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GASTROINTESTINAL PARASITE COMMUNITY IN A NEW POPULATION OF THE PRZEWALSKI'S HORSE (*EQUUS FERUS PRZEWALSKII*) IN THE ORENBURG STATE RESERVE, RUSSIA

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Gastrointestinal Parasite Community in a New Population of the Przewalski's Horse (*Equus ferus przewalskii*) in the Orenburg State Reserve, Russia. Kuzmina, T. A., Zvegintsova, N. S., Zharkikh, T. L. —

The Przewalski's horse (*Equus ferus przewalskii*, Poljakov, 1881) is an endangered subspecies of wild horses (*Equus ferus*) native to steppes of Central Asia. In 2015, the Program of Establishing of a Semi-Free Population of the Przewalski's Horse in the Orenburg Reserve was launched by FGFI "Orenburg Reserves". The first group of 6 Przewalski's horses (2 males and 4 females) born in the semi-reserve Le Villaret, France, was transported to the Orenburg Reserve. The aim of this work was to investigate the species composition of the intestinal parasite community and to monitor the dynamics of the parasite infection of the newly established Przewalski's horse population. The level of infection by gastrointestinal parasites within the horses was examined by the McMaster method. Gastrointestinal parasites were collected *in vivo* after deworming of the horses with macrocyclic lactone drug "Univerm" (0.2 % aversectin C, PharmBioMed, Russia). Totally, 20 species of parasites were found: 19 species of nematodes (species of the family Strongylidae and *Habronema muscae*) and one species of botflies from the genus *Gasterophilus*. The widest species diversity (18 species from 8 genera) was observed in strongylids: 2 species from the subfamily Strongylinae and 16 species from Cyathostominae. Distribution of strongylid species between ten prevalence classes revealed a bimodal structure ("core-satellite" mode) of the strongylid community. The results obtained in this study are to be considered as the initial data for the further parasitological monitoring of Przewalski's horses at the Orenburg State Reserve.

Key words: Przewalski's horse, *Equus ferus przewalskii*, strongyles, Strongylinae, Cyathostominae, *Gasterophilus*, *Habronema*, Orenburg State Reserve, Pre-Urals Steppe.

Introduction

The Przewalski's horse (*Equus ferus przewalskii*, Poljakov, 1881) is a rare and endangered subspecies of wild horses (*Equus ferus*) which are native to steppes of Central Asia. Until the late 18th century, the natural area of the Przewalski's horses ranged from the Russian Steppes east to Kazakhstan, Mongolia and northern China. In the 19th century the world population of the species went into catastrophic decline due to the combined effects of pasture competition with livestock and over-hunting. The species has been extinct in the wild in 1969; yet, a breeding population has been preserved in zoos since 1899 (Przewalski's horse..., 1994). In 1992, the first two re-introduction projects were launched in Mongolia to restore a population the Przewalski's horse within its native area. At present, 12 large breeding and re-introduction centers for the Przewalski's horse have been established in Europe (France, Hungary, Ukraine) and in Asia (China, Mongolia, Uzbekistan, Kazakhstan, Russia) (Zimmermann, 2004; Bakirova and Zharkikh, 2015, 2016).

In 2015, the "Program of Establishing of a Semi-Free Population of the Przewalski's Horse in the Orenburg Reserve" was launched by the FGFI "Orenburg Reserves". A former military territory of 16,538 ha named Pre-Urals Steppe in the southern part of the region of Orenburg (Russia) was joined to the Orenburg State Reserve on July, 2015, as an area for the re-introduction of Przewalski's horses (Bakirova, 2015). On October 18,

2015, the first group of 6 Przewalski's horses born at the breeding station Le Villaret, Association pour le Cheval de Przewalski: TAKH, France, was transported to the Pre-Urals Steppe. The horses successfully adapted to the harsh climate of the Orenburg region (Bakirova and Zharkikh, 2015, 2016).

Various parasitological studies of the wild Przewalski's horses kept in zoos, semi-reserves and natural reserves have been conducted in different countries (Dvojnjos, 1975; Dvojnjos and Kharchenko, 1994; Epe et al., 2001; Elias et al., 2002; Slivinska and Dvojnjos, 2006; Kuzmina et al., 2009; Painer et al., 2011; Liu et al., 2016). Most of these studies were carried out by coprological methods which determined the presence or absence of certain groups of intestinal parasites and calculating nominal levels of infection by the number of parasite eggs in one gram of feces (EPG). Only a few publications reported information on the species composition and structure of the intestinal parasites (Dvojnjos and Kharchenko, 1994; Slivinska and Dvojnjos, 2006; Kuzmina et al., 2009).

