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DYNAMICS OF INFECTION WITH STRONGYLIDAE OF THE PRZEWALSKI HORSE (*EQUUS PRZEWALSKII*) POPULATION IN THE CHERNOBYL EXCLUSION ZONE

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Dynamics of Infection with Strongylidae of the Przewalski Horse (*Equus przewalskii*) Population in the Chernobyl Exclusion Zone. Zvegintsova N. S., Zharkikh T. L., Yasynetska N. I. — In 1998 and in 1999, several Przewalski horses (PH) from the Reserve Askania Nova were transferred to the Chernobyl Exclusion Zone (CEZ), a free-roaming breeding population was therefore established. Parasitological monitoring of the population was carried out between 1998 and 2006. Before the transportation, PHs were dewormed with Albendazole. On arrival, the PHs were placed in large pasture enclosures for acclimatization, where they were kept between a few weeks and eight months before releasing into the wild. Besides PHs, some domestic working horses were kept in the enclosures. After transportation to CEZ, the level of infection with intestinal helminths in PHs increased. Probably, it was due to the following factors: 1) larvae of helminthes, which survived in intestines after deworming, developed, 2) the pasture in acclimatization enclosures was contaminated with parasites, as PHs shared the enclosures with domestic horses. Over the first three years, the prevalence of Strongylidae were 98.8%, the mean intensities varied from 248.0 ± 51.3 to 612.0 ± 278.2 eggs per gram faeces (epg). A mean intensity in bachelor males was higher than in members of harem groups, as the bachelors had closer contacts with domestic horses. After domestic horses had been transferred outside of CEZ in 2001, the overall intensity in PHs has progressively decreased. During 2004–2006, the mean intensity became stable with range 80.9 ± 25.5 to 138.9 ± 33.2 epg, with prevalence of 93.1%. In 3.5% of faecal samples, *Parascaris equorum* (Ascarididae) were found; a mean intensity was 29.0 ± 10.7 epg. A few eggs of *Anoplocephala perfoliata* (Anoplocephalidae) were found in three samples only. Due to negligible contamination of pastures excluded from farming industry long ago, there are comparatively low levels of infection in the free-roaming PHs. The present level of infection is considered harmless to the horses as clinical symptoms of helminthoses were never noticed.

Key words: Parasitological monitoring, Strongylidae, Przewalski horse, *Equus przewalskii*, Chernobyl Exclusion Zone

Динамика зараженности стронгилидами популяции лошади Пржевальского (*Equus przewalskii*) в Зоне отчуждения Чернобыльской АЭС. Звегинцова Н. С., Жарких Т. Л., Ясинецкая Н. И. — В 1998–1999 гг. в Зону отчуждения Чернобыльской АЭС (ЗО) было завезено несколько лошадей Пржевальского (ЛП) из заповедника «Аскания-Нова», из которых создана вольная популяция. Паразитологический мониторинг этой популяции проводили в 1998–2006 гг. Перед транспортировкой ЛП были дегельминтизированы альбендазолом. После завоза животные были помещены в большие загоны для акклиматизации, где их содержали от нескольких недель до 8 месяцев перед выпуском в природу. Кроме ЛП, в загонах содержали несколько домашних рабочих лошадей. После транспортировки уровень зараженности ЛП гельминтами желудочно-кишечного тракта возрос. Вероятно, это было обусловлено двумя факторами, а именно: развитием личиночной стадии гельминтов, выживших после дегельминтизации, и контаминированностью пастбищ в акклиматизационных загонах, которые ЛП делили с домашними лошадьми. В течение первых трех лет экстенсивность инвазии (ЭИ) стронгилидами составила 98,8%, средняя интенсивность (ИИ) варьировала от 248,0 ± 51,3 до 612,0 ± 278,2 яиц/г. Средняя ИИ у жеребцов-холостяков была выше, чем у членов гаремных групп, так как холостяки больше контактировали с домашними лошадьми. После того, как в 2001 г. домашних лошадей вывели за пределы ЗО, ИИ у ЛП постепенно уменьшилась. В 2004–2006 гг.

