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# Intestinal parasites in Przewalski's horses (*Equus ferus przewalskii*): a field survey at the Hortobágy National Park, Hungary

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## Research Paper

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## Abstract

The Pentezug Wildhorse Reserve, located in the Hortobágy National Park, Hungary, has one of the biggest *ex situ* populations of Przewalski's horses and aims to preserve its landscape and to study this subspecies. Between September and November 2018, 79 faecal samples were collected from Przewalski's horses. The McMaster, Willis flotation, natural sedimentation and coproculture methods were applied to all the samples. Results showed an average level of 1287 eggs per gram (EPG), which is a high faecal egg-shedding level. All the samples were positive for strongyle-type eggs (100%). There were no statistical differences regarding the EPG values between different harems of the population. The same happened when considering sexes, ages, lactating status or when bachelors are compared with harem members. Cyathostominae were dominant, when compared to Strongylinae and Tricostrongylidae, and 15 different morphological infective third-stage larvae types and/or species belonging to the order Strongylida were identified. The subfamily Cyathostominae was prevalent in 100% of the horses. *Strongylus vulgaris* was the most prevalent strongylin (40.5%). Additionally, 27.8% were positive for *Parascaris* sp. and 2.5% showed *Oxyuris equi* in their faeces. This study revealed that there is a higher prevalence of *Triodontophorus serratus* and *Poteriostomum* spp. in juveniles. Horses with *S. vulgaris* showed lower levels of EPG. This was the first study involving this population, showing 100% prevalence of intestinal parasites.

## Introduction

According to the International Union for Conservation of Nature (IUCN), Przewalski's horses are currently considered an endangered subspecies of the extinct *Equus ferus* (King *et al.*, 2017). Nowadays, *in situ* populations are restricted to some limited areas in Mongolia and China, while *ex situ* populations are located in Hortobágy National Park (HNP, Hungary), Askania Nova Biosphere Reserve, Chernobyl Exclusion Zone (CEZ, Ukraine), Cevennes National Park (France) and others.

Przewalski's horses were introduced in HNP in 1997, with the primary goal of managing the landscape in the Pentezug area inside the national park. Nowadays, the number of individuals is around 300, and besides the Przewalski's horse, a herd of domestic cattle (*Bos primigenius taurus*), carefully bred to phenotypically resemble reconstructed aurochs (*Bos primigenius*), use this area for common grazing (Zimmermann *et al.*, 2009; Kerekes *et al.*, 2019). The Pentezug population is managed without human interference or routine parasite control. Thus, deworming is only occasionally performed – for instance, during the translocation of specific individuals.

In equids, nematodes are responsible for a greater diversity of parasitic infections when compared to cestodes or trematodes – for instance, *Parascaris* spp., pinworms (*Oxyuris equi*) and many strongylids, all members of the superfamily Strongyloidea, except *Trichostrongylus axei* from Trichostrongyloidea (Lichtenfels *et al.*, 2008). Furthermore, the number of parasites present is very relevant and defines whether a health problem exists. For instance, to evaluate the level of infection, it is usual to consider the following guide: lower than 500 eggs per gram (EPG) represents a low infection, 500 to 1000 EPG a moderate infection and higher than 1000 EPG a high infection (Soulsby, 1986; Madeira de Carvalho, 2008; Madeira De Carvalho, 2014).

In Przewalski's horses, some gastrointestinal (GI) parasitological studies have been performed *ex situ* (Slivinska *et al.*, 2006; Kuzmina *et al.*, 2009, 2017; Zvegintsova *et al.*, 2019) and *in situ* (Sharkhuu *et al.*, 2000; Elias *et al.*, 2002; Painer *et al.*, 2011; Liu *et al.*, 2016). The majority of them were based on determining the presence or absence of specific parasite



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agents and calculating nominal levels of infection by the EPG (Kuzmina *et al.*, 2017). Moreover, most of the studies were performed under seminatural conditions, based in smaller populations and using an *in vivo* deworming method before the collection (Slivinska *et al.*, 2006; Kuzmina *et al.*, 2009, 2017; Zvegintsova *et al.*, 2019), which was not done in this study.

