

Horse gastrointestinal parasites in the Moscow region

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Abstract. The purpose of our work was to conduct a survey of the horse population for the presence of parasites of the gastrointestinal tract in Moscow and the Moscow region. The research included horses aged 1.5 months to 26 years from horse farms and from private owners too. Fecal samples were collected rectally from 571 horses and were examined on the day of collection by flotation method and sedimentation method. Horses are infected with parasites of the gastrointestinal tract by 47.5% in the Moscow region. The main representatives of the parasite fauna are nematodes. Strongylidae gen. sp. (44%), *Parascaris* sp. (10.3%), *Oxyuris equi* (1.4%), *Eimeria leuckarti* (0.5%) were found. Statistically revealed correlations between the level of infection and the age of the horse ($p < 0.001$), as well as between the level of infection and the conditions of keeping ($p < 0.001$). The most infected were horses in the age group from 1 to 3 years. According to the study, herd horses are significantly more likely to be infected with *Parascaris* sp. and *O. equi*. No correlation was found between the level of infection and the type of farming (horse farms or private owners) ($p = 0.252$).

1 Introduction

Over the past 10-15 years, the number of horses has increased markedly on the territory of Russia, which was facilitated by the organization of the private sector in horse breeding (stables, equestrian sports complexes, recreation centers, etc.), as well as the development of ecotourism and sports. At the same time, parasitic diseases remain an actual urgent problem, which cause significant damage to animal health and lead to economic losses. The most frequently reported parasitic diseases of horses are gastrointestinal tract nematodes caused by various strongylid species from the subfamilies *Cyathostominae* and *Strongylinae*, as well as roundworm *Parascaris* sp. Almost all grazing horses are infected with cyathostomins. At least 50 nematode species of this subfamily have been described, although it is known that 90% of detected parasitic infections in horses are caused by the 5–10 most common species [1-4]. Such infections lead to loss of appetite of animals and, consequently, to severe weight loss. *Parascaris* sp. is the largest horse intestinal nematode and the most pathogenic parasite of young animals [4]. Quite often equine gastrointestinal

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nematodes cause colic. That is why an effective parasite control in horses is so important to maintain the health of the entire herd [5-6].

In Russia, *Parascaris* sp. is registered in 17.4-80% of horses, and strongylid of the gastrointestinal tract in 14.7-94.1% [7-9]. The purpose of our work was to conduct a survey of the horse population for the presence of parasites of the gastrointestinal tract in Moscow and the Moscow region.

2 Materials and methods

2.1 Animal selections

The study protocol was reviewed and approved by the scientific and methodological commission of Federal State Budget Scientific Institution “Federal Scientific Centre VIEV” (Protocol No. 2 dated February 12, 2020).

The study included horses aged 1.5 months to 26 years from horse farms and from private owners too. The maintenance of horses is predominantly stable, less frequently herd. The stable keeping system involves the individual finding of horses in stalls, daily walking in the walking area from 60 to 90 minutes. When kept in a herd, animals spend most of the year on pastures, in winter they are transferred to indoor places. Heads of horse enterprises and horse owners were informed about the purpose and procedure of the study. They agreed to participate in the study, provided information about the animals, conditions of keeping horses, and the timing of deworming. All animals that took part in the experiment did not receive antiparasitic drugs for 3 months or more.

2.2 Study period and area

Most of the work was undertaken in 2020 and 2021. The fecal samples were collected on the territory of eight equestrian sports complexes, three equestrian clubs, two breeding farms, a hippodrome, a stud farm, a horse yard, and from private owners' horses. All the horses were kept in the central region of Russia (Moscow and Moscow region) (Figure 1).

Moscow coordinates: 55° 45' north latitude, 37° 36' east longitude. Moscow and the Moscow Region form the core of the Central Federal District of the Russian Federation. Moscow is situated on the Moskva River in the center of the East European Plain, between the Oka and Volga rivers. Moscow is located at the junction of three large physical and geographical regions: the Smolensk-Moscow moraine hill, the Moskvoretskaya-Oka moraine-erosive plain and the Meshcherskaya outwash lowland.

