

UDC 595.132:599.723.2(477)

## STRONGYLID COMMUNITY STRUCTURE OF THE PRZEWALSKI'S HORSES (*EQUUS FERUS PRZEWALSKII*) FROM THE BIOSPHERE RESERVE "ASKANIA-NOVA", UKRAINE

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Accepted 13 April 2009

**Strongylid Community Structure of the Przewalski's Horses (*Equus ferus przewalskii*) from the Biosphere Reserve "Askania-Nova", Ukraine.** Kuzmina T. A., Zvegintsova N. S., Zharkikh T. L. — Species composition and the structure of strongylid community of the Przewalski's horse (*Equus ferus przewalskii* Poljakov, 1881) semi-free population at the "Askania-Nova" Biosphere Reserve were studied by the *in vivo* method of diagnostic deworming. After deworming of 24 adult Przewalski's horses by the anthelmintic "Univerm" (0.2% aversectin C, Russia), 25,815 strongylid specimens were collected and identified. Thirty-one strongylid species of 12 genera were found including 6 species of the subfamily Strongylinae and 25 — of Cyathostominae. Nine to 18 strongylid species (average  $14.5 \pm 2.5$ ) were found per horse. Cyathostomes (Cyathostominae) dominated in the Przewalski's horse strongylid community: 9 species (*Cyathostomum catinatum*, *C. pateratum*, *Cylcocyclus nassatus*, *C. insigne*, *C. leptostomum*, *C. ashworthi*, *Cylicostephanus longibursatus*, *C. goldi* and *C. minutus*) were found in more than 80% of horses examined and composed 94.1% of the total number of strongylid collected. Strongyles (Strongylinae) were found in 100% of the *E. ferus przewalskii* examined; prevalence of separate species was from 4.2% to 58.3%. The general structure of the strongylid community was multimodal with dominant (9 species), subdominant (4), background (4) and rare (14) species. The list of strongylid species found in the Przewalski's horses in Ukraine was enlarged to 37 species.

**Key words:** Przewalski's horses, *Equus ferus przewalskii*, strongylida, nematoda, diagnostic deworming, Ukraine.

**Структура сообщества стронгилид лошадей Пржевальского (*Equus ferus przewalskii*) из Биосферного заповедника «Аскания-Нова», Украина.** Кузмина Т. А., Звегинцова Н. С., Жарких Т. Л. — Приживленным методом диагностической дегельминтизации исследован видовой состав и структура сообщества стронгилид лошадей Пржевальского (*Equus ferus przewalskii* Poljakov, 1881) полувольной популяции Биосферного заповедника «Аскания-Нова». После дегельминтизации 24 взрослых лошадей Пржевальского препаратом «Универм» (0,2% аверсектин С, Россия) собрано и определено до вида 25 815 экз. стронгилид. Зарегистрирован 31 вид стронгилид 12 родов: 6 видов стронгилин и 25 видов циатостомин. У одной лошади паразитировали от 9 до 18 видов стронгилид (в среднем  $14,5 \pm 2,5$ ). Циатостомины (Cyathostominae) доминировали в сообществе стронгилид лошадей Пржевальского: 9 видов (*Cyathostomum catinatum*, *C. pateratum*, *Cylcocyclus nassatus*, *C. insigne*, *C. leptostomum*, *C. ashworthi*, *Cylicostephanus longibursatus*, *C. goldi* и *C. minutus*) были обнаружены у более 80% исследованных лошадей и в сумме составляли 94,1% общего количества собранных стронгилид. Стронгилины (Strongylinae) обнаруживали у 100% исследованных *E. ferus przewalskii*; экстенсивность инвазии лошадей отдельными видами составляла от 4,2% до 58,3%. Установлен мультимодальный тип структуры сообщества стронгилид лошадей Пржевальского с доминантными (9 видов), субдоминантными (4), фоновыми (4) и редкими (14) видами. Расширен список видов стронгилид, обнаруженных у лошадей Пржевальского в Украине до 37 видов.