In the social organization of the Przewalski's horses, as in other equids, strong dominance hierarchies are essential for the formation of a harem. The establishment of the harem space is crucial to reduce the aggressive episodes mainly between harem stallions, grouped stallions and bachelors (Keiper & Receveur, 1992). In contrast, this Przewalski's horse population has a different organization. Even though the harems are considered single stallion, they are close to each other, forming a big group in the 2388 hectares of the Pentezug reserve. This social structure is not commonly described in different populations of the subspecies, but it has been described in Camargue horses (Duncan, 1992; Brabender *et al.*, 2016).

Consequently, the main goal of this study was to perform a survey of the GI parasites of the Przewalski's horses from the Pentezug Wild Horse Reserve, at the HNP, in Hungary, through the collection of faecal samples. Additionally, we tried to understand if there were any critical differences between age, sex, social groups, harems and other individual factors to better understand the health status of this *ex situ* population.

## Materials and methods

### Plan and objectives

The main goal of this study was to conduct a detailed survey of the GI parasites of the Pentezug population as a tool for future parasite regular monitoring plans of these Przewalski's horses. From the 79 collected samples (79 of the 280 horses, meaning 28% of the population), 62 were members from identified harems, two were from identified bachelors and 15 were from unknown bachelors. Moreover, it was possible to obtain samples from 24 of the 29 harems. This study was performed between September and November 2018.

The nomenclature used in our study is similar to the one used by other feral horses studies (Rubenstein & Hohmann, 1989). In the following paragraph we present the categories used, the number of samples collected ($n$) and the approximate total number of horses from that social group ($t$). Considering harem members, infant males ($n = 5$; $t = 12$) and females (IF; $n = 2$; $t = 8$) are less than one year old; young males ($n = 6$; $t = 20$) and females ($n = 1$; $t = 15$) are one to three years old; stallions ($n = 12$; $t = 29$) are mature males more than three years old that lead harems; non-lactating females ($n = 28$; $t = 42$) are adult females more than three years old; lactating females ($n = 8$; $t = 20$) are adult females more than three years old, having a lactating foal. Bachelors ($n = 17$; $t = 130$) are mature male horses, non-members of any harem. Fluctuations between social groups were possible due to harems changes. In some cases, when infants and young horses are studied together, the term juvenile ($n = 16$) (less than three years old) is used in comparison with adults ($n = 63$) (more than three years old).

### Individual identification and sample collection

The identification was possible for most individuals due to the strong harem connections and proximity that defines the wild horses (Rubenstein & Hohmann, 1989). The identification process occurred while horses were defecating in a range of 50–100 m, mainly at resting moments. Sampling was performed on a convenience basis. When an animal started defecating, the animals surrounding it were observed, the harem was determined. Then, using binoculars if necessary, it was possible to sex it and watch its body details, identifying the individual. However, most of the bachelors were not likely to be identified since they do not establish strong connections (as found between harem members), which leads them to have irregular positions surrounding distinct harems. Subsequently, a small piece of fresh faeces belonging to each horse was collected from the middle of the faecal material, using individual identified plastic bags and held at 4–5°C until reaching the laboratory. The analysis started immediately after the arrival.

### Coprological methods

To assess the EPG and evaluate the level of parasite infection, we used the McMaster Technique (Madeira de Carvalho *et al.*, 2014). The Willis flotation and natural sedimentation methods were performed afterwards to identify the light and heavy parasite eggs present in the samples, respectively (Madeira de Carvalho, 2001). Coprocultures were performed in order to obtain infective third-stage larvae (L3), which allow the differentiation of parasites of this group. To determine the proportion of infection by Strongylinae, Cyathostominae and Trichostrongylidae, a total of 100 L3 larvae were counted and identified in each sample in order to obtain the percentages of each mentioned family, subfamilies, genera and species, whenever possible, of the order Strongylida. *Cyathostomum sensu lato* (*s.l.*) L3 morphotypes were identified. Published identification keys and guides were used for these purposes (Madeira de Carvalho *et al.*, 2004, 2007, 2008; Santos *et al.*, 2018).