The objective of this study was to investigate the species composition of the intestinal parasite community and to monitor the dynamics of the parasite infection level of the newly established Przewalski's horse population for future ecological and parasitological monitoring of these horses in the Orenburg Reserve. A comparison of the biodiversity of the strongylid community of the recently introduced Przewalski's horse population with the available parasitological data collected from different geographical locations also was of special interest for this study.

Material and methods

Study area

This study was carried out in the Pre-Urals Steppe area (16,538 ha) of the Orenburg Reserve, Russia (51°11'–51°26' N and 56°09'–56°29' E; WGS 84 / UTM zone 40N) in 2015–2017. Before June 2015, the territory was used as pasture for livestock (sheep, cattle, horses) for decades; then it became a part of the Orenburg State Reserve. Since then, domestic animals have not been allowed to enter the area; however, numerous wild animals including roe deer (*Capreolus pygargus*) inhabit it. The Pre-Urals Steppe area was fenced totally in September 2016. The Centre of Reintroduction of the Przewalski's Horse was established in the Pre-Urals Steppe in October 2015. The Centre includes two acclimatization enclosures of 49 ha each with natural steppe vegetation, and several quarantine pens without vegetation with a total area of 1,200 sq. m (Bakirova, 2015; Bakirova and Zharkikh, 2015, 2016).

Przewalski's horses and treatment history

A breeding group of 6 Przewalski's horses consisting of one adult male, 4 adult females and one young colt was studied (table 1). The horses were born at the breeding station Le Villaret, Association pour le Cheval de Przewalski: TAKH (France) where they were kept under semi-free conditions in a fenced pasture of 600 ha (<http://www.takh.org/en/>). All of these horses were identified and examined individually during the study.

These horses had never been treated with any anthelmintics before they were placed into the quarantine pen on 27 August 2015, and then transported to Russia. During the quarantine period in France all the horses were treated with ivermectin on 10 September, 2015 and sprayed with Butox on 17 October, 2015. Further treatments of the horses were not given until our study in August, 2016. On 18 October, 2015, the horses were transported to the Centre of Re-introduction of the Przewalski's Horse. Upon arrival, the horses were placed in a quarantine pen for 5 weeks. Following the quarantine, they were released into an acclimatization enclosure to graze freely on 24 November, 2015. The group was released out of their enclosure into the main territory of Pre-Urals Steppe on October 3, 2016. This was the only group of horses dwelled on the area until the end of the study.

Parasitological methods and statistical analysis

In the Orenburg State Reserve, all horses were regularly examined for the presence of gastrointestinal parasites by several coprological techniques. First, fecal samples were sent to the Belyaevka District Veterinary Office and to the Tatarstan Republic Veterinary Laboratory where they were examined by the qualitative Baermann–Orlov and Fülleborn's flotation techniques (Kotelnikov, 1984). After August, 2016, all coprological examinations were performed using the McMaster method with sensitivity of 25 eggs per 1 g of feces (EPG) (Herd, 1992).

Gastrointestinal parasites were collected from all the Przewalski's horses by the *in vivo* method of diagnostic deworming (Kuzmina et al., 2004, 2005) after treatment of the horses with the macrocyclic lactone drug "Univerm" (0.2 % aversectin C, PharmBioMed, Russia) on 29 August, 2016. The anthelmintic was mixed with oats and provided to the horses individually. Fecal samples (200 g each) were collected from every horse 24, 36 and 48 hours after the treatment. Fecal samples were first washed in isotonic saline solution. Thereafter, all the expelled parasites were collected manually, fixed in 70 % ethanol, and identified under light microscope using morphological criteria (Dvojnjos and Kharchenko, 1994; Lichtenfels et al., 2008).

The prevalence frequency distribution was determined for all strongylid species according to Bucknell et al. (1996). The proportion of each species in the strongylid community of each host was calculated as the number of specimens of the particular species in relation to the total number of strongylids found. Comparison of the biodiversity of strongylid communities was performed using previously published data collected from semi-free Przewalski's horses at the Askania Nova Biosphere Reserve and free-ranging horses from the Chernobyl Exclusion Zone, Ukraine (Kuzmina et al., 2009; Slivinska and Dvojnjos, 2006).

