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Analysis of the strongylid nematodes (Nematoda: Strongylidae) community after deworming of brood horses in Ukraine

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Abstract

Communities of intestinal helminths in horses are commonly studied post mortem. The study objectives were here to examine the species composition of the strongylid community in brood horses in Ukraine after deworming with an aversectin drug Univerm. The site distribution of the strongylid species was analysed according to dynamics of their expulsion in faeces. Forty-four horses of different ages from Poltavaska oblast (22 horses), Kyivska oblast (17 horses) and Sumska oblast (5 horses) of Ukraine were included in the study. Horses were treated with Univerm anthelmintic (0.2% aversectin) at a dose rate of 0.5 mg aversectin preparation per kg body weight. Faecal sampling (200 g each) was performed at 24, 36, 48 and 60 h post treatment, and all nematodes expelled were collected and identified.

The largest numbers of strongylids were expelled at 24–36 h after treatment. Twenty-five nematode species from the subfamilies Strongylinae and Cyathostominae were identified. The number of strongylid species found per horse ranged from 7 to 20, on an average 11 ± 3.6 (S.D.). The number of cyathostomin species found per horse ranged from 7 to 16, on an average 10 ± 2.3 (S.D.). *Cylicocycylus nassatus* and *Cyathostomum catinatum* were the most dominant species were found in 100% of horses, amounting to 36.3% and 17.6% of the total number of strongylids collected, respectively. *C. longibursatus*, *C. ashworthi*, *Cylicostephanus calicatus*, *C. leptostomus* and *C. minutus* were identified in more than 80% horses and represented 39.9% of the total number of strongylids collected. The dynamics of the different strongylid species expelled was irregular. Correlation between the time of cyathostomin species expulsion in faeces and their predicted localisation inside the horse intestine was found. Species mainly localised in the caecum were found in faeces later than those species localised in the dorsal and ventral colons. Larvae and adult *Parascaris equorum*, *Oxyuris equi* and botfly larvae from the genus *Gasterophilus* were also found in horse faeces.

This investigation shows that is possible to study the horse strongylid community after deworming with aid of an aversectin drug. The results obtained here correspond to those recorded in previous autopsy surveys in other countries.

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Keywords: Strongylida; Cyathostominae; Horse; Nematode parasites; Anthelmintic; Ukraine

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1. Introduction

Horses are parasitized by more than 90 different helminth species (Dvojnjos and Kharchenko, 1994). Regardless of the fact, that most of these parasites occur in subclinical infections, they can cause health problems in some horses. Communities of intestinal horse helminths are commonly studied post mortem, using autopsy methods (Eydal, 1983; Ivashkin and Dvojnjos, 1984; Reinemeyer et al., 1984; Mfitlodze and Hutchinson, 1985; Lyons et al., 1985, 2000; Dvojnjos and Kharchenko, 1994; Bucknell et al., 1995, 1996; Gawor, 1995). This approach allows helminth species identification, but has limitations. Necropsy studies are costly, and horse examination at abattoirs often deals with old animals with low parasitic burdens and diverse or unknown grazing histories (Osterman Lind et al., 2003). Furthermore, the investigation of intestinal parasite communities of valuable brood horses by autopsy methods is almost impossible; one can never obtain a representative host sample for study.

Examination of intestinal helminths of horses and ruminants after deworming was conducted in the USSR more than 50 years ago (Petrov and Gagarin, 1953). However, at that time the anthelmintic drugs had low efficacy, or had no effect on some parasites. The use of modern anthelmintic drugs with higher efficacy enables collection and identification of all luminal stages of horse intestinal nematodes (Osterman Lind et al., 2003).

In Ukraine, studies on the species composition of horse intestinal nematodes by autopsy were carried out in the second half of the 20th century (Ivashkin and Dvojnjos, 1984; Dvojnjos and Kharchenko, 1994). Thirty-five strongylid species were found. Since then, no investigations of the strongylid community of horses have been carried out in Ukraine. The current study was aimed at the examination of the species composition of strongylid nematode communities in Ukrainian brood horses following deworming with aversectin drug. A site distribution analysis of various strongylid species was also performed according to the dynamics of their occurrence in faeces.