The Smolensk-Moscow moraine hill is located in the north-west of Moscow with coniferous-broad-leaved and birch forests, small oak and pine forests on loamy soils. The Moskvoretskaya-Oka moraine-erosion plain, deeply dissected by ravines and gullies, is a ridged erosional surface with absolute heights of 200 m, composed of Mesozoic rocks overlain by mantle loams. The Meshcherskaya outwash lowland is located in the east of Moscow. It is a flat sandy lowland with pine forests on sands and sandy loams. The absolute heights of the relief reach 160 meters. In some areas, swamp-podzolic soils with patches of peat swamps can be seen. The climate of Moscow is temperate continental; the annual temperature difference is 28 degrees. Winters are long and severe. In 2020, the average annual air temperature exceeded its last year's historical maximum and amounted to +8.0°C, and a record amount of precipitation fell 890 mm [10].

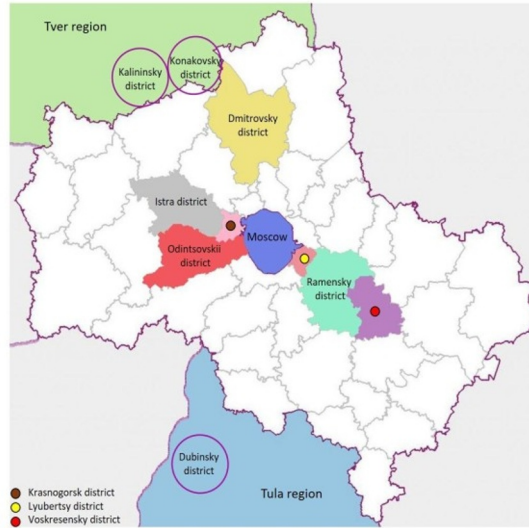


Fig. 1. The area of research on the territory of the Moscow region of Russia.

2.3 Collection and examination of fecal samples

Fecal samples were collected from 571 horses. Feces were taken individually from each animal, directly from the rectum into a disposable glove. All samples were labeled with the age of the horse, breed and conditions of keeping.

The study was carried out in the laboratory on the day of sampling. The flotation method was used with sodium nitrate solution (NaNO_3 , SG 1.38) and with centrifugation at 120g for 3 min (laboratory centrifuge Elmi SM-6M, Latvia). A separate study was carried out on the fecal sediment obtained after flotation. The eggs per gram of feces (EPG) or oocysts per gram of feces (OPG) were counted using the modified McMaster technique. For this, a counting chamber with a capacity of 0.15 ml was used. Sodium nitrate solution (NaNO_3 , SG 1.38 g/cm^3) was used as the flotation solution in this test. Take 4 g of feces, mix with 26 ml of flotation solution to make a total volume of 30 ml. Mixed and filtered through a sieve, both chambers were immediately filled with the suspension and left for 10 minutes. Examined under a 10x lens and counted the number of eggs. To determine the EPG, the result obtained was multiplied by 25 [11].

Microscopy research was made on Motic BA410T microscopes and Zeiss AxioImager Z.1 microscope with an associated digital camera and supplied software. The detected parasites were identified by morphological and morphometric parameters according to veterinary parasitological atlases and guidelines [11-12].

2.4 Data analysis

All calculations and statistical data analysis were performed using Microsoft Excel and SPSS 26.0 software. The Wilson method was used to calculate the confidence interval (CI) [13]. To analyze the relationship of infection rates with the characteristics of keeping and age of horses, the Chi-square test and the Bonferroni-adjusted z-test for multiple comparisons (in the case of 3 or more compared groups) were used. The animals were divided into 4 age groups: from 1.5 to 12 months, from one to three years, from three to 10 years, from 10 to 26 years accordingly. We compared infection rates depending on the type

of management: keeping on the farm or with private owners. We also compared the infection in different conditions for keeping horses - herd or stable. For the (χ^2) test, p-value <0.05 was considered significant, whereas p-value >0.05 considered non significant.

3 Results

Parasites of the gastrointestinal tract were registered by screening of 271 horses, which accounted for 47.5% of the total number of examined animals. The results are presented in Table 1. Nematodes Strongylidae gen. sp. were detected in 44% of horses (251 positive samples) (Figure 2), *Parascaris* sp. in 10.3% of animals (60 samples) (Figure 3), *Oxyuris equi* was found in 1.4% of horses (8 samples) (Figure 4). *Eimeria leuckarti* was found in only three foals (0.5%) (Figure 5). No cestodes or trematodes as well were found.

Table 1. General data of parasitological examination of horses in Moscow and the Moscow region (n=571).