Results

All Przewalski's horses were found to be infected with gastrointestinal parasites (table 1). Coprological studies revealed only strongylid eggs in the feces.

Monitoring studies on the dynamics of strongyle egg expulsion in horse feces showed decrease in EPG levels of infection after anthelmintic treatment which was followed by a low level of infection in winter months and an increase in the EPG levels at the end of winter and in beginning of spring (fig. 1). At the time of diagnostic deworming, the level of infection was considerably high (550 to 2600 EPG). Only one horse was found to be negative for strongyle eggs.

Totally, 20 species of gastrointestinal parasites were found in these Przewalski's horses: 19 species of nematodes and one species of gastric botflies. Nematodes from the family Strongylidae dominated in the parasite community (prevalence = 100 %). Also, the second stage larvae of gastric botflies from the genus *Gasterophilus* were found in the horses (prevalence = 100 %). Gastric nematodes *Habronema muscae* were found in three of six horses (prevalence = 50 %) (table 1).

The greatest species diversity was observed in the intestinal strongylids (Nematoda, Strongylidae): 18 species from 8 genera were found in the Przewalski's horses — 2 species from the subfamily Strongylinae, and 16 species from the subfamily Cyathostominae (fig. 2).

Distribution of strongylid species between ten prevalence classes demonstrated that the general structure of the strongylid community was similar to the bimodal structure ("core-satellite" mode) with dominant and background species (fig. 3).

Comparison of the species composition of the strongylid community in Przewalski's horses from the Orenburg Reserve, Russia, with those from the Askania Nova Biosphere Reserve, and the Chernobyl Exclusion Zone, Ukraine, revealed a depletion of the strongylid community of the newly established horse population. Fewer strongylid species were registered in every genus found in the horses in Pre-Urals Steppe (table 2).

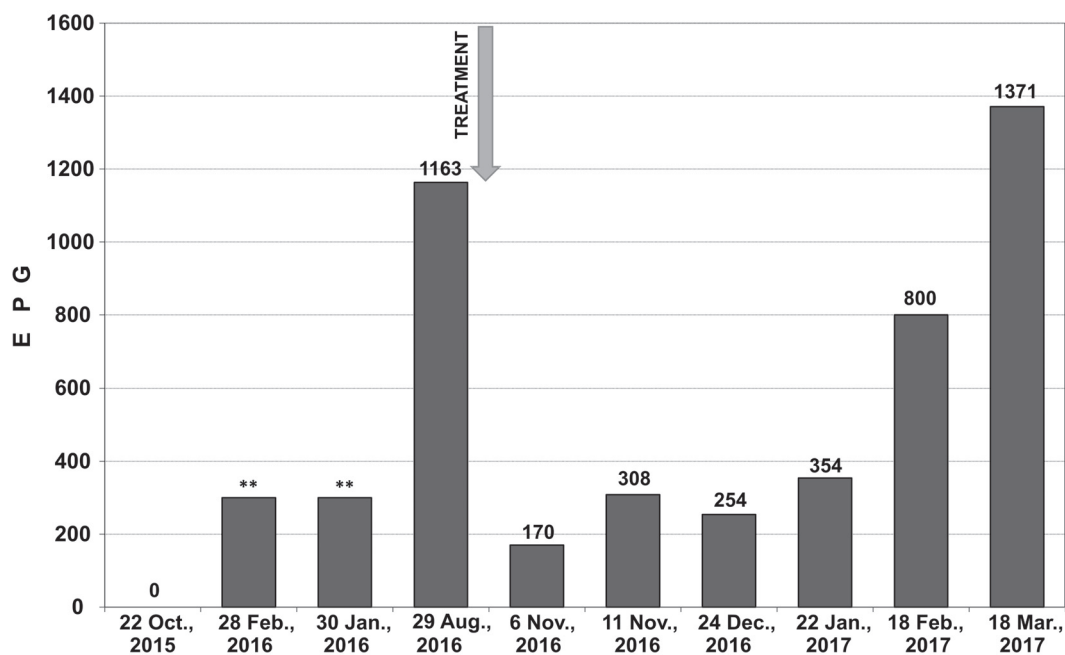


Fig. 1. Dynamics of the average strongyle egg count in the Przewalski's horses in Pre-Urals Steppe, the Orenburg State Reserve, Russia, before and following treatment.