**Ключевые слова:** лошади Пржевальского, *Equus ferus przewalskii*, стронгилиды, нематоды, диагностическая дегельминтизация, Украина.

## Introduction

The Przewalski's horse (*Equus ferus przewalskii* Poljakov, 1881) is the only surviving representative of the true wild horses (Groves, 1994). Nowadays, Przewalski's horses are kept in many zoos and natural reserves; free-ranging populations have been established in natural protected areas in Europe and Central Asia (Zimmermann, 2005). In Ukraine, there are two big population of the Przewalski's horses, i. e. the semi-free population of 89 horses at the "Askania-Nova" Biosphere reserve (Kherson region) and the free population of about 40 horses — in the Chernobyl Exclusion Zone (Kyiv region) (Zharkikh et al., 2002; Zharkikh, Yasynetska, 2008).

Biosphere Reserve "Askania-Nova" has the largest captive population of the Przewalski's horses in Europe. Most of the animals are kept in large enclosures (total area is 2330 ha) with natural steppe vegetation. A group of bachelor males are kept in an enclosure of 3.5 ha; a few individuals are kept in small paddocks of 1500 m<sup>2</sup> for the exhibition to visitors. Four equid species: Turkmenian kulans (*Equus hemionus kulan* Groves & Mazak, 1967), donkeys (*E. asinus dom.* L., 1758), Shetland ponies (*E. caballus var.* L., 1758), Burchell's zebra (*E. burchelli* Gray, 1824) are also kept at the "Askania-Nova". The population of about 120 Turkmenian kulans inhabits the same enclosures with Przewalski's horses.

Parasitological monitoring of Przewalski's horse populations in different zoos and semi-reserves has been carried out regularly; however, most of the studies on the Przewalski's horse parasitological examination are based on copromicroscopy or on the identification of *in vitro* cultured larvae (Elias et al., 2002; Epe et al., 2001; Zvegintsova et al. 2008). Species composition of gastrointestinal parasites of the Przewalski's horses was investigated only in Ukraine — at the "Askania-Nova" Biosphere Reserve in 70–80th years of XX century by *post mortem* methods (Dvojnos, 1975; Dvojnos et al., 1990; Dvojnos, Kharchenko, 1994), and in a small reproductive group of Przewalski's horses in the Chernobyl Exclusion Zone in 2004 (Slivinska, Dvojnos, 2006). No analyses of parasite community structure were carried out in those studies.

The aim of the present study was to investigate the species composition and to analyze the strongylid community structure of Przewalski's horses at the Biosphere Reserve "Askania-Nova" by *in vivo* method of diagnostic deworming.

## Material and methods

The Biosphere Reserve "Askania-Nova" is situated in the steppe zone of South Ukraine, in the Kherson region, Ukraine (46°29' North and 33°58' East). There is the largest captive population of 89 Przewalski's horses here.

The study was carried out in March 2007. Twenty-four adult Przewalski's horses — 22 bachelor males of 5 to 20 years old kept in enclosure of 3.5 ha, and 2 females (5 and 12 years old) kept in large enclosures of 2,032 ha, were examined. All horses were born at the "Askania-Nova" reserve and have never been treated with any anthelmintic drug.

The animals were treated with macrocyclic lactone drug "Univerm" (0.2% aversectinc, PharmBioMed, Russia) at an approximate dose rate of 0.1 mg aversectin per kg of body weight. Grain baits with anthelmintic (0.5 kg each) were placed at 15 m intervals the horses' enclosure of 3.5 ha to avoid eating several baits by one horse. Faecal samples of 200 g each were collected from each animal 24, 36, 48 and 60 h after treatment. All nematodes expelled were collected, fixed in 70% ethanol, clarified in 80% phenol-glycerine solution and identified under the light microscope using the published key (Dvojnos, Kharchenko, 1994). Totally 25,815 strongylid specimens were collected and identified.