### Data analysis

For descriptors regarding parasites prevalence, we used the definitions of Bush *et al.*, 1997.

For descriptive statistics, we used Microsoft Excel 365®, and for statistical inference analyses, IBM SPSS Statistics 25® was the chosen program (Abbott, 2011). Since the data distribution was not normal, Kruskal–Wallis and Mann–Whitney tests were performed to evaluate the faecal egg counts (FEC) in different groups and categories. Our observations are independent, since one observed defecation corresponds to each collected sample and to one particular result. Regarding the coprocultures, the presence or absence of specific genera/species of parasites was evaluated with the Pearson Chi-square test and Fisher's exact test. When one or more cells in the cross-table had less than five cases, only the values of the Fisher's exact test were considered due to the limitations of the Chi-square test in these conditions. Even though there was a limitation on the 'bachelor' identification when we compared adults (>3 years old) with juveniles (<3 years old), the whole 79 individuals were considered. In this case, the bachelors are all considered adults, mainly because the dispersion from the natal band occurs after the sexual maturity around 2–3 years of age. Consequently, they can be analysed with the remaining adult individuals. For all the statistical tests, we accepted a confidence level of 95% and a $P$-value < 0.05.

## Results

All the 79 analysed samples/horses were positive for strongyle-type eggs (79/79) in the Willis floatation, meaning a 100%

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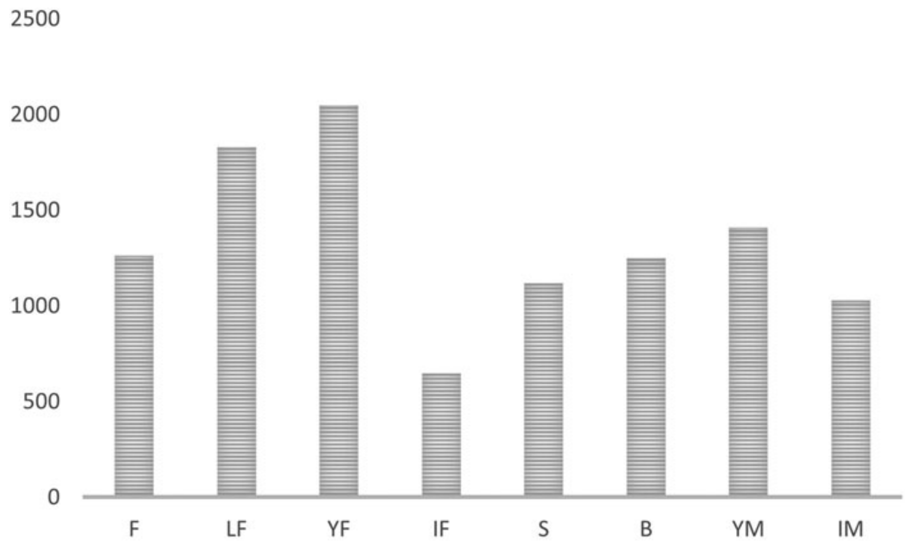


**Fig. 1.** Average level of infection (EPG) in individuals of different ages, sexes and social role. Abbreviations: IM, immature males, less than one year old; IF, immature females, less than one year old; YM, young males, 1–3 years old; YF, young females, 1–3 years old; F, females, more than three years old; LF, lactating females, more than three years old; S, harem stallions; B, bachelors.

prevalence. From the total, 27.8% were positive for *Parascaris* sp. (22/79) and 2.5% (2/79) of the analysed wild horses contained *O. equi* in their expelled faeces. There were no statistically significant associations in *Parascaris* spp. results between adults and juveniles (*P*-value = 0.360), according to Fisher's exact test. By the sedimentation method, we found a digenean egg in one horse, 1.3% (1/79).

The average EPG was 1287 (ranging from 250 to 5050) for the whole 79 samples. Furthermore, more than half of the screened population (43/79) revealed a high infection level. The median, standard error and standard deviation were, respectively, 1050, 94.2 and 836.9. Specifically, 11 samples had less than 500 EPG, indicating a low infection level, 25 samples had between 500 and 1000 EPG, a medium level of infection and, finally, 43 samples showed more than 1000 EPG, which is considered a high level of infection.