** Data obtained using the qualitative Baermann-Orlov and Fülleborn's flotation techniques.

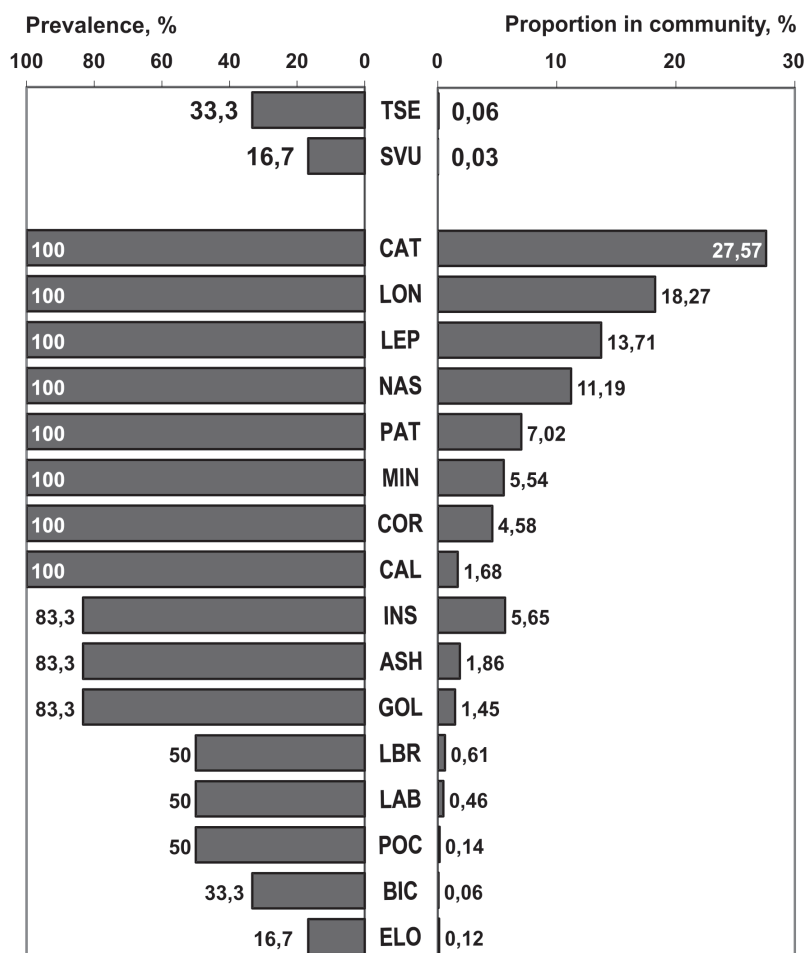


Fig. 2. Nematodes from the family Strongylidae found in Przewalski's horses in Pre-Urals Steppe, the Orenburg State Reserve. Abbreviations: TSE — *Triodontophorus serratus*, SVU— *Strongylus vulgaris*, CAT — *Cyathostomum catinatum*, PAT — *Cya. pateratum*, LON — *Cylicostephanus longibursatus*, MIN — *Cyl. minutus*, CAL — *Cyl. calicatus*, GOL — *Cyl. goldi*, NAS — *Cylicocyclus nassatus*, LEP — *Cy. leptostomus*, INS — *Cy. insigne*, ASH — *Cy. ashworthi*, ELO — *Cy. elongatus*, COR — *Coronocylus coronatus*, LBR — *Cor. labratus*, LAB — *Cor. labiatus*, BIC — *Cylicodontophorus bicoronatus*, POC — *Petrovinema poculatum*.

Table 1. Infection of the Przewalski's horses in Pre-Urals Steppe, Orenburg State Reserve, by gastrointestinal parasites

Horse name and Studbook number	Sex	Age (years) and Date of birth	EPG*	Helminthoscopic study, number of parasites per sample			
				STR	CYA	Gast.	Habr.
Aven (# 4904)	♂	10, 20.06.2006	550	—	60	2	—
Selena (# 5584)	♀	8, 12.05.2008	0	—	407	3	—
Paprika (# 6384)	♂	2, 03.07.2014	2600	1	289	4	—
Sangria (# 5924)	♀	5, 16.05.2011	1650	12	668	1	1
Lavande (# 5956)	♀	4, 30.04.2012	925	9	1095	7	1
Olive (# 6007)	♀	4, 26.05.2012	1250	10	961	3	2
Total				32	3480	20	4

* Only eggs of strongylids were detected in the horses by coprological method.