средняя ИИ стабилизировалась на уровне (80,9 ± 25,5)–(138,9 ±33,2); ЭИ составила 93,1%. В 3,5% исследованных проб были обнаружены *Parascaris equorum* (Ascarididae); средняя ИИ составила 29,0 ± 10,7. В трех пробах были обнаружены единичные яйца *Anoplocephala perfoliata* (Anoplocephalidae). Сравнительно низкий уровень инвазии вольных ЛП обусловлен незначительностью контаминирования пастбищ, давно исключенных из сельскохозяйственного использования. Учитывая, что в популяции не наблюдается клинических признаков гельминтозов, данный уровень зараженности можно оценить как не представляющий опасности для животных.

Ключевые слова: паразитологический мониторинг, стронгилиды, лошадь Пржевальского, *Equus przewalskii*, Зона отчуждения Чернобыльской АЭС

Introduction

In 1998, the Biosphere Reserve Askania Nova launched the Programme on the Establishment of the Free-roaming Population of the Przewalski Horse (*Equus przewalskii* Poljakov, 1881) in the Chernobyl Exclusion Zone (CEZ). The aim of the Programme was to enrich the biodiversity of the biocenoses damaged after human activities and to maintain ecological balance in such biocenoses. In 1998 and in 1999, the Askania Nova Reserve transferred several groups of Przewalski horses (PH) to CEZ, with financial backing of the State Forest Enterprise Chernobylles. Thirty-one PHs were transported to CEZ; out of them 28 came from Askania Nova and 3 came from a local zoo of Lozovski stud-farm. After acclimatization, the PHs were released into the wild (Zharkikh et al., 2002). Over the next nine years authors observed the development of the PH free-roaming population in CEZ.

As a parasitical status of animals could influence their well-being to some extend, a monitoring of the parasitological situation in the new-formed population was a necessary tool to estimate the success of PHs' adaptation to the new environment. The main difference between this study and earlier parasitological investigations (Slivinska, 2004) was that it had been conducted three times as much longer and, respectively, more animals were examined.

Materials and methods

Study area and study population. CEZ (2600 km^2) is situated in the northern part of Kyiv Region. The climate is temperate continental, with a mean annual temperature of +7.2° C; in July +18° C (max +32° C) and in January −6.1° C (min −25° C). The annual precipitation is about 604 mm.

Several groups of PHs were transported to CEZ in May, June, September, November 1998, and in October 1999 (Zharkikh et al., 2002). Before their transportation to CEZ, Askanian PHs were dewormed *per os* with Albendazol, using a group method of giving the preparation (horses were not separated from each other during deworming). There was no information about anthelmintic treatment of the only survived stallion from the Zoo of Lozovski stud-farm before its transportation. On arrival, the PHs were placed in large pasture enclosures for acclimatization; harem groups and a bachelor group were formed. PHs were kept in the enclosures between a few weeks and eight months. Besides PHs, some domestic working horses were kept in the enclosures. Two groups of PHs transported to CEZ in 1998, were released into the wild in January and March 1999; the PHs transported in October 1999, were released in December. In addition, some PHs from Odessa Zoo were transported to CEZ in September 2004 unknown to the Reserve Askania Nova. Upon arrival, Odessa's PHs were released into the forest without any acclimatization; and there were no information about their anthelmintic treatment before their transportation.

PHs began breeding in 1998; 17 adults and 3 foals resided in CEZ on 1 January 1999 (Zharkikh et al., 2002). Till 2004, the population size annually increased by 20 to 40%; the number of PHs was at the high of 65 specimens in 2004. Free-roaming PHs were organized into harem groups and bachelor groups; the number of the groups ranged from 3 to 8 in different years.