2. Material and methods

Forty-four horses of different ages from Poltavaska oblast (22 horses), Kyivska oblast (17 horses) and

Sumska oblast (5 horses) of Ukraine were included in the study. The site distribution of strongylid species was investigated in 22 horses from Kyivska oblast (17 horses) and Sumska oblast (5 horses). All experimental horses belonged to Orlovsky trotter breed. Nineteen males and 25 females were used in experiment. Seven of them were 1-year-old foals, 10 horses were from 2 to 3 years old, 24 horses were from 4 to 8 years old and 3 horses were older than 8 years old. All horses were naturally infected and had not been treated with any anthelmintics for at least 4 months prior to the start of the study. Horses were treated with Univerm (0.2% aversectin, PharmBioMed, Russia) at a dose rate of 0.5 mg aversectin preparation per kg body weight. Anthelmintic treatments were performed in November, 2002 (22 horses), February, 2003 (5 horses) and March, 2003 (17 horses).

Faecal egg counts were carried out on the day before treatment (day 0) and 14 days after treatment (day 14) using the McMaster technique (Herd, 1992) with a sensitivity of 25 eggs per gram (EPG).

Faecal sampling for expelled parasites (200 g in each sample) was performed 24, 36, 48 and 60 h after treatment. Samples were washed with tap water and examined for adult and larval strongylids under a stereomicroscope (Ivashkin et al., 1971). The mean number, minimum and maximum of worm specimens per sample were calculated for each strongylid species. For each horse, the total number of each strongylid species individuals (N) was calculated as

$$N = \left(\frac{W_f}{200} \right) \times N_s,$$

where W_f is the total weight of faeces produced by the horse during the experiment and N_s is the mean number of worm individuals in all samples collected from the horse. Based on these data the mean intensity was calculated for each strongylid species in experimental horse group.

From 100 to 790 worms per horse were collected and identified. All nematodes were fixed in 4% formalin solution in physiological saline, then clarified in 80% phenol–glycerine solution and identified under the light microscope Amplival (Zeiss). Strongylids were identified according to identification keys by Dvojnjos and Kharchenko (1994) and Lichtenfels (1975).

3. Results

3.1. Faecal egg counts

On day 0 the overall mean output of strongylid eggs was 937 EPG with individual variation from 350 to 2000 EPG. On day 14 after treatment, no strongylid eggs were found in the faecal samples from any horse.

3.2. Worm expulsion

The total number of strongylids expelled per horse varied from 5000 to 118,500 with an average of 28,100. The positive correlation was observed between the EPG value on the day 0 and the total number of strongylid worm collected from faeces ($r^2 = 0.57$, $P < 0.01$).

3.3. Species identification

The total number of strongylid parasites collected and identified after deworming was 17,050 specimens. Twenty-five nematode species from the subfamilies Strongylinae and Cyathostominae were identified. The number of strongylid species per horse ranged from 7 to 20, with an average of 11 ± 3.6 (S.D.). Most the species found (19 species) belonged to the subfamily Cyathostominae and to the genera *Cylicocyclus* (6), *Cylicostephanus* (5) and *Coronocyclus* (4). Species from the subfamily Strongylinae were less numerous: three species from the genus *Strongylus* and three species from the genus *Triodontophorus*. The number of Cyathostominae species per horse ranged from 7 to 16, with an average of 10 ± 2.3 (S.D.). The number of Strongylinae species per horse ranged from 1 to 5, with an average of 1.2 ± 1.5 (S.D.). Moreover, larval and adult *Parascaris equorum* and *Oxyuris equi* as well as botfly larvae from genus *Gasterophilus* were also found in horse faeces.

3.4. Analysis of the strongylid community

A significant correlation ($r^2 = 0.66$, $P < 0.05$) between the prevalence of 25 nematode species and the mean intensity of these species was found. The data on prevalence and the number of specimens of strongylid species extracted from horse faeces are shown in Table 1.

According to prevalence value, all strongylid species (25) were ranged in 10 prevalence classes

(0–10%, ..., 91–100%). The number of taxa corresponding to each prevalence class was determined (Fig. 1). Strongylid species distribution allows us to separate core species (those occurring at prevalence >50%) from satellite species (those occurring at prevalence <50%).

Nine species were identified as core: *C. nassatus*, *C. catinatum*, *C. longibursatus*, *C. ashworthi*, *C. calicatus*, *C. leptostomus*, *C. minutus*, *C. coronatus* and *C. goldi*. Specimens belonging to these species were more numerous in the samples collected; the mean intensity of *C. nassatus* was 6903.3 (29.5% of total strongylid number), *C. catinatum* 3216.4 (13.7%), *C. longibursatus* 2163.4 (9.2%), *C. ashworthi* 1460.2 (6.2%), *C. calicatus* 1161.4 (4.9%), *C. leptostomus* 1967.5 (8.4%), *C. minutus* 966.4 (4.1%), *C. coronatus* 667.5 (2.9%), *C. goldi* 408 (1.8%) (Fig. 2).