Parasites detected	PS	Prevalence, % (CI 95%)	EPG/OPG [average (min-max)]
Total	271	47.5 (43 – 51)	-
Nematodes:			
Strongylidae gen. sp.	251	44 (39 – 48)	34.5 (0.75 - 625)
<i>Parascaris</i> sp.	60	10.5 (8 – 13)	83.1 (0.25- 817)
<i>O. equi</i>	8	1.4 (0.7 – 2)	108 (2 – 405)
Protozoa:			
<i>Eimeria leuckarti</i>	3	0.5 (0.1 – 1.5)	12 (6-18)
Combined infections:			
Strongylidae gen. sp., <i>Parascaris</i> sp.	41	7.2 (5.3 – 9.6)	-
Strongylidae gen. sp., <i>O. equi</i>	7	1.2 (0.6 – 2.5)	-
Strongylidae gen. sp., <i>Parascaris</i> sp., <i>O. equi</i>	1	0.2 (0 – 0.9)	-
<i>Parascaris</i> sp., <i>E. leuckarti</i>	2	0.4 (0.1 – 1.2)	-

PS - number of positive samples, pcs. CI - Confidence Interval.



Fig. 2. Strongylidae gen.sp. eggs. Scale bars = 20 μ m.

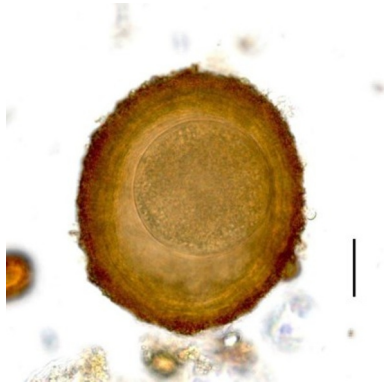


Fig. 3. *Parascaris* sp. egg. Scale bars = 20 μ m.



Fig. 4. *Oxyuris equi* eggs. Scale bars = 20 μ m.



Fig. 5. *Eimeria leuckarti* oocyst. Scale bars = 20 μ m.

Combined infections were recorded quite often: in 7% of samples (from 41 animals) a combined infection of *Parascaris* sp. and Strongylidae gen. sp., was noticed by six animals of Strongylidae gen. sp. and *O. equi* (1%). Two foals co-infected with *Parascaris* sp., *E. leuckarti* (0.4%) and one adult horse had Strongylidae gen. sp., *Parascaris* sp. and *O. equi*. The strongylid EPG averaged 34.5 eggs, *Parascaris* sp. EPG averaged 83.1 specimens. The *Oxyuris equi* EPG average was 108 specimens, *E. leuckarti* OPG - 12.

Table 2 shows the results of a parasitological research across different age groups of horses.

Table 2. Results of parasitological examination of horses of different age groups (n=571).

Parasites detected	Foals up to 12 months, n=34		Young animals aged from 1 to 3 years, n=71		Horses aged from 3 to 10 years, n=152		Horses over 10 years of age, n=314	
	PS	Prevalence, % (CI 95%)	PS	Prevalence, % (CI 95%)	PS	Prevalence, % (CI 95%)	PS	Prevalence, % (CI 95%)
Total:	15	44.1 (28 – 60)	48	67.6 (56 – 77)	84	55.3 (47 – 52)	124	39.5 (34 – 44)
Nematodes:								
Strongylidae gen. sp.	10	29.4 (16 – 46)	44	62.0 (50 – 72)	78	51.3 (43 – 59)	119	37.9 (32 – 43)
<i>Parascaris</i> sp.	9	26.5 (14 – 43)	22	31.0 (21 – 42)	20	13.2 (8 – 19)	9	2.9 (1 – 5)
<i>Oxyuris equi</i>	0	-	6	8.5 (3 – 17)	0	-	2	0.6 (0.1 – 2.2)
Protozoa:								
<i>Eimeria leuckarti</i>	2	5.9 (1 – 1.9)	1	1.4 (0.2 – 7)	0	-	0	-

PS - number of positive samples, pcs. CI - Confidence Interval.