Statistically, the Kruskal–Wallis test showed no significant differences between the distinct categories presented in fig. 1 (*P*-value = 0.454). According to the Man–Whitney test, between sexes (*P*-value = 0.784), adults vs. juveniles (<3 vs. >3 years old) (*P*-value = 0.985), lactating vs. non-lactating females (*P*-value = 0.370), bachelors vs. stallions (*P*-value = 0.183), bachelors vs. members of a harem (*P*-value = 0.567), there were no statistical differences regarding the EPG values. In the same way, according to the Kruskal–Wallis test, there are no differences in the EPG values between the different harems of the whole group (*P*-value = 0.238), meaning that their average faecal egg-shedding counts were probably homogeneous.

According to the coprocultures, the strongylid infections in these faecal cultures were composed, on average, of approximately 96.3% Cyathostominae, 2.4% Strongylinae and 1.3% Trichostrongylidae. They also provided evidence of parasite infections by multiple genera/species, 15 different strongylids *s.l.*, which was possible to analyse in different social groups (table 1).

In our study, the L3 of *Cyathostomum s.l.* type A (fig. 2a) were the most frequent, occurring in 100% of the analysed samples. In the subfamily Strongylinae, *Strongylus vulgaris* (fig. 2b) was the most common parasite of this group, with a prevalence of 40.5%, followed by *Triodontophorus serratus* (fig. 2c), with a prevalence of 12.7%. We also found *T. axei* infective larval stages, a parasite that horses share with ruminants.

Moreover, two parasites show a significant difference between adults and juveniles: *Poteriostomum* spp. (*P*-value = 0.013) and *T. serratus* (*P*-value = 0.025), according to the Fisher's exact test. Specifically, 31.3% of the juveniles are positive for *T. serratus*, comparing to 7.9% of adults. In the same way, *Poteriostomum* spp. has a prevalence of 56.3% in young horses and 22.2% in adults. In order to correlate the association of *T. serratus* and *Poteriostomum* spp. with age (adults vs. juveniles) already mentioned above, we performed a three-way crosstabulation and a Fisher's exact test, and we concluded that there is a significant association between *T. serratus* and *Poteriostomum* spp. in the juveniles (*P*-value = 0.034), that also exists, as previously mentioned, when we consider the entire population (*P*-value = 0.005), but not specifically in the adults (*P*-value = 0.307).

Finally, according to the Mann–Whitney test, the EPG values were significantly different between animals positive and negative for *S. vulgaris* (*P*-value = 0.001). Animals that were positive for *S. vulgaris* (*n* = 32) had an average level of 875 EPG and, contrarily, animals that were not infected by *S. vulgaris* (*n* = 47) had a mean value of 1567 EPG (fig. 3).

## Discussion

All the 79 analysed Przewalski's horses were infected with GI parasites, and all of them were positive for Strongylidae. Our findings agree with those of Slivinska *et al.* (2006), Kuzmina *et al.* (2009) and Zvegintsova *et al.* (2019), who also found a 100% prevalence for this taxonomic order in other Przewalski's horse populations in different Ukrainian regions. Regarding other helminths, 27.8% were positive for *Parascaris* sp. (22/79). Slivinska *et al.* (2006) presented a similar prevalence for Polish primitive horses (27.4%) in four different regions of Poland. Moreover, 2.5% (2/79) of the analysed wild horses contained *O. equi* in their faeces.

Kuzmina *et al.* (2009, 2017) also reported high infection levels in populations of the same subspecies. However, EPG level does not always correlate with the worm burden, depending, for instance, on the egg productivity of each adult worm (Carstensen *et al.*, 2013). It is essential to consider that the sample collection occurred mainly at the beginning of the autumn, in a time of low quality and quantity of food. These horses were