3.5. The dynamics of worm expulsion

The largest portion of strongylids was found in faeces 24–36 h after treatment (Fig. 3). Only a few nematodes and many gastric bots (*Gasterophilus sp.*) were found in faeces 60 h after treatment and beyond.

Individual strongylid species expulsion varied (Fig. 4). Strongylids such as *C. goldi*, *P. imparidentatum*, *C. aswhorti*, *C. hybridus*, *C. calicatus*, *C. nassatus* and *C. catinatum*, were usually expelled in faeces after 24 h. *C. leptostomus*, *C. pateratum*, *C. coronatus*, *T. nipponicus*, *T. brevicauda*, *C. labratus*, *C. insigne*, *C. labiatus*, *C. minutus* were expelled after 36–48 h. Most of *C. elongatus*, *P. poculatum*, *S. vulgaris*, *S. edentatus*, *S. equinus* and *T. serratus* were expelled 48–60 h after treatment.

Parasitic larvae of various Cyathostominae species were expelled almost simultaneously, during 24–48 h, and less than 17% of them were expelled 60 h after treatment. Most larvae were identified as fourth-stage larvae or those undergoing the fourth moult. They composed 0.4% of the number of strongylids found. The identification of the parasitic larvae to the species level was not possible.

4. Discussion

In this study an investigation of the helminth community expelled by horses after aversectin

Table 1
Prevalence and number of nematode species from order Strongylida Diesing, 1851 expelled in faeces from 44 horses examined

Species	Prevalence (%)	Number of specimens per sample			
		Mean	Minimum	Maximum	S.D.
<i>Strongylus edentatus</i>	13.6	1.25	1	3	0.8
<i>S. equines</i>	15.9	1.3	1	4	1.3
<i>S. vulgaris</i>	27.3	1.3	1	3	0.6
<i>Triodontophorus serratus</i>	15.9	0.9	1	2	0.7
<i>T. brevicauda</i>	15.9	2.9	1	7	2.5
<i>T. nipponicus</i>	6.8	1.5	1	2	1.1
<i>Cyathostomum catinatum</i>	100	65.9	1	526	90.4
<i>C. pateratum</i>	45.5	3.8	1	21	4.5
<i>Coronocylus coronatus</i>	72.7	8.4	1	80	14.3
<i>C. labiatus</i>	50.0	5.3	1	20	4.8
<i>C. labratus</i>	38.6	5.3	1	13	4.1
<i>Cylicocylus nassatus</i>	100	107.6	11	365	68.2
<i>C. ashworthi</i>	93.2	16.9	1	79	16.5
<i>C. leptostomus</i>	84.1	23.9	1	88	28.4
<i>C. elongatus kotlani</i>	25.0	2.1	1	5	1.2
<i>C. insigne</i>	36.4	3.8	1	10	2.5
<i>C. radiatus</i>	4.5	1.1	1	2	0.1
<i>Cylicostephanus calicatus</i>	84.1	16.3	1	59	14.6
<i>C. longibursatus</i>	93.2	27.1	1	98	28.3
<i>C. minutes</i>	81.8	11.6	1	81	15.8
<i>C. hybridus</i>	11.4	6.1	1	9	2.4
<i>C. goldi</i>	75.0	8.1	1	57	13.1
<i>Petrovinema poculatum</i>	27.3	3.6	1	10	3.2
<i>Poteriostomum imparidentatum</i>	18.2	1.5	1	4	0.9
<i>Gyalocephalus capitatus</i>	2.3	3	3	3	

treatment was performed. Absence of nematode eggs observed in our studies after the anthelmintic treatments of horses proved that all luminal stages of intestinal strongylids were expelled from the intestine of all experimental horses.