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

The data were summarized and descriptive analyses were calculated using Microsoft™ Excel. No significant difference in the infection rates was found between Przewalski's horses kept in zoo paddocks and those from steppe enclosures during earlier studies at the "Askania-Nova" Biosphere Reserve (Study..., 2005). Therefore, data on the strongylid species composition from males and females were analyzed altogether.

The shape of the prevalence frequency distribution was determined for all strongylid species according to description of Bucknell et al. (1996). The proportion of each species of the strongylid community of each host was calculated as the number of specimens of the particular species in relation to the total numbers of strongylids found.

## Results

All Przewalski's horses examined were infected with strongylids; an average horse infectivity level was 1016.7 EPG (375–2075 EPG). Eggs of *Parascaris equorum* (in 9 horses), *Oxyuris equi* (in 6 horses) and *Habronema* spp. (in 2 horses) were also found in faecal samples. No strongyles' or other nematodes' eggs were found in the faecal samples collected on the 14th day after dehelminthization.

Totally 31 strongylid species from 12 genera were registered in the Przewalski's horses including 6 species of the subfamily Strongylinae and 25 species of Cyathostominae. Nine to 18 strongylid species (average  $14.5 \pm 2.5$  S. D.) were found in each horse. Two species: *T. minor* and *C. tetracanthum* were first found in the Przewalski's horses from Ukraine (tabl. 1).

Large strongyles (Strongylinae) were found in 100% of the Przewalski's horses; their prevalence varied from 4.2% to 58.3% (fig. 1). However, proportion of large strongyles in the strongylid community was minor — only 0.57%. One to 3 Strongylinae species (average  $1.5 \pm 0.7$  S. D.) have been detected in each animal.

Cyathostomes (Cyathostominae) dominated in the Przewalski's horse strongylid community; they were found in all horses and consist more than 99.4% of total strongylid number (fig. 1). Eight to 16 cyathostome species (average  $13.3 \pm 2.4$  S. D.) were found in each horse.

The shape of the prevalence frequency distribution of strongylid species was multimodal (fig. 2). Four groups of species including dominant (prevalence > 80%), sub-

**Table 1. Strongylid species found in the *E. ferus przewalskii* in Ukraine by various authors**

**Таблица 1. Виды стронгилид, зарегистрированные у *E. ferus przewalskii* в Украине разными авторами**

Species	"Askania-Nova", (Dvojnos, Khar-chenko, 1994)	Chernobyl (Slivinska, Dvojnos, 2006)	"Askania-Nova", (present study)
<i>Strongylus edentatus</i> (SED)	+	+	+
<i>S. equinus</i> (SEQ)	+	-	-
<i>S. vulgaris</i> (SVU)	+	+	+
<i>Triodontophorus serratus</i> (TSE)	+	+	+
<i>T. brevicauda</i> (TBR)	+	+	-
<i>T. tenuicollis</i> (TTE)	+	+	+
<i>T. nipponicus</i> (TNI)	+	-	-
<i>T. minor</i> (TMI)	-	-	+
<i>Craterostomum acuticaudatum</i> (ACU)	+	+	+
<i>Cyathostomum catinatum</i> (CAT)	+	+	+
<i>C. pateratum</i> (PAT)	+	-	+
<i>C. tetracanthum</i> (TET)	-	-	+
<i>Coronocyclus coronatus</i> (COR)	+	+	+
<i>C. labiatus</i> (LAB)	+	+	+
<i>C. labratus</i> (LBR)	+	+	+
<i>C. sagittatus</i> (SAG)	+	-	+
<i>Cyllicocyclus nassatus</i> (NAS)	+	+	+
<i>C. radiatus</i> (RAD)	+	-	-
<i>C. ashworthi</i> (ASH)	+	+	+
<i>C. elongatus</i> (ELO)	+	-	+
<i>C. leptostomum</i> (LEP)	+	+	+
<i>C. insigne</i> (INS)	+	+	+
<i>C. ultrajectinus</i> (ULT)	+	+	+
<i>Cylicostephanus calicatus</i> (CAL)	+	+	+
<i>C. longibursatus</i> (LON)	+	+	+
<i>C. minutus</i> (MIN)	+	+	+
<i>C. hybridus</i> (HYB)	+	-	+
<i>C. goldi</i> (GOL)	+	+	+
<i>C. bidentatus</i> (BID)	+	+	+
<i>C. asymmetricus</i> (ASY)	+	-	-
<i>Cylcodontophorus bicoronatus</i> (BIC)	+	+	+
<i>Parapoterostomum mettami</i> (MET)	+	+	+
<i>P. euproctus</i> (EUP)	+	+	+
<i>Petrovinema poculatum</i> (POC)	+	+	+
<i>Poteriostomum imparidentatum</i> (IMP)	+	-	+
<i>P. ratzii</i> (RAT)	+	+	-
<i>Gyalcephalus capitatus</i> (CAP)		+	+