Abbreviations: EPG — number of eggs per 1g of feces; STR — Strongylinae; CYA — Cyathostominae; Gast. — larvae of *Gasterophilus* sp.; Habr. — *Habronema muscae*.

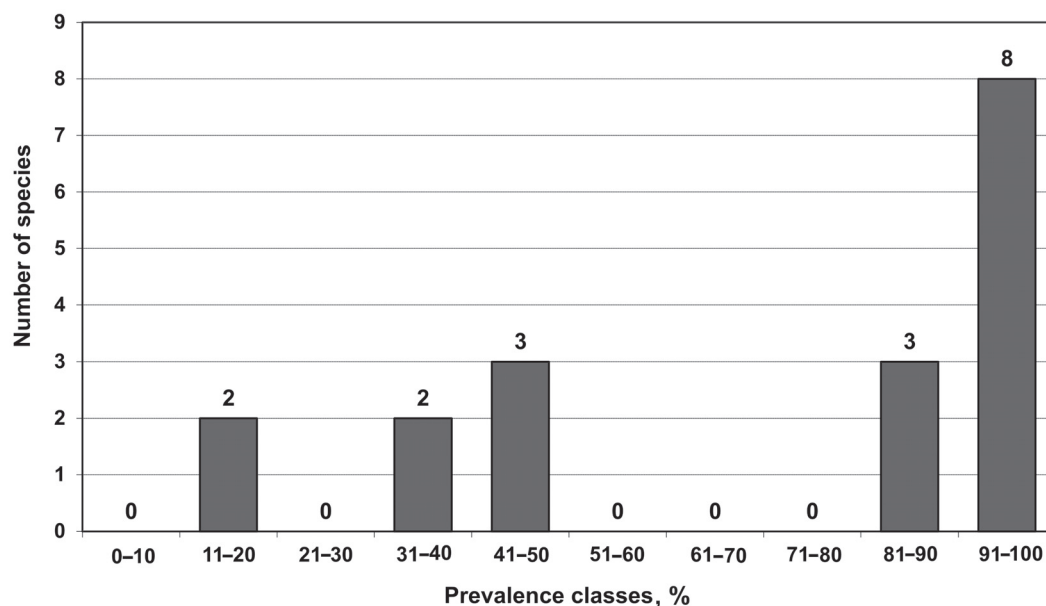


Fig. 3. Distribution of strongylid species from the Przewalski's horses in Pre-Urals Steppe, Orenburg State Reserve, on ten prevalence classes.

Table 2. Strongylid species found in Przewalski's horses in Pre-Urals Steppe, Orenburg State Reserve, Russia, compared to the Askania Nova Biosphere Reserve and the Chernobyl populations, Ukraine

Genera of the Strongylidae	Number of species found		
	Orenburg Reserve (present study)	Askania Nova Biosphere Reserve (Dvojnos, Kharchenko, 1994; Kuzmina et al., 2009)	Chernobyl Exclusion zone (Slivinska, Dvojnos, 2006)
1. <i>Strongylus</i> Müller, 1780	1	3	2
2. <i>Triodontophorus</i> (Looss, 1900) Looss, 1902	1	5	3
3. <i>Craterostomum</i> Boulenger, 1920	—	1	1
4. <i>Cyathostomum</i> Molin, 1861 Hartwich, 1986	2	3	1
5. <i>Coronocyclus</i> Hartwich, 1986	3	4	4
6. <i>Cylicocyclus</i> Ihle, 1922	5	7	5
7. <i>Cylicostephanus</i> Ihle, 1922	4	7	5
8. <i>Cylicodontophorus</i> Ihle, 1922	1	1	1
9. <i>Petrovinema</i> Ershov, 1943	1	1	1
10. <i>Poteriostomum</i> Quiel, 1919	—	2	1
11. <i>Parapoteriostomum</i> Hartwich, 1986	—	2	2
12. <i>Gyalocephalus</i> Looss, 1900	—	1	1

Discussion

The results of this study document the first parasitological data collected from the new population of Przewalski's horses reintroduced to the Pre-Urals Steppe (the Orenburg State Reserve, Russia). Despite the small number of horses in this study, the data reveal the initial status of the gastrointestinal parasite community in the Przewalski's horses inhabiting the Pre-Urals Steppe. All horses arrived to the Reserve were infected with strongylids. Despite strongylid eggs had not been found in the fecal samples of one horse (Selena, # 5584) before the diagnostic deworming study, more than four hundred nematodes were collected from this horse. These data confirm the fact that the number of strongylid eggs expelled with feces does not correlate with the number of nematodes dwelling in the horse intestine (Kuzmina et al., 2012).