In general, the prevalence of Cyathostominae was in agreement with previous necropsy surveys performed in other countries (Ogbourne, 1976; Reinemeyer et al., 1984; Silva et al., 1999). The mean number of cyathostomin species expelled per horse was 10; this

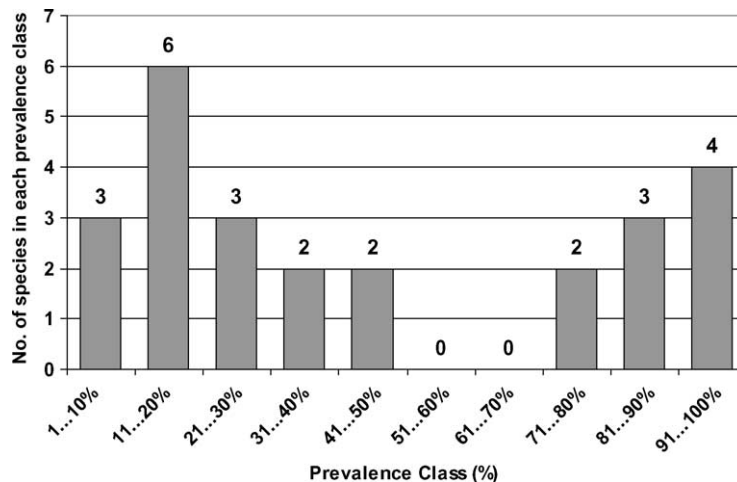


Fig. 1. Number of strongylid nematodes species in 44 horses from Ukraine divided into the different prevalence classes.

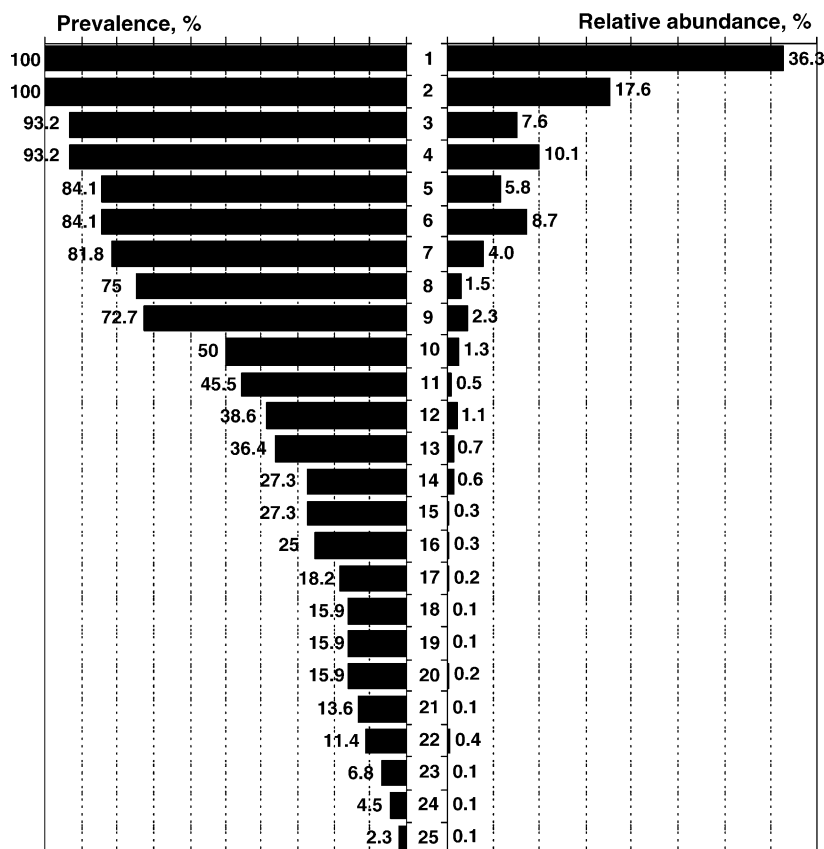


Fig. 2. Prevalence and mean intensity of various species in strongylid community in 44 horses from Ukraine: (1) *C. nassatus*, (2) *C. catinatum*, (3) *C. longibursatus*, (4) *C. ashworthi*, (5) *C. calicatus*, (6) *C. leptostomus*, (7) *C. minutus*, (8) *C. coronatus*, (9) *C. goldi*, (10) *C. pateratum*, (11) *C. labratus*, (12) *C. insigne*, (13) *C. labiatus*, (14) *S. vulgaris*, (15) *P. poculatum*, (16) *S. edentatus*, (17) *T. serratus*, (18) *P. imparidentatum*, (19) *T. brevicauda*, (20) *C. elongatus kotlani*, (21) *C. hybridus*, (22) *T. nipponicus*, (23) *S. equinus*, (24) *C. radiatus*, (25) *G. capitatus*.