Data collecting. Thirteen expeditions to CEZ were carried out between 1998 and 2006; parasitological samples were collected in October 1998, in January and August 1999, in March 2000, in January and August 2001, in March and December 2002, in December 2003, 2004, and 2005, in March and December 2006. The most samples were collected without identification of animal-producers; yet, some samples were identified. Due to the above-mentioned identified samples, the dynamics of helminthic infection in 7 adult PHs from one of the harems was studied between 1999 and 2002. Twelve samples from foals aged 4 to 11 months were collected in a period between December and March in 1999–2002; a sample from a 3.5-month-old foal was collected in August 1999. Totally, 339 samples from PHs were collected and examined. In addition, helminthic infection in domestic horses kept in acclimatization enclosures between 1998 and 2000, was monitored; 13 samples were collected.

Two grams from each collected sample were put into Barbagallo's solution (3% formalin in physiological saline); they were examined within a week after fixing. Faecal egg counts were performed using Fulleborn's technique (Kotelnikov, 1984). The number of intestinal helminths' eggs per gram faeces (epg) was calculated (Trach, 1992).

Statistical methods. Statistical analyses were performed using standard methods (Ivanov, 1990). Mean intensity (average number of eggs per gram faeces per infected animal) and prevalence (percentage of animals infected) for each examined group of PHs were calculated; then significance of differences between the groups examined during the same expedition was verified. For comparison of infection between adults and young animals, only the data from identified animals were used. Data on helminthic infection in PHs kept on natural pastures at the Reserve Askania Nova (the steppe zone of southern Ukraine) during the same period (between 1998 and 2006) were used as comparative material. Nonparametric Wilcoxon test (W) was used. All tests are two-tailed, with $P \leq 0.05$ established as the criterion for rejection of null hypotheses.

Results

In examined samples, the vast majority of helminth eggs were the eggs of a strongyloid type. The dynamics of infection with strongylids (Nematoda: Strongylidae) of the PHs in the Chernobyl Exclusion Zone is presented in figure 1. A mean intensity of infection was 161.9 $\pm$ 23.9 epg per horse in the first Askanian PHs (n = 13, two harem groups) prepared for transportation to CEZ in 1998, with a prevalence of 20%. In October 1998, the intensity increased to 204.0 $\pm$ 48.7 epg, with the prevalence of 36.4%. The rates increased until the PHs had been released from their acclimatization enclosures; the mean intensity was 314.6 $\pm$ 46.6, with the prevalence of 100%.

In August 1999, 6 months after the PHs had been released into the wild, a mean intensity decreased to 217.8 $\pm$ 48.7 epg in a harem group; a mean intensity of 520.0 $\pm$ 251.1 epg in a bachelor group was greater. Such differences between members of harem groups and bachelors were recorded up to 2001; yet, these differences were not significant in all cases ($P > 0.05$). A mean intensity in Przewalski stallions which formed harems with domestic mares and roamed over in surroundings of acclimatization enclosures in 2000 and 2001, was 612.0 $\pm$ 278.2 epg in January 2001. This was the highest mean among all the data in this study. Since 2001, the overall intensity has progressively decreased (fig. 1)

