

PARASITES OF PRZEWALSKI'S HORSES (*EQUUS FERUS PRZEWALSKII*) IN ASKANIA NOVA BIOSPHERE RESERVE (UKRAINE) AND ORENBURG STATE NATURE RESERVE (RUSSIA)

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Received: 15.02.2019. Revised: 04.05.2019. Accepted: 08.05.2019.

The results of multi-year parasitological studies of the Przewalski's horse *Equus ferus przewalskii* from Askania Nova Biosphere Reserve, Ukraine (1972–2018) and from Orenburg State Nature Reserve, Russia (2015–2018) are presented. Multi-year coprological studies on the horses have revealed significant relations between the level of horse infection with intestinal parasites and the type of horse-keeping conditions (zoo exhibition paddocks or steppe enclosures). Two peaks (spring and autumn) of the horse infection with strongylid nematodes were documented. Forty-five species of helminths (two species of cestodes and 43 of nematodes) as well as two species of gastric botflies of the genus *Gastrophilus* were found in the Przewalski's horses in Askania Nova Biosphere Reserve. The structure of the parasite community was multimodal, with dominant, subdominant, background and rare species. A comparison of the Przewalski's horse parasite communities from Askania Nova Biosphere Reserve and Orenburg State Nature Reserve revealed low species diversity (20 species) and a bimodal structure of the parasite community in horses in Orenburg State Nature Reserve. We suppose that regular examinations of the Przewalski's horse infection with parasites are necessary to monitor the parasitological situation in both Protected Areas to prevent an increase in horse infection with these parasites to clinically significant levels.

Key words: cyathostomes, Gastrophilidae, level of horse infection, parascarids, parasite fauna, strongilides

Introduction

The Przewalski's horse *Equus ferus przewalskii* Poliakov, 1881 is a wild species of Equidae that previously lived in a large area of Central Asia from southern Russia to Kazakhstan, Mongolia and northern China. From the end of the XIX century, the population of Przewalski's horses went into rapid decline due to the expansion of livestock pastures and over-hunting; the last Przewalski's horses vanished from the wild in the late 1960s (Boyd & Houpt, 1994). A small number of horses, preserved in several European and American zoos, were used to restore this species in the wild. Nowadays, the Przewalski's horses are kept in 12 large breeding and re-introduction centres in Europe and Asia (Zimmermann, 2005; Bakirova & Zharkikh, 2019).

The Askania Nova Biosphere Reserve (Khererson Region, Ukraine) is the oldest and one of the largest breeding centres for Przewalski's horses. The number of Przewalski's horses in Askania Nova Biosphere Reserve ranged from 9 (in 1972) to 126 individuals (in 1993). Now the population is maintained at about 60–70 individuals.

The Federal State Funded Institution «Joint Directorate of State Nature Reserves «Orenburg» and «Shaitan-Tau»» started a Programme on establish-

ing a semi-free population of Przewalski's horse in Orenburg State Nature Reserve in 2015 (Bakirova & Zharkikh, 2019). Totally 38 horses transported from the breeding centres of France (Association pour le Cheval de Przewalski: TAKH) and Hungary (Hortobagy National Park) in 2015–2017 are kept in Orenburg State Nature Reserve (Russia) on 31 March, 2019.

Regular monitoring parasitological studies of Przewalski's horses kept in zoos, state reserves and breeding centres are conducted in various countries (Elias et al., 2002; Kuzmina et al., 2009, 2017; Painer et al., 2011; Zvegintsova & Yasynetskaya, 2018). However, most of these studies were carried out using only coprological methods, which do not allow examination the species composition of these parasites. Detailed studies of the species composition of a Przewalski's horse parasite community have been carried out from the 1970s in Askania Nova Biosphere Reserve (Dvojnjos & Kharchenko, 1994; Kuzmina et al., 2009) and in the Chernobyl Exclusion Zone (Slivinska & Dvojnjos, 2006). Pilot studies of the intestinal parasites of a new semi-free Przewalski's horse population were conducted in Orenburg State Nature Reserve in 2016 (Kuzmina et al., 2017).

