Field study on the survival, migration and overwintering of infective larvae of horse strongyles on pasture in central Ukraine

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Abstract

Experimental studies on the survival of infective stage larvae of horse strongyles and their ability to overwinter on pasture were carried out in central Ukraine (Poltavska oblast). Faecal pats (1.5 kg each) of naturally infected horses were placed on pasture, and samples of faeces and surrounding vegetation (10 g each) were collected each month, excluding the winter months, from November 2002 until April 2004. The number of infective third stage larvae was calculated in each sample and compared with that from the control faecal samples cultivated in the laboratory. In the control samples, the ratio of infective third stage larvae to the initial number of eggs was from 54.7% in June up to 84.2% in November. This ratio depended on the presence of nematophagous fungi growing in the faeces. On pasture, the development of larvae to the infective third stage took approximately 4 weeks in the warm season, from April until September. In October, a percentage of the eggs (25% to EPG value) did not hatch. No larval development was observed in faeces in November. A minute quantity of larvae, about 0.03% of their initial number, was observed to survive on pasture for the 12 months. Migration of infective larvae from the faeces to vegetation was not intensive, between 71% and 89% of larvae remained in the faeces 4 weeks after deposition of the faecal pats, the percentage related to soil humidity in each month. The proportion of larvae successfully surviving during winter appeared to be maximal in faecal pats deposited on pasture in September of the previous year (up to 42.0% of the initial number of larvae). Some larvae were observed surviving winter in soil beneath the faecal pats. The results of the study demonstrated that horse pastures in the central part of Ukraine are never free from the infective third stage larvae of strongyles.

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Keywords: Strongylidae; Horse parasites; Larval development; Ukraine

1. Introduction

Nematodes from the family Strongylidae Baird, 1853, are common and the most veterinary important group of horse parasites in Ukraine. Almost 100% of horses are infected with strongylids; the intensity of infection often reaches hundreds of thousands of nematodes per horse (Dvojnos and Kharchenko, 1994). The main and usually the only method of horse strongylid control in Ukraine is anthelmintic treatment aimed at adult nematode stages within the horse. Free-living larval stages of strongylids on pasture are not affected by the anthelmintics and are the source of grazing horses’ infection. In Ukraine the role of infective larvae in pasture contamination is usually neglected. As a result, alternative control
methods for pasture management (Herd, 1986a), biological control using predacious fungi (Larsen, 1999) and others are not used.

A number of studies on the development of the free-living stages of strongyles and their survival on pasture have been carried out in various regions of the world: in Australia (English, 1979), Canada (Polley, 1986), in the USA (Baudena et al., 2000), in Western Europe (Ogbourne, 1972; Lindberg, 1976; Grelk et al., 1977; Genchi et al., 1978; Hasslinger, 1981; Mirck, 1981; Ramsey et al., 2004). In the former USSR ecological studies of horse strongylids were carried out in the dry steppe conditions of Turkmenia (Shagalin, 1960), Kazakhstan (Shumakovich, 1968) and Bashkiria (Kadyrov, 1979). No field investigations on the ecology of the free-living stages of strongyles have been carried out under the climatic conditions of the Ukraine. The aim of our work was to investigate the survival and overwintering of third stage infective larvae of strongyles on pasture in the central part of Ukraine.

2. Material and methods

2.1. Time and place of the study

The study was carried out between October 2002 and April 2004 on the Dubrovsky State Horse farm situated in Poltavska oblast (ca. 50° North and 33–34° East) of Ukraine. The total area of the Dubrovsky horse farm is 7200 ha. Grassland covers 600 ha, of which 340 ha are used as horse pasture. The grazing season starts in April and ends in October or November; all pasture is continuously grazed during the season.

The experiments were carried out on a small plot (approximately 50 m × 20 m) of pasture which had been continually grazed by horses although they did not graze on the experimental plot throughout the study. The predominant vegetation on the pasture was wheat grass (Agropyran repens). The level of grass coverage on the pasture was from 6 cm (in April) to 22–35 cm (in June).

2.2. Experimental design

The faecal material was obtained from mature horses with naturally acquired strongylid infections. During the experimental period horses were housed at night and grazed on the permanent pasture during the daytime. Seventeen to 20 faecal samples (1.5 kg each) were collected monthly from each experimental horse. All samples were examined for strongyle egg numbers (EPG) using the McMaster technique with a sensitivity of 25 strongyle eggs per gram of faeces (Herd, 1992). The number of third stage infective larvae in each faecal sample was calculated after culturing the faeces for 10 days at room temperature (+23 to +25 °C) (Kotelnikov, 1984). Each experimental faecal pat was cultured separately in the laboratory.

