

The effects of seasonality and group size on fecal egg counts in wild Przewalski's horses (*Equus ferus przewalskii*, Poljakov, 1881) in the Chernobyl Exclusion Zone, Ukraine during 2014 – 2018

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Summary

The aim of this study was to investigate the difference in Fecal Egg Counts (FEC) with regard to group size, age, sex and body condition of wild free-roaming Przewalski's horses in the Chernobyl Exclusion Zone (Ukraine), across different seasons, over a five-year period (2014 – 2018). We hypothesized that horses from larger group sizes would have higher faecal egg counts (FECs). The relationship between FECs and the year and season of sample collection, and age, sex and group size of the horses was analyzed. Generalized linear model using positive strongylid FEC's as a variable response, was used to investigate the differences in FECs between the groups. Nematode (Strongylidae, *Parascaris* spp., Habronematidae) and cestode (Anoplocephalidae) eggs were also identified. Strongylids were the most prevalent helminth egg, and had the highest FECs. The model for egg counts of strongylids showed that season and group size of horses were statistically significant. Presence of strongylid eggs was not dependent on age and sex of horses. We suggest that this could be a result of parasite transmission between individuals and groups in places where animals aggregate around water sources or collective farms.

Results obtained in this current study broaden the knowledge of gastrointestinal parasites in free-roaming horses under wild natural conditions.

Keywords: gastrointestinal parasite; fecal egg counts; strongylids; Przewalski's horse; *Equus ferus przewalskii*; Chernobyl Exclusion Zone

Introduction

Przewalski's horses (*Equus ferus przewalskii*, Poljakov, 1881) are a rare and endangered subspecies of wild horse (*Equus ferus*), which are native to the steppes of Central Asia. Until the late 18th century, the natural range of Przewalski's horses was from the Russian Steppes east to Kazakhstan, Mongolia and northern China. Przewalski's horses are threatened by small population sizes, their restricted range, potential hybridization with domestic horses, loss of genetic diversity and disease. In 1969 they were

officially declared extinct (Bouman & Bouman, 1994), but successful reintroductions have qualified this species for reassessment. The number of living captive and reintroduced animals in the International Studbook was estimated at 2444 (1087 males, 1343 females, and 14 unknown sex) on 01.01.2018 (Jaroslav Simek *personal comm.*). Currently *Equus ferus przewalskii* qualifies as Endangered (King *et al.*, 2015).

In 1992 the first two reintroduction projects were launched in Mongolia, to restore a population of Przewalski's horse within their native area. At present, 12 large breeding and reintroduction centers

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for Przewalski's horses have been established in Europe (France, Hungary, Ukraine) and in Asia (China, Mongolia, Uzbekistan, Kazakhstan, Russia) (Zimmermann, 2004; Bakirova & Zharkikh, 2016). In 1998 – 1999 the first captive bred wild horses (n=21) were introduced to the Chernobyl Exclusion Zone (CEZ) from the Askania Nova Biosphere Reserve in Ukraine. The purpose of this introduction was breeding of this unique equid species as well as to re-wild the area through grazing by wild horses.

Currently there are about 130 Przewalski's horses roaming freely on the grasslands of CEZ (Slivinska *et al.*, 2007; 2017). Groups comprise of either harem or bachelor groups. The number of horses per group ranges from 2 to 20 individuals. Before the transportation to CEZ, Przewalski's horses were dewormed with Albendazole (Zvegintsova *et al.*, 2008). Gastrointestinal parasite monitoring has been conducted from the date of introduction of horses into the CEZ (Slivinska, 2004; Slivinska *et al.*, 2006; Zvegintsova *et al.*, 2008). There were 19 domestic horses which are bred by local services in the same area and the same time and may pose the potential epidemiological hazard for endangered species (Slivinska *et al.*, 2006).

However, over the last decade, no data regarding gastrointestinal parasites within the population of wild Przewalski's horse in the Chernobyl zone has been reported.