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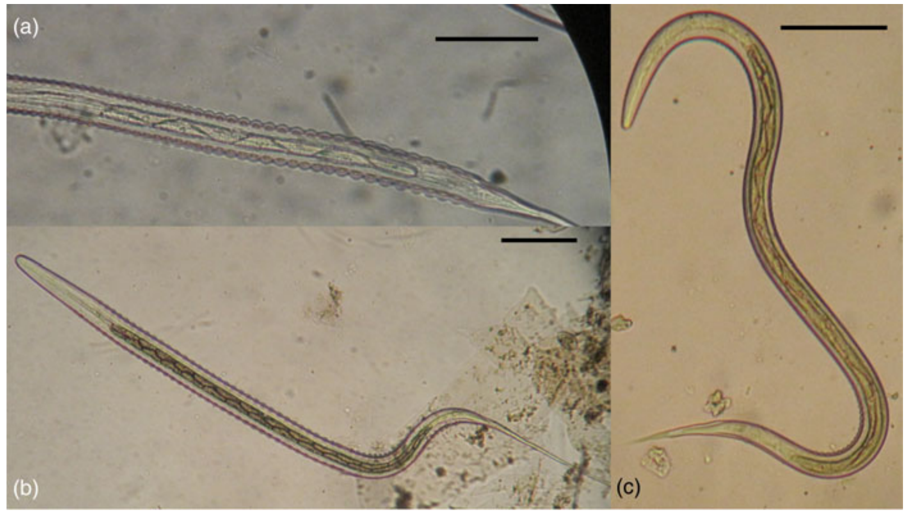


**Fig. 2.** Three of the 15 different strongylid L3 found in our study: *Cyathostomum sensu lato* morpho-type A (a), *Strongylus vulgaris* (b) and *Trichostrongylus axei* (c). *Cyathostomum sensu latum* morpho-type A has eight cells (2 + 6), the first two in a double row and the following six form a single line (medium total size: 812 µm); *S. vulgaris* has more than 20 cells, well-defined and very dark (medium total size: 936 µm; width: 32 µm); *T. axei* has a very short tail (it does not have a 'whip' form) measuring 115 µm from the anus to the end of the tail (medium total size: 738 µm) (Madeira de Carvalho et. al., 2008). Scale bars: 100 µm.

**Table 1.** Positive cases and prevalence levels of each identified parasite (according to Madeira de Carvalho *et al*., 2004, 2007, 2008; Santos *et al*., 2018) divided by categories in the population.

| | No. of positive animals | | | | | Percentage of positive animals | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | B | J | F | S | Total | B | J | F | S | Total |
| Order ASCARIDIDA | | | | | | | | | | |
| *Parascaris* spp. | 3 | 7 | 8 | 4 | 22 | 17.6 | 50 | 22.2 | 33.3 | 27.8 |
| Order OXYURIDA | | | | | | | | | | |
| *Oxyuris equi* | 0 | 0 | 2 | 0 | 2 | 0 | 0 | 5.6 | 0 | 2.5 |
| Order STRONGYLIDA | | | | | | | | | | |
| Subfamily CYATHOSTOMINAE | | | | | | | | | | |
| Cyathostomins type A | 17 | 14 | 36 | 12 | 79 | 100 | 100 | 100 | 100 | 100 |
| Cyathostomins type C | 2 | 6 | 11 | 5 | 24 | 11.8 | 42.9 | 30.6 | 41.7 | 30.4 |
| Cyathostomins type D | 7 | 5 | 18 | 9 | 39 | 41.2 | 35.7 | 50.0 | 75.0 | 49.4 |
| Cyathostomins type E | 0 | 2 | 2 | 0 | 4 | 0 | 14.3 | 5.6 | 0 | 5.1 |
| Cyathostomins type F | 4 | 1 | 8 | 4 | 17 | 23.5 | 7.1 | 22.2 | 33.3 | 21.5 |
| Cyathostomins type H | 2 | 1 | 2 | 3 | 8 | 11.8 | 7.1 | 5.6 | 25.0 | 10.1 |
| *Gyalocephalus capitatus* | 0 | 1 | 2 | 1 | 4 | 0 | 7.1 | 5.6 | 8.3 | 5.1 |
| *Poteriostomum* spp. | 4 | 8 | 9 | 2 | 23 | 23.5 | 57.1 | 25.0 | 16.7 | 29.1 |
| Subfamily STRONGYLINAE | | | | | | | | | | |
| *S. vulgaris* | 9 | 6 | 10 | 7 | 32 | 52.9 | 42.9 | 27.8 | 58.3 | 40.5 |
| *S. equinus* | 1 | 0 | 0 | 0 | 1 | 5.9 | 0 | 0 | 0 | 1.3 |
| *S. edentatus* | 1 | 0 | 1 | 0 | 2 | 5.9 | 0 | 2.8 | 0.0 | 2.5 |
| *Craterostomum acuticaudatum* | 0 | 2 | 2 | 1 | 5 | 0 | 14.3 | 5.6 | 8.3 | 6.3 |
| *Triodontophorus serratus* | 1 | 5 | 4 | 0 | 10 | 5.9 | 35.7 | 11.1 | 0.0 | 12.7 |
| *Triodontophorus* spp. | 1 | 0 | 3 | 0 | 4 | 5.9 | 0 | 8.3 | 0.0 | 5.1 |
| Family TRICHOSTRONGYLIDAE | | | | | | | | | | |
| *Trichostrongylus axei* | 4 | 3 | 14 | 5 | 26 | 23.5 | 21.4 | 38.9 | 41.7 | 32.9 |