Horse faecal pats (1.5 kg each) were placed on the experimental pasture plot every month during the grazing season (in November 2002, and from April to November 2003). Faecal pats were placed in rows at 1.5 m intervals and labelled. Samples (10 g) were extracted from the faecal pats monthly so that the total weight of samples extracted did not exceed 5% of the pat weight. This method minimized any error in the estimation of the general population of nematode larvae.

Faecal samples (10 g each) and grass samples (10 g each) were collected from and around all faecal pats every 4 weeks during the grazing season (in April–November 2003 and in April 2004). Pasture sampling was usually conducted between 7 and 8.30 a.m. No samples were collected in the cold winter period (December–March), when pasture was not used for horse grazing. The grass was cut from quarter section 0–10 cm above and at 0–10 cm around the edge of each faecal pat. Soil samples (10 g each) were collected in April 2004 at a depth of 1–2 cm under all experimental faecal pats. Infective larvae were collected from faecal, grass and soil samples using the Baermann procedure (Kotelnikov, 1984).

2.3. Mycological study of the horse faeces

All laboratory faecal cultures were examined for the presence of soil predacious fungi. Five samples of fungal mycelium, if present, were taken from the surface of the faecal samples on 10th day of cultivation. The predacious fungi were identified by the shape of fungal conidia using the identification keys by Mekhtieva (1979).

2.4. Meteorological data

The meteorological data were provided by the Central Hydrometeorological Record Office of the Ukraine from a station approximately 20 km west of the experimental pasture.

2.5. Statistical analysis

Data analysis was performed using Microsoft™ Excel. Descriptive statistics and one-way analysis of variance (ANOVA) were performed using the Statsoft™ Statistica V6.
3. Results

3.1. Meteorological data

Monthly mean, minimum and maximum soil temperatures, air humidity, and precipitation in the region of investigation are shown in Fig. 1.

3.2. Strongyle egg counts and viability of infective larvae in faecal cultures

The changes in the number of eggs in faeces examined corresponded to the typical seasonal pattern described in horse Strongylida (Duncan, 1974; Ogbourne, 1975) with prominent maximum in summer and decrease in autumn (Fig. 2).

In laboratory faecal cultures, the ratio of the infective larvae to the previously calculated EPG decreased from May (74.4%) to June (54.7%) and increased during the period from June to November (84.2%) (Fig. 3). The differences between EPG and strongyle larvae found was significant ($p < 0.05$) for all months of experiment (Appendix 1).

The reduction in numbers of infective larvae in the warm season coincided with the presence of the nemathophagous fungi *Arthrobothrys* spp. and *Monacrosporium* spp. These fungi were observed in laboratory cultures from May to October (Table 1).

![Fig. 1. Monthly soil temperatures (A) and total monthly precipitation and average relative humidity (B) during the period of investigations.](image-url)
In June and July contamination of the faecal cultures with fungi was at its highest: up to 65% and 80% of cultures were contaminated, respectively. No predacious fungi were observed in faecal cultures in April and November.

In the faecal cultures contaminated with predacious fungi the number of nematode larvae was lower than in clean cultures (Fig. 4). From June till September the number of larvae in laboratory faecal cultures containing the predacious fungi (FP—fungi-positive, Group 1) was significantly different \( (p < 0.05) \) from that in the cultures lacking the predacious fungi (FN—fungi-negative, Group 2) based on the \( t \)-test for independent samples (Appendix 2). In May and October those differences were not statistically significant due to the small number of faecal samples contaminated with the fungi. Thus we consider the predacious fungi as an important agent of larval elimination both in cultures and on pasture.

3.3. Recovery of the infective larvae and their migration on pasture

During the warm season (from April to September) development of larvae on pasture was relatively successful. In this period, all strongyle eggs successfully hatched; no eggs were found in the faecal pats 4 weeks after deposition on the pasture. This contrasts with the relatively large numbers of strongyle eggs (average EPG = 85.7) found after 4 weeks in the faecal pats deposited on pasture in October, when average daily temperature was below +6 °C.