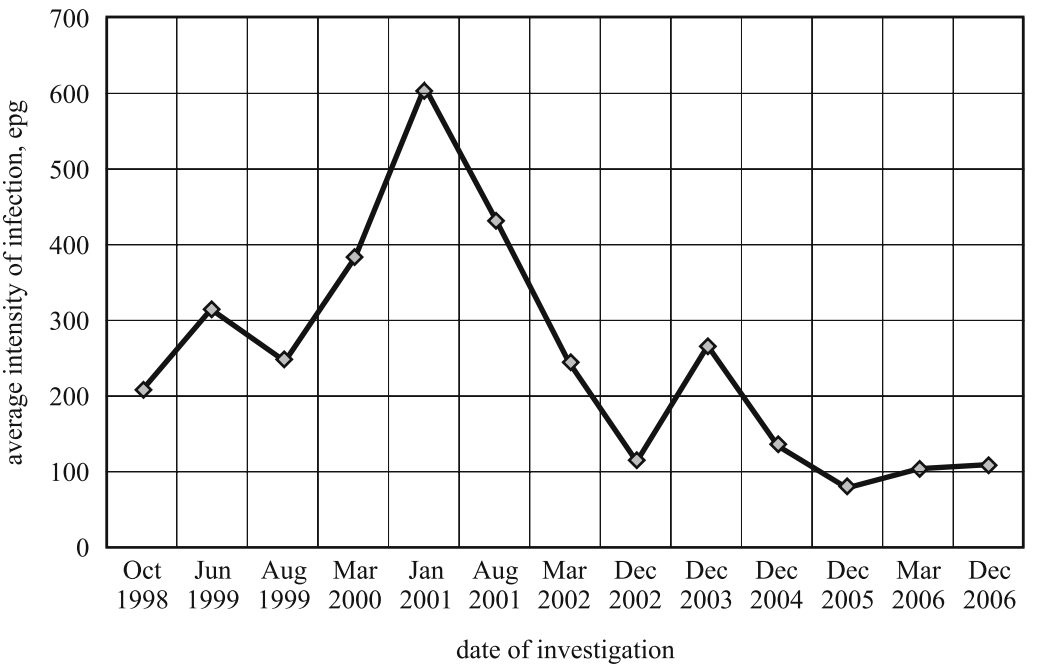

Fig. 1. The dynamics of infection of Przewalski horses with strongylids in the Chernobyl Exclusion Zone.

Рис. 1. Динамика зараженности стронгилидами лошадей Пржевальского в Зоне отчуждения Чернобыльской АЭС.

Table 1. Mean intensities of Strongylidae infection in bachelor males and members of harem groups of Przewalski horses

Таблица 1. Средняя интенсивность инвазии стронгилидами жеребцов-холостяков и членов гаремных групп лошадей Пржевальского

Years	Mean ± S. E. M.		W	U_f*	P
	bachelor groups	harem groups			
2004	27.3 ± 5.5	202.7 ± 47.0	105	2.14	$P < 0.05$
2005	26.9 ± 4.1	115.3 ± 40.1	194	1.21	$P < 0.05$
2006	130.7 ± 51.1	90.4 ± 35.4	61	32	$P > 0.05$

* Ut = 1.13

A comparison between members of harem groups and bachelors showed that over the last three years, the bachelors were infected less than the members of the harem groups (tabl. 1). No significant differences ($P > 0.05$) in mean intensities were found between all the harem groups inhabiting different areas.

In general, the overall intensity in all PHs' groups has become stable with range of 80 to 140 epg over the last years (fig. 1).

Since 1999, the strongylid prevalence has been 96.8% (range 82.5–100%). There were no strongylids in some samples collected in August 1999, December 2002, 2004, and 2005. In December 2004, samples from 3 harem groups and one bachelor group were examined. A prevalence was 12.5% in one of the harem groups consisting of 7 adults (out of them, 3 horses from Odessa Zoo, others were born in CEZ), an 18-month-old filly and a foal born in 2004. The low prevalence in the group indicated that the horses from Odessa Zoo had been dewormed before their transportation to CEZ. Prevalences were 100% in other groups examined.

In 5 out of 7 Askanian PHs examined individually within 3 years after they had been transported to CEZ, mean intensities became stable within one year after their arrival to CEZ. One of the PHs (mare studbook No 2438) had a permanent low intensity within subsequent 2.5 years, with mean of 77.3 ± 51.0 epg; another mare (studbook No 2309) had a permanent high intensity with mean of 725.0 ± 142.7 epg. Others had medium mean intensities ranged from 222 to 424 epg. In a harem stallion (studbook No 1719), an intensity slightly varied from 188 to 314 epg, with mean of 271.0 ± 26.8 epg.