B, bachelors; F, adult females, more than three years old; S, harem stallions; J, juveniles, females or males less than three years and members of a harem.

grazing close to the soil, and this behaviour could perhaps increase the ingestion of faecal material and, consequently, the possibility of infection, at least of parasites with a short prepatent period (for instance, cyathostomins or *T. axei*) (Nielsen & Reinemeyer, 2018).

According to the literature, several social and metabolic factors tend to influence wild horse parasitism. The social status of the individual and its role in the harem is also an influence factor, although complex and distinctive. Dominance behaviours, as group defence, are associated with high testosterone and cortisol

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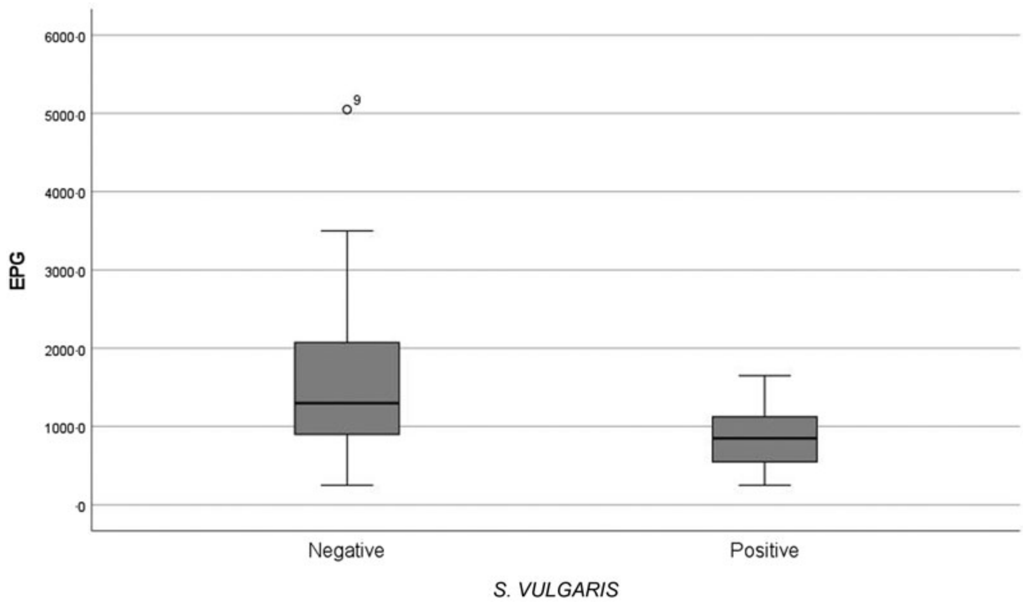


**Fig. 3.** EPG results according to the presence or absence of *Strongylus vulgaris* (positive group: mean = 875; min. value = 732; max. value = 1018; *N* = 32; negative group: mean = 1567; min. value = 1291; max. value = 1843; *N* = 47).