To estimate the changes in number of infective larvae surviving on pasture we expressed the number observed in the samples collected as the mean percentage to the number of larvae observed in 10-day-old laboratory cultures considering the latter as 100% (Table 2), since the absolute quantities of larvae depended on the EPG values. Comparatively large numbers of larvae were found on the pasture and in disintegrated faecal pats 1

### Table 1

Presence of the predacious fungi in the faecal laboratory cultures

<table>
<thead>
<tr>
<th>Number of cultures</th>
<th>Month of faecal laboratory cultures established</th>
</tr>
</thead>
<tbody>
<tr>
<td>--------------------</td>
<td>---------------</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
</tr>
<tr>
<td>With <em>Arthrobothrys</em> spp.</td>
<td>0</td>
</tr>
<tr>
<td>With <em>Monacrosporium</em> spp.</td>
<td>0</td>
</tr>
<tr>
<td>% of cultures infected with fungi</td>
<td>0</td>
</tr>
</tbody>
</table>
month after deposition during the period from May to September (6–11%). During this period, however, comparatively small numbers of larvae survived for a month from July to August. Moreover, a comparatively lower proportion of larvae survived for 2 months, from June to August.

Very low numbers of infective larvae were found to be able to survive on pasture for more than 12 months. In April 2004 larvae were observed in the disintegrated faecal pats deposited on the pasture in April of the previous year; no larvae were found on grass around the disintegrated faecal pats.

Calculation of infective larvae within and outside the 1-month-old faecal pats on pasture demonstrated that the larger percentage of larvae remained within the faecal pats (Fig. 5). On the other hand, comparatively larger numbers of larvae were observed on the grass around the faecal pats in July (faeces deposited in June) and in the autumn months of 2003. Obviously, larvae migrated more successfully from the faeces to the grass under conditions of higher humidity and precipitation (see Fig. 1).

During the warm season the percentage of larvae on the grass has increased and reached a maximum, up to

Table 2
Percentage of strongyle infective larvae (L3) recovered from pasture and faeces during the experimental period

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
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<th></th>
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<th></th>
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</tr>
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<tr>
<td>November 2002</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>April 2003</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 2003</td>
<td>0.0</td>
<td>5.51</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2003</td>
<td>0.0</td>
<td>3.98</td>
<td>7.97</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July 2003</td>
<td>0.0</td>
<td>2.11</td>
<td>5.59</td>
<td>11.06</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>August 2003</td>
<td>0.0</td>
<td>0.79</td>
<td>3.17</td>
<td>4.69</td>
<td>5.97</td>
<td>100.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>September 2003</td>
<td>0.0</td>
<td>0.45</td>
<td>1.84</td>
<td>3.56</td>
<td>4.11</td>
<td>9.27</td>
<td>100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October 2003</td>
<td>0.0</td>
<td>0.37</td>
<td>1.33</td>
<td>2.23</td>
<td>3.33</td>
<td>6.67</td>
<td>7.08</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>November 2003</td>
<td>0.0</td>
<td>0.24</td>
<td>0.85</td>
<td>1.65</td>
<td>2.46</td>
<td>4.01</td>
<td>4.22</td>
<td>1.31</td>
<td>100.0</td>
</tr>
<tr>
<td>April 2004</td>
<td>0.0</td>
<td>0.03</td>
<td>0.43</td>
<td>0.53</td>
<td>0.57</td>
<td>1.51</td>
<td>2.97</td>
<td>0.07</td>
<td>0.0</td>
</tr>
</tbody>
</table>

Fig. 5. Proportion of the strongyle infective larvae found inside horse faecal pats and on pasture herbage around faecal pats during the period of study (■) in faecal pats; (■) on pasture herbage.
35% of all larvae recovered, in autumn. This percentage decreased again in the samples collected in April of the next year. In our opinion, larvae survive the winter mainly in disintegrated faecal pats, from where 90–97.6% of larvae were observed (Fig. 5).

3.4. Overwintering of the strongyle infective larvae on pasture

Calculation of infective larvae in faecal pats in April 2004 enabled the estimation of the percentage of larvae which survived in the pats deposited on the pasture in various months of the previous year. The number of infective larvae observed in April was compared with that in the corresponding 1-month-old faecal pats (100%). The results are shown in Fig. 6. The proportion of larvae which successfully survive the winter is a maximum of 42.0% in the pats deposited on pasture in September of the previous year. The percentage of overwintered larvae was significantly lower in pats deposited on pasture in summer and late autumn. No infective larvae were found in the pats deposited on pasture in November. Eggs and early stage larvae were also absent in those faecal pats.

Infective larvae were also found in soil samples collected directly beneath the disintegrated faecal pats in April (Table 3). No larvae were observed in these samples under the faecal pats deposited on pasture in April and November of the previous year. The largest number of larvae (12–117) was observed under the pats deposited on pasture in September of the previous year.