The purpose of this study was to analyse the long-term monitoring data on the Przewalski's horse infec-

tion with various groups of parasites in Askania Nova Biosphere Reserve, as well as to compare the species composition and the strongylid nematodes community structure in horses from Askania Nova Biosphere Reserve and Orenburg State Nature Reserve.

Material and Methods

Multi-year monitoring data on the Przewalski's horse infection with main groups of parasites collected in Askania Nova Biosphere Reserve from 1984 to 2018 were used for this study. In Askania Nova Biosphere Reserve, the horses are kept in groups of different age and sex composition; several groups are kept in small zoo exhibition paddocks ranging 500 m² to 0.1 km² without grass. Several breeding groups and one only-female group are kept under semi-free conditions in large enclosures (0.75–15 km²) with natural steppe vegetation. The Askanian Przewalski's horses have not been treated with anthelmintic medication over the past 30 years.

In Orenburg State Nature Reserve, several breeding and single-sex groups Przewalski's horses are kept in a fenced area called Pre-Urals Steppe of 165.38 km² with natural steppe grassland vegetation. All horses are dewormed with the «Univerm» (0.2% aversectin C, PharmBioMed, Russia) every 8–10 months.

In Askania Nova Biosphere Reserve, regular coprological examinations of the horses were carried out by Fülleborn and McMaster flotation methods (Kotelnikov, 1984; Herd, 1992). The level of horse infection was calculated as the number of helminth eggs per 1 g of feces (EPG – eggs per gram). In Orenburg State Nature Reserve, only the McMaster method was used.

In Askania Nova Biosphere Reserve, the species composition of the parasite community was studied using the «post mortem» method of full and partial helminthological autopsy (Dvojnjos & Kharchenko, 1994) for 48 horses, as well as the *in vivo* diagnostic deworming method (Kuzmina et al., 2009, 2017) for 24 horses.

In Orenburg State Nature Reserve, only the diagnostic deworming method was used (see Kuzmina et al., 2017). The larvae of gastric botflies of the genus *Gastrophilus* Leach, 1817 (Diptera: Gastrophilida) were collected from 105 Przewalski's horses from three months to 32 years old. Helminths and botflies larvae were identified under a light microscope using morphological criteria (Grunin, 1953; Ivashkin & Dvojnjos, 1984; Dvojnjos & Kharchenko, 1994).

The prevalence (P) of horse infection with separate parasites was determined as the proportion of infected horses to their total number in per cents (%). The intensity of horse infection (I) was calculated only for horses studied by the «post mortem» method of helminthological autopsy. All strongylid species of Askanian Przewalski's horses were distributed into ten prevalence classes (1–10%, 11–20%, 21–30%, 31–40%, 41–50%, 51–60%, 61–70%, 71–80%, 81–90%, 91–100%).

Results

According to the results of multi-year coprological studies (1984–2018), the intestinal strongylides (Nematoda: Strongylidae) were the dominant group of parasites of Przewalski's horses in Askania Nova Biosphere Reserve. The prevalence (P) of horse infection was 100%; the average level of infection was 665.7 ± 10.1 EPG (lim. 25–4200 EPG) (Table 1).

The level of Przewalski's horse infection with parascarisids *Parascaris equorum* Goeze, 1782 (Nematoda: Ascarididae) was low (132.5 ± 33.0 EPG); this species parasitised mainly in young horses (6 months to 2 years old) with P up to 76%; in adult horses, P was 1.9–8.3% (Table 1). The highest level of infection with both groups of parasites was observed in the horses kept in small exhibition paddocks of Askania Nova Zoo.

Table 1. The level of Przewalski's horse infection with the main helminth groups in Askania Nova Biosphere Reserve, Ukraine (coprological data of 1984–2018)

Helminths	Age group	Zoo exhibition paddocks		Breeding group		Only-female group		Only-male group			
		EPG	P, %	EPG	P, %	EPG	P, %	Enclosures №3, №4 (0.708–0.898 km ²)		Enclosure №10 (0.0349 km ²)	
Strongylidae	adult	888.7 ± 42.7	100	705.5 ± 22.0	100	656.3 ± 46.3	100	600.9 ± 24.3	100	632.8 ± 21.1	100
	juvenile	768.1 ± 109.0	100	646.6 ± 60.2	100	751.9 ± 262.7	100				
Parascarisidae	adult	41.7 ± 6.1	7.5	45.3 ± 7.6	8.3	97.8 ± 48.1	5.5	52.1 ± 14.6	2.6	109.4 ± 44.3	1.9
	juvenile	848.1 ± 248.0	72.2	181.1 ± 35.4	65.2	169.3 ± 99.6	76.0				

Note: EPG (average ± SD); P – prevalence in %.

