## The faeces contamination by Strongylidae eggs and <sup>137</sup>Cs of the wild Przewalski's horses in the Chornobyl Exclusion Zone, Ukraine: preliminary study

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In 1998–1999, first captive bred wild horses (n=21) were introduced to the Chornobyl Exclusion Zone (CEZ) from the Askania-Nova Biosphere Reserve in Ukraine for their breeding as well as protection of the areas damaged after human activities through the horse' grazing. At present, near 130 Przewalski's horses roam freely in the grasslands of CEZ (Slivinska et al. 2007, 2017). Adaptation and survival of wild animals in natural conditions with constant chronic radiation exposure are influenced by various factors, including parasites (citation). For this reason, a parasitological survey has been conducted from the moment of introduction of horses into the CEZ. The monitoring showed that Przewalski's horses keep their typical biological features high resistance to parasitic infections (citation). A substantial growth of the population was observed as well as good clinical health state of horses (Slivinska et al. 2004, 2006; Zvegintsova et al. 2008; Slivinska et al. 2020).

The higher susceptibility of hosts on parasites in the radiated environment previously studied (Morley 2012; Pelgunov 2005). Revealed, that as the radiation load increases, the level of infection with parasites increases in rodents (Pelgunov 2005) and in birds (Schyhobalova, Paruzhynskaja 1963). Moreover, a distinct effect of radiation on nematodes was stated in the definitive mammalian hosts, resulting in reduction of parasites (Ishii et al. 1986; Sazykina and Kryshev 2006), and an effect of mutation related to radiation was stated in this taxonomic group (Genchi et al. 1987; Schyhobalova, Paruzhynskaja 1963; 1968). In former studies conducted by Slivinska et al. (2006) in 2004 year no influence of radiation on parasite infestation in Przewalski's horses were stated. However, horses studied in 2014–2018 of the faeces contamination by Strongylidae eggs and <sup>137</sup>Cs of the wild Przewalski's horses in the CEZ, Ukraine in current radiation level conditions.

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 km<sup>2</sup>, and falls entirely within the Polesie Lowland, Russian Plain. Climate of the CEZ is humid, with relatively mild winter and warm summers. The mean annual temperature is 5–7°C. The mean temperature in July is 18°C (max. 32°C), while in January –6.1°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 (Chornobyl disaster, 1996).

The CEZ is fenced with metal nets. Along with c. 50 peasants are still living in the CEZ, only few other persons have constant access to this area. Before the nuclear disaster, the CEZ consists of farmlands with forest fragments. At present, c. 60% the area is covered with forests (50% of which is pine forest) and the remaining consists of abandoned arable grounds, meadows, pastures and



Figure 1. Location of the five reproductive groups of the wild Przewalski's horses in the Chornobyl Exclusion Zone during field study in 2019

## human settlements.

Samples of faeces of wild Przewalski's horses were collected during field trips in 2019, March. During each trip survey was conducted using vehicles. During each survey the same method was used. When horses were sighted, they were observed with the aid of binoculars or telescope. The number of individuals was recorded in each group. External negative signs of parasitosis among the wild horses such as body condition (slim; fat; regular) and poor coat condition, were noted during the study. After the basic census horses were observed for longer time to the moment of excretion of feaces of all or at least some of individuals. After the group moved to graze in the other part of the meadow the samples of faeces were collected and recorded was the location with GPS receiver. The samples were cooled in portable refrigerator, transported to laboratory and stored in refrigerator at +3 Celsius degrees until analysis. In total, we have collected individual samples from 26 wild horses belonging to the five reproductive groups. Groups were located in two places of higher radiation level: Benjovka (group N4, 14 horses), Stari Shepelichi (group N5, over 3 horses), and lower radiation level: Korogod (group N1, 10 horses), Chernobyl (group N2, 9 horses), and Cherevach (group N3, 13 horses) (Fig. 1).

Faecal egg counts (FECs) were performed using the McMaster technique, with a sensitivity of 25 Eggs Per Gram faeces (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 reproductive groups. A total of 26 horses from 5 reproductive groups were examined.

To analyze the relation of Strongylidae infection (faecal egg counts, EPG) and  $^{137}$ Cs concentration in faecal samples we build regression model where faecal egg counts (EPG) was a dependent variable and  $^{137}$ Cs concentration was an explanatory variable. In the model we did not divide the samples on age or sex group, becasue most of animals where not known regarding tha sex and foals constituted a marginal percentage. The Curve Estimation procedure in SPSS software to find the most fit regression line basing on R<sup>2</sup> values, visual estimation of the curve and model assumptions.

Strongylidae eggs were found in faeces of wild Przewalski's horses. The prevalence of strongylids infections was 100% in all observed groups of horses. The mean FEC of strongylids in observed groups of horses fluctuated between  $58.3\pm14.4$  $-865.6\pm508.5$  EPG. In all analyzed samples the mean FEC of strongylids equaled  $455.8\pm431.1$  EPG. Among the identified groups of horses, the infection increased in order N5 – N2 – N4 – N1 – N3 (58.3± 14.4; 233.3±255.4; 317.9±211; 65±273.1; 865.6 ±508.5, respectively).

External negative signs of parasitosis among the wild horses has not been identified during the study.

Faecal egg count (EPG) presented statistically significant relation with  $^{137}Cs$  concentration in faeces (F=9.215, p=0.006). The most fit line presented the inverse function, but with relative low R<sup>2</sup> value (0.286) indicating that the  $^{137}Cs$ 



Figure 2. Relation of faecal egg count of the Strongylidae gastrointestinal parasites (EPG) and <sup>137</sup>Cs concentration in faecal samples.

concentration explain less than 30% of the faecal egg count (EPG) variability. The faecal egg count (EPG) was increasing with 1/137Cs values increase in the faeces (for  $B_{1/137Cs}=285576\pm94077$  SE, t=3.036, p=0.006;  $B_{intercept}=5.6\pm153$  SE, t=0.231, p=0.819) (Fig. 2).

This work represents the first extensive analysis of a dependence of infections of strongylides gastrointestinal parasites in wild Przewalski's horses with regard to the radiation level of their faeces.

Radiation level was one of the factor influencing the strongylids infection of Przewalski's horses within the CEZ. The lower infection in higher radiation level is surprising in light of the numerous facts indicating higher susceptibility of hosts on parasites and in the radiated environment (Morley 2012; Pelgunov 2005). As the radiation load increases, the level of infection with parasites increases in rodents (Pelgunov 2005) and in birds (Schyhobalova, Paruzhynskaja 1963). At the same time, a recent publication on influence of radiation on parasite infection in Przewalski's horses indicated no correlated effect (Slivinska et al. 2006). In contrast, a distinct effect of radiation on nematodes was stated in the definitive mammalian hosts, resulting in reduction of parasites (Ishii et al. 1986, Sazykina and Kryshev 2006), and an effect of mutation related to radiation was stated in this taxonomic group (Genchi et al. 1987; Schyhobalova, Paruzhynskaja 1963; 1968).

Results obtained in the current study broaden the knowledge on existence of parasites systems in horses under chronicle radionuclides influence. This is also the first study that present drivers that can influence on the wild horses parasite infection level in natural conditions.