Parasitological studies of wild Przewalski's horses kept in zoos, semi-reserves and natural reserves have been previously reported (Dvojnjos, 1975; Dvojnjos & Kharchenko, 1994; Epe *et al.*, 2001; Elias *et al.*, 2002; Slivinska *et al.*, 2006; Zvegintsova *et al.*, 2008; Painer *et al.*, 2011; Liu *et al.*, 2016; Kuzmina *et al.*, 2009, 2017). Most of these studies were performed with coprological methods which determined the presence or absence of certain groups of intestinal parasites and estimated the intensity of infection by Fecal Egg Counts (FECs). A few publications have reported information on species composition and structure of intestinal parasites population (Dvojnjos & Kharchenko, 1994; Slivinska & Dvojnjos, 2006; Kuzmina *et al.*, 2009, 2017). Nematodes from the family Strongylidae are the most prevalent and pathogenic group of intestinal parasites of wild and domestic horses worldwide (Dvojnjos & Kharchenko, 1994; Love *et al.*, 1999; Lichtenfels *et al.*, 2008). Coprological examination of intestinal helminths of wild horses is the only ante-mortem method available to study parasites of free-roaming Przewalski's horses in the CEZ (Zvegintsova *et al.*, 2008) as well as wild horses worldwide (Rubenstein & Hohmann, 1989; Debafe *et al.*, 2016; Cain *et al.*, 2018; Harvey *et al.*, 2019).

Previously, differences in strongyle faecal egg counts between adults wild Przewalski's horses and their social groups (bachelors/harems) in CEZ were recorded during the first years of the existence of the population (Slivinska, 2004; Zvegintsova *et al.*, 2008). Higher (Slivinska, 2004) or lesser (Zvegintsova *et al.*, 2008) strongyle faecal egg counts were found in bachelor groups compared to harem groups of wild Przewalski's horses in CEZ. Zvegintsova *et al.* (2008) speculated that the differences in FEC between some adult wild Przewalski's horses in CEZ might be affected by

their different immune responses to helminthiases. However, yet no systematic study has been performed the parasite loads with regard to social and environmental factors in CEZ. This is important because the population of the Przewalski's horse in CEZ may be a reservoir for free populations of this species. The species has still endangered status (King *et al.*, 2015), and the population is exposed to many threats, including environmental ones (e.g. Kaczensky *et al.*, 2011). Moreover, it is important to assess the threat from parasites for Przewalski's horses in the forest-meadow landscape.

The aim of this study was to investigate the difference in Fecal Egg Counts (FECs) with regard to group size, age, sex and body condition of wild free-roaming Przewalski's horses in the Chernobyl Exclusion Zone (Ukraine), across different seasons, over a five-year period (2014 – 2018). We hypothesized that horses from larger group sizes would have higher FEC's.

Materials and Methods

Study Area

The CEZ is located c. 200 km N of Kiev, Ukraine (51.3°N; 30.005°E), 123 m above sea level. This zone covers an area of 2,600 km² and falls entirely within the Polesie Lowland, Russian Plain. The climate in the CEZ is humid, with relatively mild winters and warm summers. The mean annual temperature is 5 – 7°C, with a mean temperature in July of 18°C (max. 32°C), and in January of -6°C (min. -25°C). The annual precipitation ranges from 550 to 750 mm. The snow cover lasts on average c. 50 days per year. The mean depth of the snow cover is 12 – 13 cm (Barjachtar *et al.*(ed), Chernobyl disaster 1996).

The CEZ is surrounded by metal fencing two meters high. Approximately c. 50 peasants also are still living in the CEZ; only few other persons have constant access to this area. Before the nuclear disaster, the CEZ consisted of farmlands with patchy areas of forest. Currently, ca. 60 % of the area is covered with forests, 50 % of which is pine forest and the remaining consists of abandoned arable grounds, meadows, pastures and human settlements.

Data collection

Faecal samples from wild Przewalski's horses were collected during five field trips between April 2014 and September 2018. Each survey was performed over 7 – 10 days, using a car to travel between sampling sites. When horses were sighted, they were observed with the aid of binoculars or a telescope. The number of individuals was recorded in each group. Where possible, the age class and sex of each individual were recorded (Zharkikh 2003). Clinical signs that may be associated with parasitism, such as body condition (slim; fat; regular) and poor coat condition, were noted. The age of each horse was assessed visually and assigned to one of four groups: a) <1 year, b) 1 – 3 years, c) 3 – 10 years, and d) >10 years. After the basic census data were collected, the individual horses were observed during defecation.