An analysis of the level of Przewalski's horse infection with strongylides in different seasons revealed a prominent peak of horse infection in spring (736.0 ± 17.5 EPG); it was significantly higher than in winter (625.6 ± 22.5 EPG), in summer (615.1 ± 25.1 EPG), and in autumn (633.7 ± 17.0 EPG). The highest level of infection with *P. equorum* was registered in autumn (517.2 ± 202.3 EPG). The highest level of infection with parascarids (6225 EPG) was registered in a foal up to 1-year-old; however, no clinical signs of such high infection were observed.

All Przewalski's horses from Orenburg State Nature Reserve were infected with strongylides ($P = 100\%$). The highest level of infection with strongylides was observed in July and August (1833.0 ± 240.9 , lim. 250–3525 EPG), the lowest in November and December (790.3 ± 151.4 , lim. 50–1900 EPG). The level of infection increased in March and April after the snow cover had melted (892.9 ± 189.2 , lim. 50–2150 EPG). Although a rather high level of helminthic infection, no clinical signs of strongylidosis were observed. To date, no parascarid eggs were found in the faecal samples.

Forty-five species of helminths (two species of cestodes and 43 species of nematodes) and two species of botflies from the genus *Gastrophilus* were documented in the Przewalski's horses from Askania Nova Biosphere Reserve from 1972 to 2018.

Cestode species *Anoplocephala perfoliata* Goeze, 1782 (Cestoidea: Anoplocephalidae) was found in 41.7% of the horses with an intensity (I) from 2 to 177 specimens. This species was not registered in Askania Nova Biosphere Reserve before 1999. In our opinion, it might be transported to the Reserve in the late 1990s with Przewalski's horses and/or with other species of equids from other zoos. A larval stage of *Echinococcus granulosus* Rudolphi, 1801 (Cestoidea: Taeniidae) was found in two Przewalski's horse females (13 and 31 years old) in the number of four and one specimens respectively. This cestode is very rare for Przewalski's horses; in Askania Nova Biosphere Reserve, it was found only in a camel, *Camelus bactrianus* Linnaeus, 1758.

Nematodes parasitised all Przewalski's horses examined ($P = 100\%$; $I = 1313.9 \pm 292.2$). Strongylids were found to be the main group of nematodes; 37 species from two subfamilies: Strongylinae Railliet, 1893 (six species) and Cyathostominae Nicoll, 1927 (31 species) were found (Table 2).

From the subfamily Strongylinae, the two species from the genus *Strongylus* (*Strongylus vulgaris* Looss, 1902 and *S. edentatus* Looss, 1900) (Table 2). In 1970s, the Przewalski's horses were infected with *Strongylus equinus* Muller, 1780; however, in recent studies (Kuzmina et al., 2009; Zvegintsova & Yasynetskaya, 2018), this species was not detected. Four species from the genus *Triodontophorus* (*T. serratus* Looss, 1900, *T. brevicauda* Boulenger, 1916, *T. nipponicus* Yamaguti, 1943 and *T. minor* Looss, 1900) and *Craterostomum acuticaudatum* Kotlan, 1919 were found.

The highest species diversity (31 species from nine genera) was documented for cyathostomes (subfamily Cyathostominae). From one to eight species per one genus were detected in various cyathostome species (Table 2). Cyathostominae were the dominant group of parasites in the Przewalski's horses – according our data they composed more than 99.4% of the total strongylid number (Kuzmina et al., 2009; present data).

An analysis of prevalence classes reveals that the multimodal strongylid community with dominant ($P = 80\text{--}100\%$), subdominant (50–80%), background (30–50%) and rare (< 30%) species was observed. The distribution presents a multimodal type of the parasite community.