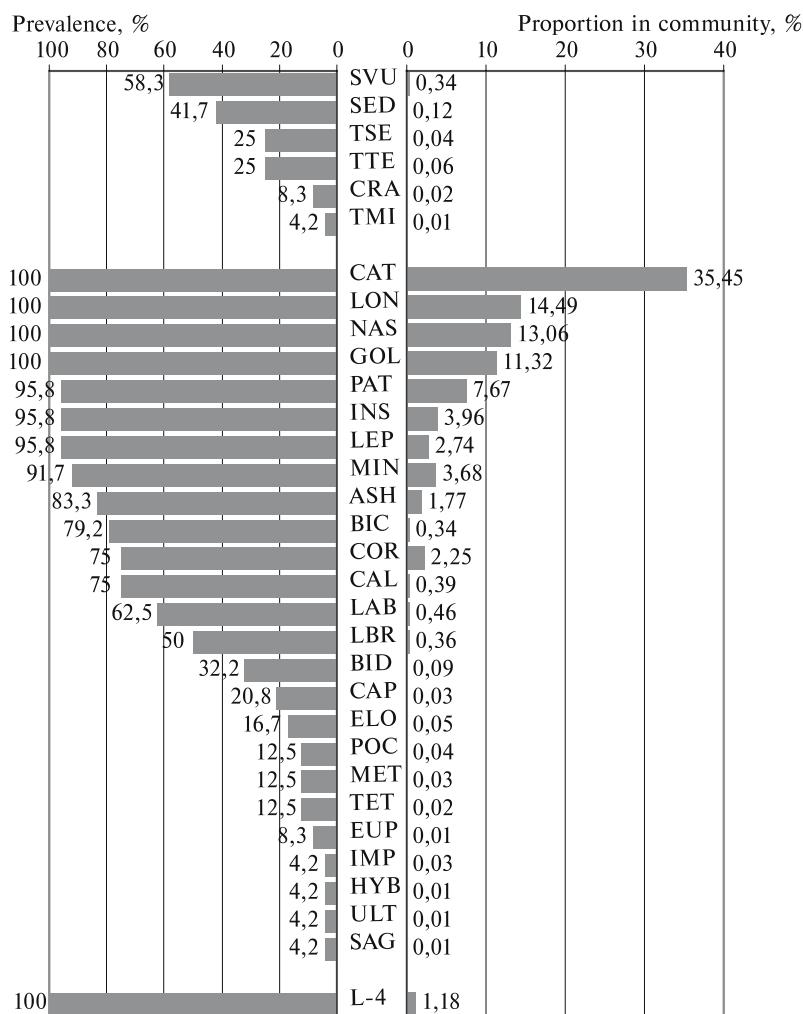


Fig. 1. Prevalence (%) and proportion (%) of various species in the strongylid community of the Przewalski's horses at "Askania-Nova" Biosphere Reserve.