Comparison of parasitic infection levels depending on the age of the horse was carried out using the chi-square test, which showed the presence of a relationship ($\chi^2(15) = 156.922, p < 0.001$). A pairwise comparison of infection rates using a Bonferroni-corrected multiple comparison z-test showed that among horses older than 10 years, the infection rate was generally lower than in the 1–3 year old groups ($p < 0.001$) and in the 3–10 year old group ($p = 0.008$). The infection with Strongylidae gen. sp and *Parascaris* sp. was higher among horses in the 1-3 year old group. Comparing with the horses under the age of 1 year ($p = 0.011$) and older than 10 years ($p = 0.001$), the infection with Strongylidae gen. sp was higher among horses in the 1-3 year old group. For *Parascaris* sp. compared to horses aged 3-10 years ($p = 0.009$) and older 10 years ($p < 0.001$), the infection in the 1-3 year old group also was higher. Similarly, infection with *O. equi* is significantly higher in horses aged 1-3 years compared with horses older than 10 years ($p < 0.001$). Horses under 12 months of age were significantly more likely to have *Parascaris* sp. compared to horses aged 3-10 years and compared to horses over 10 years of age ($p < 0.001$). Also, among horses aged 3-10 years, Strongylidae gen. sp. ($p = 0.036$) and *Parascaris* sp. ($p < 0.001$) has been discovered.

In Table 3 we conducted the results of a parasitological study of horses kept in horse farms and kept by private owners as well. Comparison of infection rates depending on the type of enterprise using the Chi-square test did not reveal the relationship of these variables ($\chi^2(5) = 6.598, p = 0.252$).

Table 4 shows the results of a parasitological examination of horses of different conditions for keeping horses - herd or stable ones. The results of the Chi-square test showed a relationship between the level of infection in horses and the conditions of their keeping ($\chi^2(10) = 39.908, p < 0.001$). Thus, herd horses are statistically significantly more likely than those living in stables to be infected with *Parascaris* sp. ($p < 0.001$) and *Oxyuris equi* ($p = 0.002$).

Table 3. Formatting sections, subsections and subsections. Results of a parasitological examination of horses kept in horse farms and by private owners (n=571).

Parasites detected	Type of management			
	Private owners, n=78		Horse farms, n=493	
	PS	Prevalence, % (CI 95%)	PS	Prevalence, % (CI 95%)
Total	40	51.3 (40 – 62)	231	46.9 (42 – 51)
Nematodes:				
Strongylidae gen. sp.	37	47.4 (36 – 58)	214	43.4 (39 – 47)
<i>Parascaris</i> sp.	3	3.8 (1 – 10)	57	11.6 (9 – 14)
<i>O. equi</i>	2	2.6 (0.7 – 8)	6	1.2 (0.5 – 2.6)
Protozoa:				
<i>Eimeria leuckarti</i>	0	-	3	0.6 (0.2 – 1.7)

PS - number of positive samples, pcs. CI - Confidence Interval.

Table 4. Formatting sections, subsections and subsections. Results of a parasitological study of horses under different conditions (n=571).

Parasites detected	Conditions for keeping horses			
	Herd, n=153		Stable, n=418	
	PS	Prevalence, % (CI 95%)	PS	Prevalence, % (CI 95%)
Total:	73	47.7 (39 – 55)	198	47.4 (42 – 52)
Nematodes:				
Strongylidae gen. sp.	63	41.2 (33 – 49)	188	45.0 (40 – 49)
<i>Parascaris</i> sp.	33	21.6 (15 – 28)	27	6.5 (4 – 9)
<i>O. equi</i>	6	3.9 (1 – 8)	2	0.5 (0.1 – 1.7)
Protozoa:				
<i>Eimeria leuckarti</i>	2	1.3 (0.3 – 4.6)	1	0.2 (0 – 13)

PS - number of positive samples, pcs. CI - Confidence Interval.

4 Discussion

According to the results of the work carried out, we revealed the infection of the gastrointestinal tract with parasites in 47.5% of the examined horses. The main representatives of the parasite fauna are nematodes, among them strongylids and parascarids are the most frequently recorded. In our study, 44% of all examined horses were infected with strongylids. The peak of horses' infections was noted in the age group between 1 to 3 years (62%), less often recorded in the group from three to ten years (51.3%), in horses older than 10 years in 37.9% of samples and most rarely in young animals up to one years - 29.4%.