result corresponds with those obtained in studies based on post mortem examination of horse intestines in Ukraine (Dvojnos and Kharchenko, 1994), Poland (Gawor, 1995), USA (Reinemeyer et al., 1984), Australia (Mfitlodze and Hutchinson, 1985; Bucknell et al., 1996) and with results of cyathostomin community investigation after deworming in Sweden (Osterman Lind et al., 2003). The core species ratio observed in this study is almost the same as that obtained in Ukrainian post mortem studies (Dvojnos and Kharchenko, 1994). Seven species were reported previously as predominant in parasite communities: *C. nassatus*, *C. catinatum*, *C. ashworthi*, *C. longibursatus*, *C. calicatus*, *C. leptostomus* and *C. minutus*. Up to 90% of all strongylid nematodes observed in post mortem studies belonged to these species. In our

study, we did not find 10 species previously reported in Ukraine: *Triodontophorus tenuicollis*, *Oesophagodontus robustus*, *Cylicodontophorus bicoronatus*, *Coronocylcus sagittatus*, *Cylicostephanus asymetricus*, *C. bidentatus*, *Cylicocylcus ultrajectinus*, *Poteriostomum ratzii*, *Parapoteriostomum mettami* and *P. euproctus*. All these are relatively rare in the region. The abundance of these species as calculated from the data of Dvojnos and Kharchenko (1994) was from 0.03 to 1.73. Presumably, they were absent in samples due to the comparatively small number of parasites collected per horse (100–790). This quantity may be not enough for rare species to be found (Chapman et al., 2003).

The prevalence of Strongylinae infection observed in this study was much lower than that reported

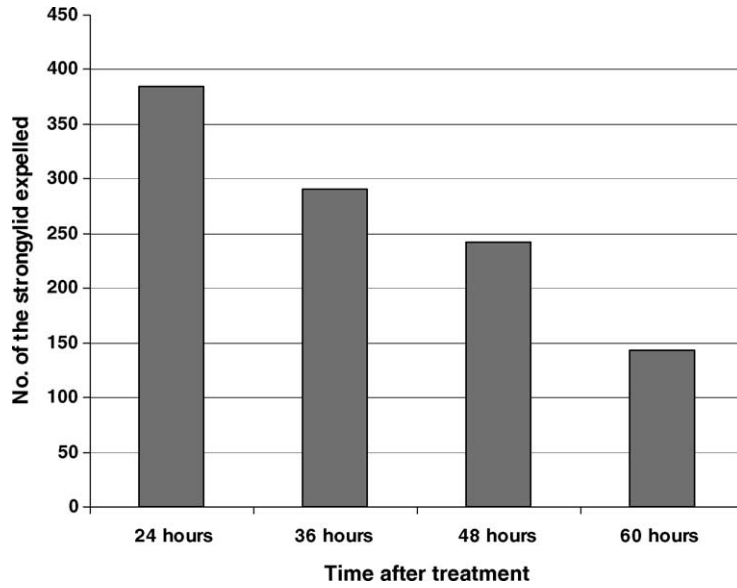


Fig. 3. The mean number of strongylid nematodes expelled in horse faeces after 24, 36, 48 and 60 h.

previously by Dvojnok and Kharchenko (1994). In these investigations, *S. vulgaris* and *S. edentatus* were found in 100% of horses, and *T. serratus* had a prevalence 90%. In our opinion, such difference might

be a result of intervening frequent anthelmintic treatments with aversectin drugs recently made available in Ukraine. Under such conditions, strongylines have no possibility to fulfil their developmental