No helminth eggs were found in a sample collected in August from 3.5-month-old foal. In samples collected in December from foals aged between 5 and 6 months, the intensity averaged 118.7 ± 76.9 epg, with the prevalence of 66.7%. In samples collected in March from foals aged between 7 and 11 months, the intensity averaged 229.7 ± 63.2 epg, with the prevalence of 85.7%. In adults examined at the same time in March, a mean intensity of 353.5 ± 36.3 epg was higher than in foals; yet, this difference was not significant ($P > 0.05$, $W = 62$, $U_f = 1.09$, $U_t = 1.13$).

Among other helminth species found in PHs of the Chernobyl population, horse ascarids *Parascaris equorum* (Nematoda: Ascarididae) and tapeworms *Anoplocephala perfoliata* (Cestoidea: Anoplocephalidae) were recorded. The overall prevalence of ascarids was 3.5%; 40% of young PHs were infected. The intensity was low: mean of 29.0 ± 10.7 epg (range 4–128); the highest number of ascarid eggs was found in a sample in March 2002. Besides, a few eggs of tapeworms (range 4–16 epg) were found in 3 samples in 2002 and 2003.

Over all the time of existing of the PH population in CEZ, no clinical symptoms of helminthiases were observed; the PHs have bred successfully.

Discussion

The finding that strongylids dominated among species of intestinal helminthic fauna of PHs of the Chernobyl population has been reported before (Slivinska, 2004). The taxon is also prevalent in equids at the Reserve Askania Nova (Dwoinos, Sweginzowa, 1990; Dvojnos, Kharchenko, 1994; Zvegintsova, 1998).

It is known that intensity of infection usually decreases during winter due to drop in helminth egg production (Trach, 1986; Baudena et al., 2000). However, after PHs had been transported to CEZ and placed into acclimatization enclosures, the level of their helminthic infection steady increased over winter months. Probably, the rapid increase of infection was due to the following factors: 1) larvae of helminths which survived in intestines after deworming, developed, 2) the pasture in acclimatization enclosures was contaminated with parasites, as PHs shared the enclosures with domestic horses. The domestic horses were infected in spite of their regular deworming; their mean intensity was 213.7 $\pm$ 66.6 epg, with a prevalence of 53.8%.

Differences in intensities of strongylid infection between harem groups and bachelor groups were recorded over the first years of the existence of the population. K. A. Slivinska (2004) speculated that the high intensity in bachelors was due to the fact that the males experienced more physiological stress than members of harem groups. In our opinion, the bachelors were higher infected because they often came to acclimatization enclosures and contacted with domestic horses; i. e., the bachelors lived in a territory contaminated with helminths since the initial stage of the re-introduction project. Our assumption is also supported by data on a high intensity in the Przewalski stallions which formed harems with domestic mares. In contrast to bachelors, harem groups of pure-bred PHs widely dispersed over CEZ; yet, they avoided places visited by humans (and pastures for domestic horses). In 2001, the total area of home ranges of two harem groups (38 specimens) was about 240 km^2 (Zharkikh et al., 2002). As contamination of the pastures was negligible due to low density of PHs, a decrease in intensity of infection in members of harem groups was predictable. After domestic horses had been transferred outside of CEZ in April 2001, the overall mean intensity gradually decreased in PHs (fig. 1). At the Reserve Askania Nova, in PHs with exposure to highly helminthic-contaminated pastures (60–80 horses were kept on area of 2330 ha), a mean intensity of 679.6 $\pm$ 20.2 epg (range 497.2–849.2), and a prevalence of 100% have been recorded over the last 9 years. It is the much higher level of infection than in PHs of the Chernobyl population.