Of other groups of nematodes registered in Askanian Przewalski's horses, *Oxyuris equi* Schrank, 1788 (Oxyurata: Oxyuridae) ($P = 54.2\%$, $I = 1\text{--}828$), which parasitises in the large intestine, was the most common. The nematode *Habronema microstoma* Schneider, 1866 (Spirurata: Habronematidae) ($P = 37.5\%$, $I = 3\text{--}1752$) which parasitises the gastric mucosa was one of the most abundant; these nematodes accounted for up to 8.2% of the total number of nematodes found in a horse. The pulmonary nematode *Dictyocaulus arnfieldi* Rudolphi, 1809 is a rare parasite in Przewalski's horses. It was found only once in a number of seven specimens. *Setaria equina* Abildgaard, 1789 (Filariata: Setariidae) was found in 29.2% of horses in one specimen.

Larval stages of gastric botflies were found in all Przewalski's horses examined ($P = 100\%$; $I = 177.9 \pm 14.0$). *Gastrophilus intestinalis* (De Geer, 1776) parasitised 88.5% of horses; *G. haemorrhoidalis* (Linnaeus, 1758) – 75%. No significant differences in level of infection with gastric botflies were found between horses of different age groups ($p > 0.05$).

Table 2. Strongylid species found in the Przewalski's horses (*Equus ferus przewalskii*) in the Askania Nova Biosphere Reserve and Orenburg State Nature Reserve

Species	Askania Nova Biosphere Reserve (Ukraine)		Orenburg State Nature Reserve (Russia)	
	P, %	I	P, %	I
Subfamily Strongylinae				
1. <i>Strongylus vulgaris</i> Looss, 1902	58.3–95.8	287	16.7	ND
2. <i>S. edentatus</i> Looss, 1900	41.7–87.5	41	–	–
3. <i>S. equinus</i> * Muller, 1780	12.5	4.5	–	–
4. <i>Triodontophorus serratus</i> Looss, 1900	25–62.5	64	33.3	ND
5. <i>T. brevicauda</i> Boulenger, 1916	31.3	5	–	–
6. <i>T. nipponicus</i> Yamaguti, 1943	6.3	2	–	–
7. <i>T. minor</i> Looss, 1900	4.2	ND	–	–
8. <i>Craterostomum acuticaudatum</i> Kotlan, 1919	8.3–31.3	14	–	–
Subfamily Cyathostominae				
9. <i>Cyathostomum catinatum</i> (Mehlis, 1831)	75–100	427.1	100	ND
10. <i>C. pateratum</i> (Yorke et Macfie, 1919) Cram, 1924	31.3–98.5	7	100	ND
11. <i>C. tetracanthum</i> (Mehlis, 1831) Molin, 1861	12.5	ND	–	–
12. <i>Coronocyclus coronatus</i> (Looss, 1900)	75–81.3	7	100	ND
13. <i>C. labiatus</i> (Looss, 1902) Hartwich, 1986	50–62.5	20	50	ND
14. <i>C. labratus</i> (Looss, 1900) Hartwich, 1986	31.3–50	38	50	ND
15. <i>C. sagittatus</i> (Kotlan, 1920) Hartwich, 1986	4.2–12.5	4	–	–
16. <i>Cylicocyclus nassatus</i> (Looss, 1900) Foster, 1936	62.5–100	283	100	ND
17. <i>C. leptostomus</i> Kotlan, 1920	56.3–98.5	6	100	ND
18. <i>C. insigne</i> (Boulenger, 1917) Chaves, 1930	56.3–98.5	50	83.3	ND
19. <i>C. ashworthi</i> (LeRoux, 1926) McIntosh, 1933	62.5–83.3	55	83.3	ND
20. <i>C. radiatus</i> (Looss, 1900) Chaves, 1930	50	4	–	–
21. <i>C. elongatus</i> (Looss, 1900) Ershov, 1943	16.7–43.8	10	16.7	ND
22. <i>C. ultrajectinus</i> (Ihle, 1920) Ershov, 1943	6.3	1	–	–
23. <i>C. brevicapsulatus</i> Ihle, 1929, Ershov, 1943	12.5	4	–	–
24. <i>Cylicostephanus longibursatus</i> (Yorke & Macfie, 1918) Cram, 1924	68.8–100	60	100	ND
25. <i>C. goldi</i> (Boulenger, 1917) Lichtenfels, 1975	62.5–100	39	83.3	ND
26. <i>C. minutus</i> (Yorke and Macfie, 1918) Cram, 1924	62.5–91.7	50	100	ND
27. <i>C. calicatus</i> (Looss, 1900) Ihle, 1922	62.5–75	18	100	ND
28. <i>C. bidentatus</i> (Ihle, 1925) Lichtenfels, 1975	6.3–32.2	6	–	–
29. <i>C. hybridus</i> (Kotlan, 1920) Cram, 1924	4.2–12.5	5	–	–
30. <i>C. asymmetricus</i> (Theiler, 1923) Ihle, 1925 Cram, 1925	6.3	7	–	–
31. <i>Cylicodontophorus bicoronatus</i> (Looss, 1900)	75–79.2	141	33.3	ND
32. <i>Poteriostomum imparidentatum</i> (Quiel, 1919)	4.2–50	2	–	–
33. <i>P. ratzii</i> (Kotlan, 1919) Yorke and Macfie, 1920	12.5	4	–	–
34. <i>Parapoteriostomum mettami</i> Hartwich, 1986	12.5–37.5	8	–	–
35. <i>P. euproctus</i> (Boulenger, 1917) Hartwich, 1986	8.3–31.3	3	–	–
36. <i>Petrovinema poculatum</i> (Looss, 1900) Ershov, 1943	12.5–37.5	6	50	ND
37. <i>Gyalocephalus capitatus</i> Looss, 1900	20.8–75	38	–	–