Рис. 1. Экстенсивность инвазии (%) и доля разных видов (%) в сообществе стронгилид диких лошадей Пржевальского Биосферного заповедника «Аскания-Нова».

dominant (prev. = 60–80%), background (prev. = 30–60%) and rare (prev. < 30%) species can be separated in the strongylid community.

Nine cyathostome species (29.0% of the total species number): *Cyathostomum catinatum*, *C. pateratum*, *Cylicocyclus nassatus*, *C. insigne*, *C. leptostomum*, *C. ashworthi*, *Cylicostephanus longibursatus*, *C. goldi* and *C. minutus* dominated in the strongylid community (fig. 1); they were found in more than 80% of horses and amounted more than 94.1% of the total number of strongylid collected.

Four subdominant species (12.9% of the total species number): *Coronocyclus coronatus*, *C. labratus*, *Cylicostephanus calicatus* and *Cylicodontophorus bicoronatus* were found in 60–80% of the horses and amounted to 3.4% of the total strongylid number. Four background species: *Strongylus vulgaris*, *S. edentatus*, *Coronocyclus labiatus* and *Cylicostephanus bidentatus*, amounted to 0.9% of total strongylid number. Fourteen rare species (48.4% of total species number) amounted to 0.5% of total number of strongylid collected.

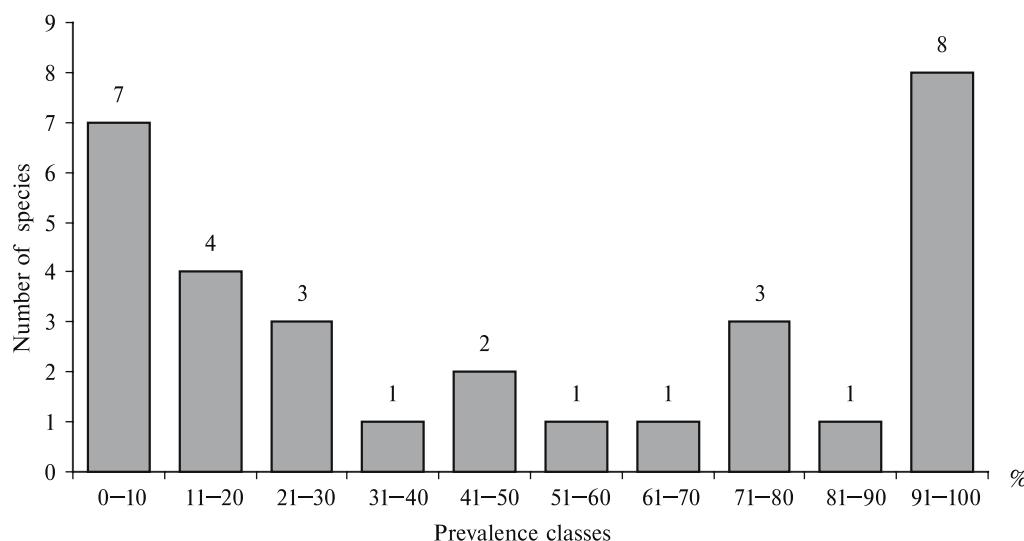


Fig. 2. Distribution of the strongylid species from the Przewalski's horses on the prevalence classes.

Рис. 2. Распределение видов стронгилид лошадей Пржевальского по классам экстенсивности инвазии.

## Discussion

The present work is the first *in vivo* study of the Przewalski's horse strongylid community performed simultaneously in the "Askania-Nova" reserve. Intestinal nematodes have been collected from 24 adult animals during one week. This approach allowed analyzing results obtained without fallibilities related to the seasonal variations in Przewalski's horse infection level, in contrast to previous studies at the Biosphere Reserve (Dvojnos, 1975; Dvojnos et al., 1990; Dvojnos, Kharchenko, 1994).