Differences in intensities of infection between some adult PHs in CEZ might be affected by their different immune responses to helminthiases. A latent period of development of most parasites occurs in foals during their first months of life; the present data supports that the level of intensity progressively increases as various species of helminths pass their developmental stages.

The weight of other potentially important species in helminthic fauna of the PH population in CEZ was not significant, as it was also reported by Slivinska (2004). The similar situation was seen in PHs at the Reserve Askania Nova over the same period, as *Parascaris equorum* had a prevalence of 6.2% and a mean intensity of 26.8 $\pm$ 1.2 epg in the total population, and a prevalence of 50% and a mean intensity of 45.2 $\pm$ 12.8 epg in young animals. *Anoplocephala perfoliata* was recorded in Askania Nova in 2002–2004, and 2006 (a prevalence of 2.4% and a mean intensity of 8.0 $\pm$ 2.4 epg with range of 4 to 20).

Conclusions

In PHs of the Chernobyl population, the intensity of infection with intestinal helminths has become stable on a low level over the last few years. The data indicate

that biogeocenosis has developed for the most part, and parasite-host relationships have established within home ranges of the PHs.

A comparatively low level of infection in free-roaming PHs was conditioned on negligible contamination of pastures excluded from farming industry long ago.

Contacts often occurring between PHs and domestic working horses over the first years of re-introduction, resulted in a rapid increase of intensity of infection in the PHs; this also affected the higher infection of bachelors in comparison with members of harem groups.

Taking into consideration that there are no clinical symptom of helminthiases in the PH population in CEZ, the present level of infection is considered to be ecologically balanced and harmless to the horses.

Baudena M. A., Chapman M. R., French D. D., Klei T. R. Seasonal development and survival of equine cyathostome larvae on pasture in south Louisiana // Veterinary Parasitology. — 2000. — **88**. — P. 51–60.

Dvojnos G. M., Kharchenko V. A. Strongylids of domestic and wild horses. — Kyiv : Naukova Dumka, 1994. — 236 p. — Russian.

Dwoinos G. M., Sweginzowa N. S. Öekologisch-helminthologische Charakteristik des Przewalskipferdes in Askania Nova // V Internationales Symposium zur Erhaltung des Przewalskipferdes. — Leipzig (Deutschland) : Zoologischer Garten Leipzig, 1990. — S. 164–165.

Ivanov A. A. Basic statistics. — Moscow : Fizcultura i sport, 1990. — 86 p. — Russian.

Kotelnikov G. A. Helminthological investigations of animals and environment. — Moscow : Kolos, 1984. — 238 p. — Russian.

Slivinska K. A. The Przewalski horse (Equus przewalskii Poljakov, 1881) under conditions of the Chernobyl Exclusion Zone (an ecological-parasitological analysis) : Abstract of Ph. D. thesis: 03.00.08 / Institute of Zoology NASU. — Kyiv, 2004. — 20 p. — Ukrainian.

Trach V. N. Ecological-faunistic characteristics of adult Strongylata of domestic ruminants in Ukraine. — Kyiv : Naukova Dumka, 1986. — 216 p. — Russian.

Trach V. N. Recommendations on using of a new method of counts of helminthic eggs and protozoa cysts in faeces of animals. — Kyiv : Izdatelstvo GU Veterinarii Ministerstva Selskogo Khozyajstva Ukrainy, 1992. — 14 p. — Russian.

Zharkikh T. L., Yasynetska N. I., Zvegintsova N. S. Przewalski horse in the Zone of Chernobyl nuclear power // Gazella. — 2002. — **29**. — P. 93–111.

Zvegintsova N. S. An experience of studying of parasitocenoses of wild ungulates in Askania Nova, and a contemporary conception of control of parasitoses at reserves // Proc. of Int. Sci. Conf. “Aktualni pytannya zberezhennya i vidnovlennya stepovykh ekosystem”. — Askania Nova (Ukraine). — 1998. — P. 52–57. — Russian.