4. Discussion

The climatic conditions of the experimental area in Poltavskaja oblast are typical for most parts of Ukraine, except the Crimea, Carpathian Mountains and arid steppes of Khersonska oblast. Thus, the observations from the present study may also be true for a larger part of the country.

It is known that strongyle larvae can develop under the temperature from +8 to +38 °C and with a soil humidity of more than 30% (Shagalin, 1960; Shumakovitch, 1968; Ogborne, 1972; Mfitilodze and Hutchinson, 1987). Analysis of the meteorological data in the present study demonstrated that climatic conditions of central Ukraine were favourable for the development and survival of strongyle infective larvae during the whole grazing season, from April to November.

Our observations on the changes in quantity of the nematode larvae in horse faeces on pasture during the grazing season are generally similar to those from the studies in temperate climatic zones of the Northern hemisphere (Kazlauskas, 1959; Ogborne, 1972; Lindberg, 1976; Gench et al., 1978; Hasslinger, 1981; Mirck, 1981; Herd, 1986b; Polley, 1986). The maximum quantity of larvae was observed in June and July, and the minimum quantity was observed in April, at the beginning of grazing season.

According to our data, all eggs and early stage larvae died in faecal pats during the cold winter period (from November to March), when the temperature was below +3 °C. Neither larvae nor eggs have survived until April (2003 and 2004) in the faecal pats deposited on pasture in November of the previous years. On the other hand, strongyle eggs were reported to survive freezing temperatures under natural conditions in Canada (Polley, 1986) and Kazakhstan (Shumakovitch, 1968). However, a high egg mortality on pasture in regions where freezing temperatures prevail for long periods had been reported by Parnell (1936), Pukhov (1941), Kazlauskas (1959), and Barus (1963). In our opinion,

Table 3
Strongyle infective larvae recovered from soil under disintegrated faecal pats

<table>
<thead>
<tr>
<th>Number of L3 per sample</th>
<th>Month of faecal disposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>0</td>
</tr>
<tr>
<td>Range</td>
<td>0</td>
</tr>
</tbody>
</table>
the complete elimination of eggs and early stage larvae during the winter period was caused by sporadic snow covering and the alternating freezing and melting of faeces on pasture in the region under investigation.

The summer period in Ukraine is generally favorable for strongyle larval development on pasture. Even in the hottest months the maximum soil temperature is usually lower than 40 °C. Under such conditions most of the larvae survive and successfully develop. In more arid conditions of Central Asia (Turkmenia), Bashkiria, strongyle larvae were reported to be eliminated in the hottest season of the year, when soil temperature is constantly higher than +40–46 °C (Shagalin, 1960; Shumakovich, 1968). In Ukraine the opposite occurs as the pastures are most heavily contaminated with strongyle infective larvae during the summer months.

It would appear that the predacious fungi may also reduce the number of strongyle larvae in faecal pats in the summer. In the present study, the predacious fungi Arthrobotrys sp. and Monacrosporium sp. were observed to more heavily contaminate faecal cultures in June (65% of samples) and July (80% of samples). In our opinion, the decrease in numbers of nematode larvae in horse faeces in summer is more likely caused by the presence of predacious fungi than by the density dependant effect on egg viability and development, as it was proposed by Ramsey et al. (2004).

According to our observations, the infective larvae tend to remain in the horse faeces and not to migrate actively onto the surrounding pasture grass. Such behaviour obviously depends on the humidity and level of precipitation in certain periods. In summer (July) only 10.9% of larvae were found to leave the faecal pats, whereas in October 28.3% of larvae were found on grass, not in the faeces. Thus, it is the horse faeces that appear to be the main reservoir of infective larvae on pasture during the grazing season. This data supports the results of Ramsey et al. (2004) regarding the influence of precipitation on larval migration. On the other hand, these authors observed a higher proportion of infective larvae migrating from the faeces under the climatic conditions of west central Scotland. Moreover, strongyle infective larvae can survive winter in disintegrated faecal pats rather than on pasture, where less than 10% of larvae were observed.