In this study, nematodes were the predominant group of parasites found in the Przewalski's horses. Cestodes from the genus *Anaplocephala*, which are frequently reported from various equids including the Przewalski's horses (Nilsson et al., 1995; Williamson et al., 1997; Elias et al., 2002; Matthews et al., 2004; Slivinska and Dvojnjos, 2006), were not found in our study. In our opinion, this may be due to the peculiarity of the management strategies and horse-keeping conditions in the Le Villaret reserve area, where the horses were initially kept, or because of the absence of intermediate hosts (oribatid ticks) for these cestodes in the steppe ecosystems of the Orenburg Reserve. However, further studies are necessary to confirm our assumptions.

In our study, nematodes from the family Strongylidae predominately infected the Przewalski's horses. These nematodes are the dominant group of parasites of wild and domestic equids worldwide (Dvojnjos and Kharchenko, 1994; Lichtenfels et al., 2008; Kuzmina et al., 2009; Kuzmina et al., 2013). However, only 18 species of strongylids were found in our study. In Przewalski's horses from the Askania Nova Biosphere Reserve, which had never been treated with any anthelmintics, from 31 to 33 species of strongylids were registered (Dvojnjos and, Kharchenko, 1994; Kuzmina et al., 2009). In the Przewalski's horses moved from the Askania Nova to the Chernobyl Exclusion Zone, 29 species were registered (Slivinska et al., 2006). Probably, the fauna of these parasites in the Le Villaret, where the studied horses were born, has been depleted even without regular anthelmintic treatments. Pastures in the Le Villaret had been used only for sheep for decades before Przewalski's horses were introduced to the area; this promoted elimination of all parasites infectious for horses from the pastures (Eysker et al., 1983).

Besides strongylids, one more nematode species, *Habronema muscae*, was detected in three of the six horses (prevalence = 50 %). Intermediate hosts of *Habronema* spp. are flies — *Musca domestica* and *Stomoxys calcitrans* (Pugh et al., 2014). Apparently, *H. muscae* is spread worldwide and infects domestic equids (horses, donkeys) in the Pre-Urals Steppe area, but data on habronematosis in horses inhabiting the Orenburg region have not been published. Other parasites such as *Dictyocaulus arnfieldi*, *Trichostrongylus axei* and *Strongyloides westeri* registered in Przewalski's horses previously (Dvojnjos and Kharchenko, 1994; Painer et al., 2011) were not found in our study. We suppose that these parasites were absent in the Le Villaret, France, where horses were kept initially. However, as there are numerous domestic horses kept in private farms in the Orenburg Region, we believe that other equine parasite species may be transferred to and found in the Przewalski's horses in future.

Parascarids (*Parascaris equorum*) are common parasites in foals and horses under 4 years of age including Przewalski's horses (Lyons et al., 1981; Dvojnjos, 1975; Gawor, 1995, 1996; Chapman et al., 2001; Elias et al., 2002; Slivinska and Dvojnjos, 2006); however these nematodes were not found in the horses in Pre-Urals Steppe, despite a young 2-year-colt was in the group. In future studies, it will be interesting to find out whether infection with *P. equorum* appears in the foals born in the Reserve, or will this species remain absent in this recently introduced population of Przewalski's horses.

The depleted species composition of gastrointestinal parasites in the first group of the Przewalski's horse re-introduced to the Orenburg Reserve might be a result of effective deworming before their transportation from France to Russia and low contamination of pastures of Pre-Urals Steppe due to the absence of livestock for a year after the site had become a part of the Orenburg Reserve. However, as there are numerous domestic horses kept in private farms bordering to Pre-Urals Steppe, we believe that other equine parasite species may be found in the Przewalski's horses of Pre-Urals Steppe in future.

We believe that continuous multiyear monitoring of the Przewalski's horse for infections by various groups of parasites is an extremely important strategy for monitoring the re-introduction of these horses into the Orenburg State Reserve. Despite the limited scientific novelty of the results obtained in this particular study, our data have to be considered as a "reference point" for subsequent parasitological studies of the free-roaming

population of the Przewalski's horses in Pre-Urals Steppe of the Orenburg Reserve and in Russian steppe ecosystems.

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