humidity is sufficient. The onset of migration of the larvae in the herbage is dependent on the temperature and humidity of the environment that is in accordance with the observations of Bürger et al. (1983) and Callinan and Westcott (1986).

The ability of infective larvae to migrate quickly in the herbage, including their ability of surviving the relatively very unfavourable environmental conditions, is a very serious epizootiological problem. Kotrlá and Koždoň (1978) observed that not even recurrent winter temperatures connected with hoarfrost decontaminated the pastures. According to these authors, 53% of the herbage samples on the experimental plot were positive from June till June of the following year. Based on observations of Overend et al. (1984) and Prosl (1986), the pastures are infested with considerable numbers of infective larvae during the whole grazing season. In their studies, Cabaret et al. (1982), Mitchell and Fitzsimons (1983) and Mitchell et al. (1984) concentrated their attention to testing various types of pastures. They found that systems of so-called „clean pastures“ (i.e. pastures which had not been grazed for 12 months, or pastures ploughed and sown in the autumn and not grazed until the following autumn) did partly reduce the hazard of infesting the grazing animals, especially the young ones, but after a certain period these pastures were infested.

From the present results and from data of other authors quoted in this study, it is apparent that also at the present time the pasture herbage is the main source of distribution of helminthiasis; pastures remain to be a very serious epizootiological problem in spite of the fact that the present methods of treating pastures have qualitatively improved as compared with the past.

Миграция инвазивных ларв глист желудочно-кишечного тракта овец

Способность миграции инвазивных ларв глист родов *Haemonchus*, *Ostertagia*, *Trichostrongylus* и *Chabertia* в травяном покрове и в почву изучали в лабораторных условиях на трех искусственно залуженных площадях с разным режимом орошения.

Было установлено, что наибольшая способность вертикальной миграции развита у инвазивных личинок *Trichostrongylus* spp., продвигающихся на высоту 27 см. Преобладающее большинство инвазивных личинок выщепленных нематод находили в травяном покрове высотой 6—15 см от поверхности почвы.

Скорость вертикальной миграции инвазивных личинок пропорциональна влажности травяного покрова. В ходе трехчасового орошения травяного покрова инвазивные личинки были обнаружены на высоте 12—15 см, между тем как после четырехчасового высушивания покрова личинки были сконцентрированы на высоте не более 4 см от поверхности почвы.

Инвазивные личинки *H. contortus*, *Trichostrongylus* spp. и *Chabertia ovina* мигрировали горизонтально на максимальное расстояние 21 см от центра образца кала в условиях, когда травяной покров орошали два раза в сутки.

Инвазивные личинки приведенных видов нематод лишь в единичных случаях находили в почве на глубине 2 см, только в одном случае (инвазивные личинки *Ostertagia* spp.) — на глубине 7 см.

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