Note: * – species found in 1970s (see Dvojnjos & Kharchenko, 1994); ND – no detected; P – prevalence, in %, I – average intensity of horse infection.

Studies on the Przewalski's horse parasite community conducted in the Orenburg State Nature Reserve showed that from the first group of horses transported to this Protected Area from France, all animals (100%) were infected with parasites within four months after their transportation. Totally, 20 species of parasites were found; larvae of gastric botflies *Gastrophilus* sp. (P = 100%), gastric nematode *Habronema muscae* (P = 50%) and 18 species of strongylid nematodes from eight genera (see Kuzmina et al., 2017). Two species of large strongylids (subfamily Strongylinae): *Strongylus vulgaris* (P = 16.7%) and *Triodontophorus serratus* (P = 33.3%) were found. From small strongylids (sub-

family Cyathostominae), eight species (*Cyathostomum catinatum*, *C. pateratum*, *Cylicocyclus nassatus*, *C. leptostomus*, *Cylicostephanus longibursatus*, *C. minutus*, *C. calicatus* and *Coronocyclus coronatus*) were found in all horses (P = 100%). Three species (*Cylicostephanus goldi*, *Cylicocyclus insigne* and *C. ashworthi*) were found in 88.3% of horses. Three species (*Coronocyclus labratus*, *C. labiatus* and *Petrovinema poculatum*) were found in 50% of horses. And two species were rare: *Cylicodontophorus bicoronatus* (P = 33.3%) and *Cylicocyclus elongatus* (P = 16.7%). The structure of the parasite community was destructed and similar to the bimodal («core – satellite mode»).

Discussion

In general, the level infection with strongylids was significantly lower in the Przewalski's horses kept in large steppe enclosures than in small zoo exhibition paddocks in Askania Nova Biosphere Reserve. In small paddocks, due to the higher number of horses the grass and ground are much more contaminated with strongylid infective larvae which facilitate the horse infection with these parasites.

Multi-year observations of the level of infection with strongylids was found to be fluctuating (the trend line $y = -4.9543x + 767.11$). Moreover, a steady tendency in decreasing of the infection level even without special antiparasitic actions was observed. We are of the opinion, that the level of the Przewalski's horse infection currently is ecologically balanced in Askania Nova Biosphere Reserve. Therefore, no clinical signs or any adverse effects of parasites to the physiological state of horses were detected.

Although the population density of Przewalski's horses in Orenburg State Nature Reserve is much lower than in Askania Nova Biosphere Reserve, the level of their infection with strongylides is quite high (Zharkikh et al., 2019). To our opinion, this is probably due to the ongoing adaptation of the horses, recently transported from European semi-reserves, to the dry climatic conditions of Orenburg State Nature Reserve.