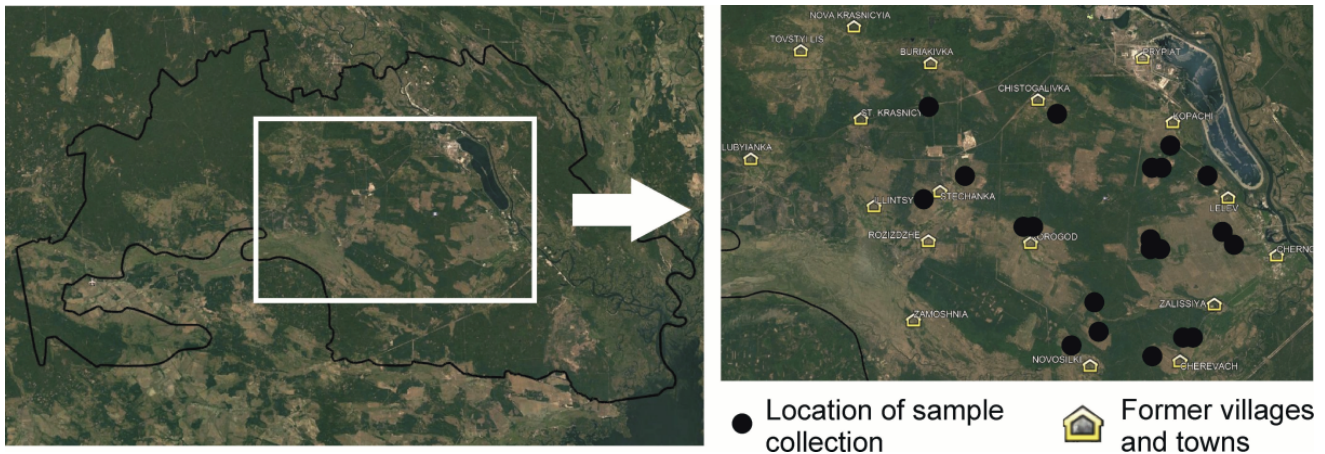


Fig. 1. Location of fecal sample collection in Chernobyl Exclusion Zone.

During this time the location of feces deposition was determined for each horse. After the group moved to graze to other sites of the meadow, samples of feces were collected and the GPS location was recorded for each collection (Fig. 1). During each survey, samples of fresh feces that were found on roads or other open places, were also collected. The samples were cooled in a portable refrigerator, transported to a laboratory and stored in a refrigerator at 3°C for 2 – 6 days, until analyzed. 72 fecal samples were performed according to the spring season (13 in 2014, 14 in 2015, 15 in 2016 and 30 in 2017, respectively) and 46 fecal samples were performed according to the autumn season (38 in 2015 and 8 in 2018, respectively). In total, 118 fecal samples were examined from April 2014 to September 2018. Although we were able to distinguish horses during collection of samples during one year, we were not able to check whether samples were collected from the same horse during the following years.

Fecal Egg Counts

Fecal egg counts (FECs) were performed using the McMaster technique, with a sensitivity of 25 Eggs Per Gram feces (EPG) (Herd, 1992). Mean FEC (average number of EPG per infected animal) and prevalence (percentage of animals infected) were calculated for each horse individually, and assigned to either 'harems' or 'bachelor groups'. Fresh feces that were opportunistically found on roads or other open places were assigned as 'unknown groups'. A total of 118 horses from 21 groups (14 harems; 4 bachelor groups; 3 unknown groups) were examined.

Data elaboration and statistics

For further analysis, we used samples that were positive for strongylids egg counts, which was the most common species observed. We excluded 8 samples where other species of parasites were more common (over 50 eggs EPG) since different parasite species can influence the FECs of other parasitic species (Paterson & Lello, 2003). To investigate the differences in strongylid parasitic infection, we used a generalized linear model with egg counts of

strongylids as a response variable. The sex, age of each known horse, season (spring and autumn) and the year of the sample collection were fixed factors; the group size was a covariate. In this model we used a Tweedie distribution with log link after comparison with the Poisson and a negative binomial distribution model using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC). The model with Tweedie distribution showed lower values of both information criteria (AIC and BIC). Selection of the models was performed with backward elimination procedure basing on the AIC values. The model was constructed with SPSS software (version 24.0, IBM Corporation, Armonk, NY).