The winter survival of the strongyle infective larvae under the climatic conditions of Ukraine is of particular interest. According to the observations made in separate parts of the world, a percentage of strongyle infective larvae can survive a cold winter period in faecal pats and soil. These larvae act as a reservoir for the re-infection of horses at the beginning of the grazing season (Polley, 1986; Slocombe et al., 1987; Ramsey et al., 2004). On most of the territory of Ukraine, winter is temperately cold, with small and sporadic snow cover. The present study demonstrated that successful survival of infective larvae in winter depends on the time of their emergence on pasture. According to our data, the maximum number of larva which survived until spring were from the faecal pats deposited on pasture in September (up to 42% of larvae survived). Thus, it could be concluded that the prophylactic treatment of the most heavily infected horses in the middle of the grazing season is necessary to minimize pasture contamination with strongyle infective larvae at the beginning of new grazing season.

In the present study we observed some infective larvae successfully overwintering in soil at a depth of 1–2 cm. Those were found under faecal pats in the beginning of the new grazing season. We observed a comparatively smaller number of horse strongyle larvae in the soil than did Dimander et al. (1999) who studied the biology of trichostrongyle infective larvae in Sweden. We consider soil as another reservoir of infective larvae on pasture in Ukraine.

Based on the data of the present study, one can conclude that horse pastures in the central part of Ukraine are never free from the strongyle infective larvae. Consequently, there is the continuing possibility of horse infection with strongylid nematodes throughout the whole grazing season. The requirement for pasture management within modern control programs for horse strongyles in the Ukraine therefore cannot be ignored.

Acknowledgements

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Appendix A

The differences between EPG and strongyle larvae found calculated for all months of experiment

General effect

Univariate results for each DV (sigma-restricted parameterization, effective hypothesis decomposition)

<table>
<thead>
<tr>
<th>Degrees of freedom</th>
<th>EPG SS</th>
<th>EPG MS</th>
<th>EPG F</th>
<th>EPG p</th>
<th>L3% SS</th>
<th>L3% MS</th>
<th>L3% F</th>
<th>L3% p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Months</td>
<td>7</td>
<td>1841689</td>
<td>263098</td>
<td>6.10</td>
<td>0.000003</td>
<td>15075.4</td>
<td>2153.6</td>
<td>4.84</td>
</tr>
<tr>
<td>Error</td>
<td>142</td>
<td>612404</td>
<td>43127</td>
<td></td>
<td></td>
<td>63132.8</td>
<td>444.6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>149</td>
<td>796573</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>78208.2</td>
</tr>
</tbody>
</table>

Appendix B

Descriptive statistic and $t$-test for predacious fungi positive (FP, Group 1) vs. fungi negative (FN, Group 2) faecal cultures

<table>
<thead>
<tr>
<th>Group 1 vs. Group 2</th>
<th>Mean, Group 1</th>
<th>Mean, Group 2</th>
<th>t-Value</th>
<th>d.f.</th>
<th>$p$</th>
<th>Valid N, Group 1</th>
<th>Valid N, Group 2</th>
<th>S.D., Group 1</th>
<th>S.D., Group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>May_FP vs. May_FN</td>
<td>277.50</td>
<td>465.23</td>
<td>−1.197</td>
<td>15</td>
<td>0.2497</td>
<td>4</td>
<td>13</td>
<td>171.41</td>
<td>294.34</td>
</tr>
<tr>
<td>Jun_FP vs. Jun_FN</td>
<td>227.69</td>
<td>534.71</td>
<td>−4.177</td>
<td>18</td>
<td>0.0005</td>
<td>13</td>
<td>114.74</td>
<td>217.78</td>
<td></td>
</tr>
<tr>
<td>Jul_FP vs. Jul_FN</td>
<td>205.19</td>
<td>439.25</td>
<td>−3.109</td>
<td>18</td>
<td>0.0060</td>
<td>16</td>
<td>4</td>
<td>136.65</td>
<td>124.21</td>
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<tr>
<td>Aug_FP vs. Aug_FN</td>
<td>214.57</td>
<td>471.08</td>
<td>−3.827</td>
<td>18</td>
<td>0.0012</td>
<td>7</td>
<td>13</td>
<td>77.78</td>
<td>166.24</td>
</tr>
<tr>
<td>Sep_FP vs. Sep_FN</td>
<td>171.20</td>
<td>308.57</td>
<td>−3.156</td>
<td>17</td>
<td>0.0057</td>
<td>5</td>
<td>14</td>
<td>73.05</td>
<td>86.53</td>
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<tr>
<td>Oct_FP vs. Oct_FN</td>
<td>195.00</td>
<td>337.50</td>
<td>−1.702</td>
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<td>0.1058</td>
<td>2</td>
<td>18</td>
<td>60.81</td>
<td>114.59</td>
</tr>
</tbody>
</table>

*Note*: Variables were treated as independent samples.

References