All Przewalski's horses examined had high numbers of strongylid eggs in their faeces — up to 2075 EPG. However, no clinical symptoms of strongylidosis, such as weight loss, chronic diarrhea, colic, subcutaneous oedema or pyrexia (Ogbourne, 1978; Love et al., 1999), have been observed in the horses. In our opinion, wild Przewalski's horses are more resistant to strongylid infection than well-bred domestic horses; the latter can have clinical signs of strongylidosis with the faecal egg counts of 400 EPG (Ogbourne, 1978; Uhlinger, 1991).

No eggs of strongylids or other gastrointestinal nematodes were found in Przewalski's horse faeces on the 14th day after anthelmintic treatment. It meant that all luminal stages of parasitic nematodes were expelled from the horse intestines. Thus, the present *in vivo* study data satisfactorily represent the strongylid community structure of the Przewalski's horses and can be comparable with results of *post mortem* examinations of the intestinal matrix performed by other researchers (Dvojnos, 1975; Dvojnos et al., 1990; Dvojnos, Kharchenko, 1994).

According to data of previous researchers (Dvojnos, 1975; Dvojnos et al., 1990; Dvojnos, Kharchenko, 1994; Zvegintsova, 2003), 35 horse strongylid species were found in Przewalski's horses at the "Askania-Nova" Biosphere Reserve. In the present study, three species of Strongylinae (*Strongylus equinus*, *Triodontophorus brevicauda* and *T. nipponicus*) and two species of Cyathostominae (*Cylicostephanus asymmetricus* and *Poteriostomum ratzii*) were not found. All these species belonged to the group of rare species; according to data of G. M. Dvojnos and V. A. Kharchenko (Dvojnos, Kharchenko, 1994), their prevalence was 6.25–12.5%. However, we found two rare strongylid species, *Triodontophorus minor* and *Cyathostomum tetracanthum* (prevalence 4.2%

and 12.5%), which have not been registered in Przewalski's horses before. Therefore, our data extended the list of strongylid species found in the Przewalski's horses in Ukraine to 37 species. The differences between the present study and the data of previous researchers may be attributed to the low proportion of rare species in the strongylid community. Intensity of horse infection with strongylids may exceed tens thousands of nematodes (Bucknell et al., 1996; Dvojnos, Kharchenko, 1994; Uhlinger, 1991). Thus, in standard samples of 1000 nematodes (Chapman et al., 2003) the rarest species numbering just dozens of individuals per host may be absent in samples collected.

Twenty-six strongylid species were found in the Przewalski's horses which had been transferred to the Chernobyl Exclusion Zone from the "Askania-Nova" Biosphere Reserve (Slivinska, Dvojnos, 2006). In our opinion, absence of such species as *Strongylus equinus*, *Triodontophorus nipponicus*, *T. minor*, *Cyathostomum pateratum*, *C. tetracanthum*, *C. sagittatus*, *Cylicocyclus radiatus*, *C. elongatus*, *Cylicostephanus hybridus*, *C. asymmetricus* and *Poteriostomum imparidentatum* in the strongylid community of the Chernobyl horse population is the result of anthelmintic treatment of all Przewalski's horses before their transportation to Chernobyl in 1998. The proportion of these species in the strongylid community was rather low; thus they disappeared from the Chernobyl population of the Przewalski's horses.

The multimodal shape of the prevalence frequency distribution of strongylid species in the Przewalski's horses is typical for strongylid communities of semi-free equid populations from the "Askania-Nova" Biosphere reserve (Kuzmina et al., 2007). Similarity of the Przewalski's horse strongylid community structure in the present study with that observed in the previous studies (Dvojnos, 1975; Dvojnos et al., 1990; Dvojnos, Kharchenko, 1994) demonstrates the stability of ecological and parasitological situation in the "Askania-Nova" Biosphere reserve for last 30–40 years.

The authors thank Dr. Natalia I. Yasynetska, the Deputy Director of the "Askania-Nova" Biosphere Reserve, for promotion of the researches. The authors also are very grateful to Dr. Iurii Kuzmin, PhD, from the Institute of Zoology NAS of Ukraine, for his valuable help in revising the manuscript.

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