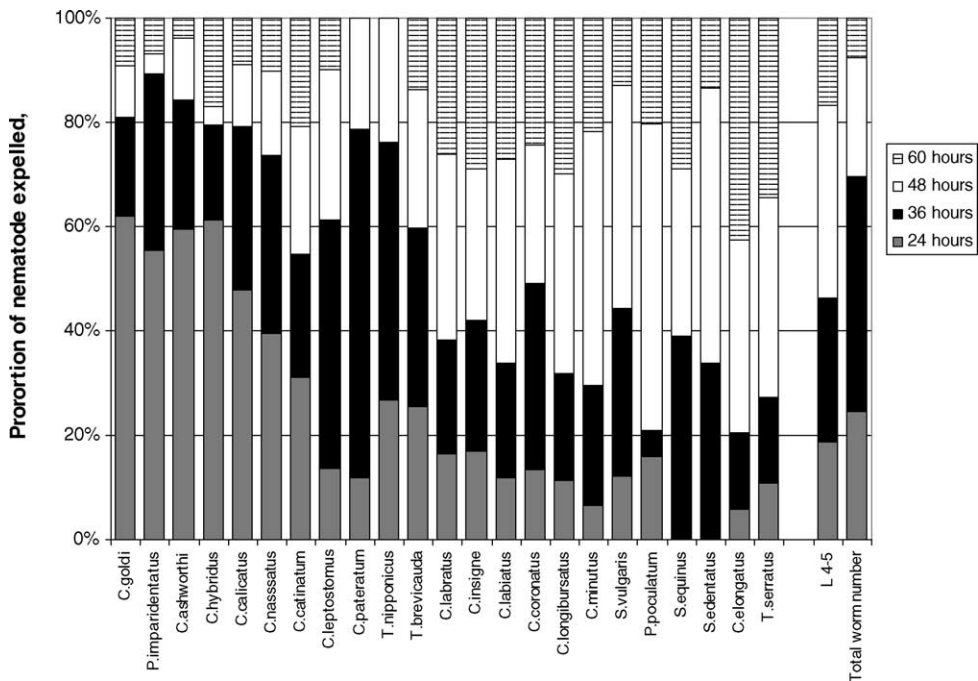


Fig. 4. Dynamics of strongylids species expulsion from horse intestine (24, 36, 48 and 60 h after treatment).

cycle, which lasts from 6 to 8 months (Velichkin, 1955; Ogbourne and Duncan, 1985).

In the present study, cyathostome larval stages (L4 and M4) were observed and composed 0.4% of all helminths found. It is known that the worm burden in horses predominantly consists of inhibited cyathostomin larvae. More than 90% of the cyathostomin burdens may be in the mucosa at specific times of the year (Eysker et al., 1984, 1989, 1990; Eysker and Mirck, 1986) and they are not affected by the anthelmintic treatment. Our studies were carried out in November, February and March, when the proportion of encysted larvae was comparatively large. However, species identification of encysted larvae is still difficult, since their morphology differs from that in adult stages (Dvojnjos and Kharchenko, 1994). Studies on horse helminth communities based on autopsy method dealt with identified luminal stages of strongylids (Ogbourne, 1976; Reinemeyer et al., 1984; Mfitlodze and Hutchinson, 1985; Dvojnjos and Kharchenko, 1994; Gawor, 1995; Bucknell et al., 1995, 1996; Silva et al., 1999; Collobert-Laugier et al., 2002). Species composition and proportion of each species larvae in mucosa are supposed to correspond those observed for luminal stages. We believe that absence of data about the species composition of the mucosal cyathostome larvae in this study did not affect considerably the data on the separate strongylid species proportion in the horse strongylid community.

The dynamics of separate strongylid species expelled from the intestine showed a correlation between the site of nematodes localised within the host intestine and the time of their emergence in the faeces. According to the data of previous authors obtained by autopsy (Ogbourne, 1976; Mfitlodze and Hutchinson, 1985; Bucknell et al., 1995; Gawor, 1995; Collobert-Laugier et al., 2002), it was established that such species as *C. goldi*, *P. imparidentatum*, *C. pateratum*, *C. ashworthi* inhabited mainly the dorsal colon; *C. nassatus*, *C. catinatum*, *C. longibursatus*, *C. leptostomus*, *C. insigne* inhabited the ventral colon, and such species as *C. elongatus*, *S. vulgaris*, *S. equinus*, *S. edentatus*, *T. serratus*, *C. coronatus* inhabited the caecum. In our study strongylid species inhabiting the dorsal colon were observed in faeces within 24–36 h after deworming. Species from the ventral colon were observed within 24–48 h; whereas

species from the caecum were found as late as 48–60 h after deworming.

On the other hand, specific nematode species attachment to the intestinal wall may also affect the rate of expulsion after treatment. In our opinion, this subject needs further experimental investigations.

Our data demonstrate that deworming methods can allow a partial study of all groups of intestinal parasites of horses, not only strongylids. Adult and larval stages of *P. equorum* and *O. equi*, as well as the botfly larvae were observed in faeces after deworming. Host treatment with aversectin anthelmintics allows conducting complete study of the luminal parasite community.

Osterman Lind et al. (2003) proposed to use benzimidazole drugs (7.5 mg fenbendazole) for the same purpose. We believe, however, that benzimidazole drugs will not give accurate results because of the widely distributed cyathostomin resistance to benzimidazole, also known in Ukraine (Borgsteede et al., 1997; Kuzmina et al., 2002).

The results obtained in this study confirm the possibility of intestine helminth community investigation without the need for necropsy. The results obtained by this method for lumen-dwelling parasites are comparable with those obtained by previous invasive methods.

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