Results

Nematode (Strongylidae, *Parascaris* spp., Habronematidae) and cestode (Anoplocephalidae) eggs were found in feces of wild Przewalski's horses (Table 1). The prevalence of strongylids in-

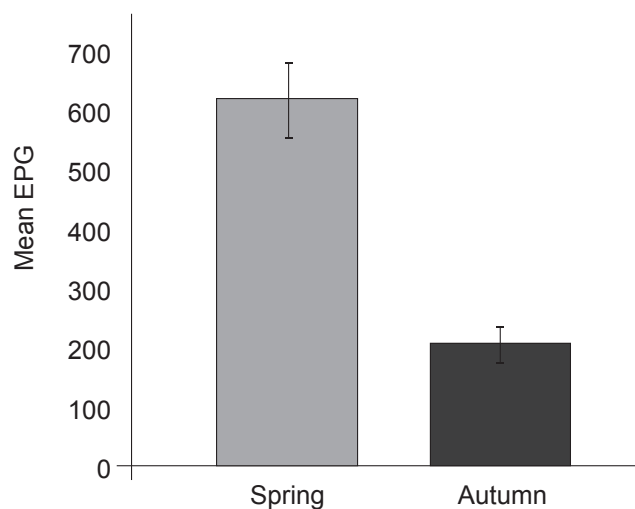


Fig. 2. Mean EPG of strongylids in feces of Przewalski's horses in two analyzed seasons and pairwise comparisons with Bonferroni adjustment ($\chi^2=45.75$, $p=0.000$, $N = 71$ for Spring and $N = 39$ for Autumn).

Table 1. Egg counts of the gastrointestinal parasites of wild Przewalski's horses in the Chernobyl Exclusion Zone between April 2014 to September 2018. (EPG – eggs per gram of feces; EI – prevalence of infections; FEC – mean fecal egg counts, SD – standard deviation)

Year	Group type (n = number of horses),	No. of feces samples	Strongylidae			Parascaris spp.			Habronematidae			Anoplocephalidae		
			EI, %	FEC, EPG	±SD	EI, %	FEC, EPG	±SD	EI, %	FEC, EPG	±SD	EI, %	FEC, EPG	±SD
2014	Harem (5)	5	100	470	258.2	-	-	-	-	-	-	60	41.7	29.9
	Harem (8)	8	100	925	512.3	-	-	-	-	-	-	-	-	-
2015	Harem (15)	3	100	158.3	52	-	-	-	-	-	-	-	-	-
	Harem (4)	3	66.7	25	14.4	-	-	-	-	-	-	-	-	-
	Harem (5)	2	100	37.5	17.7	-	-	-	-	-	-	-	-	-
	Harem (7)	6	100	125	52.4	-	-	-	-	-	-	-	-	-
	Harem (11)	3	66.7	187.5	175	-	-	-	-	-	-	-	-	-
	Bachelors (8)	8	100	33.4	166.9	-	-	-	12.5	25	-	25	25	11.6
	Unknown	27	88.9	117.1	142.1	11.1	191.7	76.2	-	-	-	7.4	25	6.7
2016	Harem (12)	7	100	660.7	677.4	-	-	-	-	-	-	-	-	-
	Harem (3)	3	100	300	201	-	-	-	-	-	-	-	-	-
	Bachelors (5)	4	100	487.5	512.6	25	25	-	-	-	-	-	-	-
	Unknown	1	100	250	-	-	-	-	-	-	-	-	-	-
2017	Harem (8)	4	100	718.8	451	-	-	-	-	-	-	-	-	-
	Harem (14)	11	100	788.6	377.9	-	-	-	-	-	-	9.1	25	-
	Harem (3)	3	100	250	106.1	33.3	25	-	-	-	-	66.6	25	-
	Bachelors (11)	11	100	479.5	237.4	18.2	112.5	123.7	-	-	-	9.1	25	-
	Unknown	1	100	600	-	-	-	-	-	-	-	-	-	-
2018	Harem (3)	2	100	250	70.7	-	-	-	-	-	-	-	-	-
	Harem (9)	2	100	112.5	17.7	50	25	-	-	-	-	-	-	-
	Bachelors (6)	4	100	193.8	104.8	25	25	-	-	-	-	-	-	-