In Askania Nova Biosphere Reserve, the multimodal type of parasite community is typical for the horses that do not get regular anthelmintic treatments (Kuzmina et al., 2009). The structure of the parasite community of Przewalski horses in Orenburg State Nature Reserve was destructed and similar to the bimodal («core – satellite mode»), what is typical for horses treated regularly with anthelmintic medication (Kuzmina et al., 2017).

In contrast to Askania Nova Biosphere Reserve where several species of equids (Przewalski's horses, kulans *Equus hemionus kulan* Groves et Mazák, 1967, donkeys *Equus asinus* Linnaeus, 1758, etc.) are kept in the same steppe pastures, the new population of Przewalski's horses from Orenburg State Nature Reserve is isolated from other equid species. In this case, these Przewalski's horses do not have the opportunity to exchange parasites with domestic horses; we assume that the species diversity of their parasite community will not increase. However, regular monitoring studies on the level of infection with different groups of parasites, as well as examination of parasite species diversity, are necessary to control the parasitological situation in the Orenburg State Nature Reserve on the whole, and to prevent any possible increasing in the Przewalski's horse infection to clinically significant levels.

Conclusions

Multi-year (1984–2018) coprological studies of the Przewalski's horses from Askania Nova Biosphere Reserve revealed the dependence of the infection level on the horse-keeping conditions: horses from small zoo exhibition paddocks were significantly more infected than horses from large steppe enclosures. A prominent peak of horse infection with strongylides was detected in spring. Despite a high level of infection with strongylides and parascaris detected in some horses, no clinical manifestations of these parasitoses were observed. This reflects the high adaptability of the Przewalski's wild horses to parasites.

Helminthological studies performed in Askania Nova Biosphere Reserve from 1972 to 2018 revealed 45 species of helminths (two species of cestodes and 43 – of nematodes), as well as two species of gastric botflies of the genus *Gastrophilus*. The intestinal parasite community was multimodal, with dominant, subdominant, background, and rare species.

A comparison of the parasite communities of Przewalski's horses from Askania Nova Biosphere Reserve and Orenburg State Nature Reserve revealed a low species diversity and bimodal structure of the parasite community in Orenburg State Nature Reserve. To our opinion, it is associated with regular de-worming of the horses. In order to control the parasitological status of horses in the Orenburg State Nature Reserve and prevent an increase in infection of the Przewalski's horses to a clinically significant level, we suggest carrying out and supporting regular monitoring studies on the level of horse infection with different groups of parasites.

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ПАРАЗИТЫ ЛОШАДЕЙ ПРЖЕВАЛЬСКОГО *EQUUS FERUS PRZEWALSKII* В ЗАПОВЕДНИКАХ «АСКАНИЯ НОВА» (УКРАИНА) И «ОРЕНБУРГСКИЙ» (РОССИЯ)

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Представлены результаты изучения паразитов лошади Пржевальского *Equus ferus przewalskii* из Биосферного заповедника «Аскания-Нова» (Украина) за весь период проведения паразитологических исследований (1972–2018 гг.) и из Оренбургского заповедника (Россия) (2015–2018 гг.). Многолетними копрологическими исследованиями установлена зависимость уровня зараженности лошадей от условий их содержания (экспозиционный вольер зоопарка по сравнению с загонами степи); также выявлен весенний пик зараженности лошадей стронгилидами. У лошадей Пржевальского в «Аскания-Нова» выявлено 45 видов гельминтов (два вида цестод и 43 вида нематод), а также два вида желудочных оводов рода *Gastrophilus*. Структура сообщества кишечных паразитов мультимодальная, с доминантными, субдоминантными, фоновыми и редкими видами. Сравнение сообществ паразитов лошадей Пржевальского из заповедника «Аскания-Нова» и Оренбургского заповедника выявило низкое видовое разнообразие и бимодальную структуру сообщества паразитов у лошадей в Оренбургском заповеднике. Регулярные исследования зараженности лошадей Пржевальского паразитами необходимы как для мониторинга паразитологической ситуации в заповеднике, так и для предотвращения повышения зараженности новой популяции лошадей паразитами до клинически значимого уровня.

Ключевые слова: гастрофилиды, паразитофауна, параскариды, стронгилиды, уровень зараженности, цистостомины