Over the past 15 years, in the territory of the central region of Russia, studies of horses have been carried out separately and locally. In horse breeding farms and enterprises of the

Kostroma and Nizhny Novgorod regions, strongylids were registered by 48% and 49.2% of horses, respectively [14]. Horses of all ages were studied in private stables in the Mozhaisk, Noginsk, and Krasnogorsk regions: strongylids were found in 35.2%, 85%, and 35% of horses [15]. In the Ryazan region, strongylids were found in 50-71.4% of horse breeding farms and in 50% of horses of a stud farm [15-16]. In the Kostroma region, 63.5% of foals under one year and 52% of horses aged 3-9 years are infected strongylids. Infection of horses older than nine years is reduced to 30% [14]. On the territory of the Moscow region, these studies were carried out only in individual farms with a small sample of the studied specimens. In spring, 31.9-47.4% of horses from private farms in the Moscow region are infected strongylids, in autumn the figure rises to 52.1% [17-18]. In private stables, infection was noted from 77% to 90.5% [19-20]. At the Moscow Hippodrome, the infection rate of horses from 1.5 to 2 years old was 75.6% [21].

The prevalence of *Parascaris* sp. according to our data was 10.3%. The most infected horses were about 1-3 years old (31%), slightly less showed the foals under 12 months old (26.5%), significantly less the horses from three to ten years old (13.2%) and much less over 10 years old (2.9%). Herd horses are more likely than those living in stable conditions to be infected with parascarids. These data are consistent with the data in the Ryazan (9.9%) and Moscow regions (3.9%) obtained earlier [14, 18]. However, in other papers we find higher infection rates in the Kostroma region - 26.6%, in the Nizhny Novgorod region - 36.5%, in the Mozhaisk region 20.5% [14-15], and in the Moscow region - 64.1% [20]. In the Ryazan region, mares 3-9 years old are more infected with *Parascaris* sp. - 16%, foals under two years old and animals over nine years old are less. In the Nizhny Novgorod region, 46% of foals under three years of age are infected, adults are infected by 21.7% [14]. In spring, parascarids infection is lower - 9.8%, in autumn it increases - 22.8% [17].

It is now believed that *Parascaris equorum* and *P. univalens* infect horses. Both species are found in the small intestine of horses. *P. equorum* is a well-known equine ascarid species, but *P. univalens* is distributed mainly in America and Switzerland so it is often overlooked. There are contradictions in the opinions of scientists because these two ascarid species are difficult to distinguish morphologically [22]. J.F. Gao et al. (2018) compared *P. equorum* and *P. univalens* mitochondrial genome data. The findings may provide evidence that *P. equorum* and *P. univalens* may represent the same species [23].

Our research showed the average values of infection of horses with strongylids and parascarids which is most likely due to a large sample of the studied animals in different farms in various territories. In some farms, the infection rates of animals are quite high and even can reach maximum values (up to 100%). However, with statistical data processing, we obtain more reliable average values applicable to the horse population commonly. The patterns we have identified are consistent with the work of other authors in different countries all over the world. Parascarids and cyatostomines are currently the most common and pathogenic parasites in horse populations [3, 4, 6, 24-26].

Oxyuris equi infection was 1.4%. However, in our work, we did not conduct a targeted study of the spread of oxyurids in horses, for this it is necessary to use the method of taking scrapings from the perianal folds or the "Scotch tape test". The cases identified by us were determined by the flotation method, which is not a typical diagnostic method for *O. equi* [12, 27].

Coccidia *Eimeria leuckarti* has been discovered and described in Russia in 2016 firstly during a study of sports horses in the Lyubertsy district of the Moscow region. This pathogen has a global range, but local focal detection and is characterized by low infection rates in animals [28]. We registered 3 cases of infection with coccidia: 2 cases in foals under 1 year and 1 case in a horse 2.5 years. In most cases, animals excreting *E. leuckarti* oocysts are not recorded clinical signs at all or temporary diarrhea was observed [29].

Clinical manifestations of parasites of the gastrointestinal tract are not specific, that is why the most reliable for lifetime diagnosis is laboratory testing of feces. Taking into account the intensity of infection becomes a key factor in the development of anti-epizootic programs. The fight against intestinal helminthiasis of horses is carried out mainly with the help of chemicals, but one should not forget about the development of anthelmintic resistance during the prophylactic use of anthelmintic agents without prior diagnosis.

5 Conclusion

By examining the number of horses in the territory of central Russia, gastrointestinal tract parasites were found in 47.5% of the animals. Nematodes Strongylidae gen. sp. were recorded in 44%, *Parascaris* sp. in 10.3%, accidental detection during flotation *Oxyuris equi* in 1.4%, *Eimeria leuckarti* was found in 0.5%. No cestodes or trematodes were found. The main representatives of the parasite fauna are nematodes, among them strongylids and parascarids are the most frequently recorded. The most infected group of horses was between 1-3 years of age.

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