fections was 66.7 to 100 % in observed groups of horses, during the whole period of observation. The mean FEC of strongylids in observed groups of horses fluctuated between 25 ± 14.4 – 925 ± 512.3 EPG. In samples collected in spring season over all study period the mean FEC of strongylids averaged 555.2 ± 414.5 EPG, with the prevalence of 100 %. In samples collected in autumn season the mean FEC of strongylids averaged 142.1 ± 95.1 EPG, with the prevalence of 89.1 %.

The prevalence of parascarides infection was also recorded and ranged from 11.1 – 50 %. The mean FEC of *Parascaris* spp. ranged from 25 – 191.7 ± 76.2 . Habronematids eggs were only detected in a single horse in 2015 (12.5 %; EPG = 25).

In additional, a few cestode eggs were detected in some groups of horses (7.4 – 66.6 %; $25 - 41.7 \pm 29.9$ EPG) (Table 1).

Externally observable clinical signs of parasitosis among these wild horses were not noted in any individual. All horses were in good clinical condition, with 'regular' body condition score and typical coat condition.

The best model for egg counts of strongylids in feces of Przewalski's horses included the season, age and group size; and was highly statistically significant (Table 2). The model selection proce-

dure excluded the variable "sex", "year" and all interactions. Horses presented a significantly higher parasitic infection level during spring than autumn ($p=0.000$). During Spring, feces contained over three times a higher number of strongylids eggs than during autumn (mean= 624.5 and 219.6, respectively) – Fig. 2. Group size was positively correlated to strongylid FECs. The bigger the group size, the higher the strongylid FECs ($p=0.045$). Age was not statistically significant in this model.

Discussion

The dominant helminth group (both in terms of prevalence and FEC) was Strongylidae. Domination by strongylids in the intestinal helminthic fauna of horses of the Chernobyl population has been previously reported (Slivinska, 2004; Slivinska *et al.*, 2006; Zvegintsova *et al.*, 2008). Other potentially important gastrointestinal parasites (*Parascaris* spp., Habronematids and Cestodes: Anoplocephalidae) in the Przewalski's horses in CEZ were not significant in this study, as also reported by Slivinska (2004) and partially by Zvegintsova *et al.* (2008). Earlier parasitological investigation (Zvegintsova *et al.*, 2008) has been recorded the mean

Table 2. Effects season, age and group size on the egg counts of strongylids in feces of Przewalski's horses ($\chi^2=56.06$; $df=4$; $p=0.000$, $N=110$). Non-significant sex and year of sample collection were excluded by AIC backward elimination procedure.

Source	Wald χ^2	df	P
Intercept*	961.1	1	0.000
Season*	52.58	1	0.000
Group size*	4.03	1	0.045
Age	1.65	2	0.439

FEC in all harem's and bachelor's groups (prevalence 93.1 %) with a range of 80 to 140 EPG over the first eight years (1998 – 2006) after transportation of Przewalski's horses in the CEZ. In our study, the mean FEC of strongylids in observed groups of horses fluctuated between 25 ± 14.4 – 925 ± 512.3 EPG (prevalence 66.7 to 100 %) during the whole period of our observation. In our opinion, the differences may be due to the use of the different methods of the fecal egg counts as well as other seasons of the sample collection.

Strongylids are prevalent in horses of different breeds and throughout different regions of the world, despite huge climatic differences (Ogbourne, 1976; Anderson & Hasslinger, 1982; Reinemeyer *et al.*, 1984; Krecek *et al.*, 1989; Bucknell *et al.*, 1995; Silva *et al.*, 1999; Gawor, 1995; Lind *et al.*, 2003; Kuzmina *et al.*, 2005).

Wild horses of the CEZ were documented to have variable strongylid FECs, which depended on the season of sample collection and horses' group size (Table 2, Fig. 2). In our survey all wild Przewalski's horses were examined in spring and early autumn when the adult strongylid populations are considered to reach their highest level (Reinemeyer *et al.*, 1986).

Mean total egg emissions differ significantly depends of season according to our research. Four times higher values of strongylid FECs were in spring than in autumn (555.2 ± 414.5 and 142.1 ± 95.1 EPG, respectively). Our findings confirm previous studies, where the level of strongylid egg shedding depended on the season (Dobrowolska & Grabda, 1951; Gawor, 1995). However, intensity of infection usually decreases during winter due to a decrease in strongyle egg production (Trach, 1986; Baudena *et al.*, 2000). In addition, we recorded that strongylid FECs increased during the rainy season and decreased during the dry season (Betlejewska, 2000; Epe *et al.*, 2001).

Group size was positively correlated to strongylid FECs. The results confirm previous studies, where the larger the group the higher the FEC (Rubenstein & Hohmann, 1989; Harvey *et al.*, 2019). We hypothesize that this could be a result of parasite transmission between individuals within group, which is observed in areas where animals aggregate (Reinemeyer & Nielsen, 2018). Moreover, transmission may be higher than stated in other studies, because we have found a lack of influence of sex and age on strongylid FECs. In general, sex and age is indicated to influence the infection level in horses (Côté & Poulin, 1995; Bucknell *et al.*, 1995; Romaniuk *et al.*, 2004; Fikru *et al.*, 2005; Francisco *et al.*, 2009; Kornas *et al.*, 2010). Significant differences in gastrointes-

tinal parasite communities associated with sex were observed for intestinal strongylids: mares had significantly higher numbers of strongylids as compared to stallions (Slivinska *et al.*, 2016). Moreover, significant differences with infection of intestinal parasites associated with age were observed for Strongylidae and *Parascaris equorum* infections in different breeds of horses (Lind *et al.*, 1999; Larsen *et al.*, 2002; Kornas *et al.*, 2010; Kuzmina, 2012; Slivinska *et al.*, 2016), and this supports the concept of the development of anti-parasite immunity in horses with age (Klei & Chapman, 1999). We suggest that, within the CEZ, wild horses may transmit the parasites within group at watering places or former collective farms. Within the CEZ, there are vast areas where watering places are scarce and some of them are small in size. The necessity of water access can be a reason for horses to aggregate in small areas near the waterholes. We also speculate, that another places which may increase parasite transmission are the former collective farms. Przewalski's horses use the abandoned buildings of former collective farms to protect themselves against environmental challenges (Klich *et al.*, 2017, Schlichting *et al.*, 2019). There is the proximity with former collective farms where animals gather and location of sample collection in our study (Fig. 1). However, there may be also other places of aggregation, that could influence the overall general infection levels of parasites in wild horses within the group regardless the sex and age. Transmission between groups in aggregation places is less probable. Groups may appear in the same areas, at least partially, however, during vegetation season harems of Przewalski's horses tend to keep distance between themselves (Klich *et al.*, 2019). Although, in the forest meadow mosaics such behavior was not described, we expect to behave horses in a similar way.

Results obtained in this study broaden the knowledge of parasite communities in free-roaming wild horses in the Chernobyl Exclusion Zone. This paper also documents factors that could influence the parasitic infections in wild horses under natural conditions.

Conclusions

Results obtained in this study broaden the knowledge of parasite communities in free-roaming wild Przewalski's horses in the Chernobyl Exclusion Zone. This paper also documents factors that could influence the parasitic infections in wild horses under natural conditions of forest-meadow landscape.

Results presented in this study gives an overview of the situation

over the years in protected animals inhabiting a peculiar niche, where the material is difficult to access and there are not many sources of knowledge regarding the health and parasitological status of these animals.

Results obtained in this study broaden the knowledge of parasite communities in free-roaming wild horses in the Chernobyl Exclusion Zone. This paper also documents factors that could influence the parasitic infestations in wild horses under natural conditions.

Conflicts of interest

The authors declare no conflicts of interest.

Ethical Approval and/or Informed Consent

For this study formal consent is not required.

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