levels and energetic costs (Habig & Archie *et al*., 2015). Testosterone and cortisol can have immunosuppressive effects that can explain the higher FEC of GI nematodes found in dominant harem stallions comparing to the bachelors of a studied population of feral horses in Sable Island, Canada (Debeffe *et al*., 2016; Jota Baptista *et al*., 2021). Mares tend to spend more time feeding than other females, increasing the probability and intensity of GI infections (Boyd, 1988). On the other hand, the fecundity of female worms may increase, and the immune expulsion is impaired, due to the progesterone effect. Furthermore, depending on the parasite's species considered, younger horses usually have more parasites than the adults (Kuzmina *et. al*., 2016; Slivinska *et al*., 2016). Nevertheless, these changes were not evidenced in our population. Since the Pentezug population usually grazes and moves together, the pasture area occupied by a harem is frequently confluent with other harems, causing cross-contamination of the food and water resources. Consequently, these horses present a very homogeneous parasite population between the different harems, ages, sexes and other factors, as statistically evidenced. However, to better compare the EPG between different social groups, it would be important to standardize the number of samples collected in each group, proportionally to the total number of animals, allowing a more accurate and detailed analysis. Similarly, a study performed on a feral horse population in North Carolina revealed no differences between stallions and bachelors, between males and females and between lactating and non-lactating females (Rubenstein & Hohmann, 1989). Moreover, a study performed in our population reported no differences before and after foaling in the female body condition score, which supports the idea of a low physiological impact of reproduction and lactation in females (Brabender *et al*., 2016).

Regarding Strongylidae as the most prevalent parasites, the coprocultures showed parasite infections by 15 different strongylids forms. The proportions of Cyathostominae, Strongylinae and Trichostrongylidae (96.3%, 2.4% and 1.3%, respectively) were in accordance with other research performed with domestic horse parasites (Lopes *et al*., 2020).

Cyathostomum type A were the most frequent larvae found, occurring in all the analysed samples. This is in accordance with a *post-mortem* study in Prezwalski horses, where three species of this morpho-type (*Cylicostephanus minutus*, *Cyathostomum catinatum* and *Cylicocyclus nassatus*) occurred at a prevalence of more than 90%, and *C. minutus* with 100%, being the most frequent parasites found (Slivinska *et al*., 2006). Since these Przewalski's horses are seldom dewormed, or only in some specific situations, this may explain the diversity of Strongylidae genera and species – namely, the wide range of *Cyathostomum s.l.*, with six different morphotypes, as observed in other domestic and feral horse populations with low levels of anthelmintic pressure (Madeira de Carvalho, 2008; Madeira de Carvalho *et al*., 2008).

*Strongylus vulgaris* was the most common parasite of the subfamily Strongylinae and the same was found with Przewalski's horses studied by Slivinska *et al.* (2006) and Kuzmina *et al.* (2009). The evidenced prevalence (40.5%) is in the middle of the prevalence rates described for this nematode in the mentioned studies. This prevalence should be considered as threatening, due to the potentially severe consequences of the larvae migration in the mesenteric arteries (Nielsen & Reinemeyer, 2018). We believe that the relation of *S. vulgaris* with the horses with low faecal egg shedding (i.e. with lower EPG) can be explained by possible antagonistic relations established by this parasite. In other words, some parasites can induce microenvironmental changes inside the host, turning it unfavourable to other parasites. These changes can be direct, caused by the parasite, or indirect, with the intervention of the immune system (Cézilly *et al*., 2014). A study performed by Poulin (2001) describes the competitive interactions between helminths – one of them is the numerical response. Numerical responses are essentially the numerical changes in one or more parasite species induced by another one. Possibly, in our population, *S. vulgaris* is creating a numerical response in some other species of parasites, leading to lower values of EPG in those hosts. However, some equid studies contradict this finding, presenting no negative interactions between strongylid species, but instead some kind of niche

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partitioning and mutualistic interactions (Bucknell *et al.*, 1996; Stancampiano *et al.*, 2010; Sallé *et al.*, 2018). Specific studies regarding this situation in Przewalski's horses must be performed in order to produce consistent conclusions.

Moreover, 31.3% of the juveniles are positive for *T. serratus*, compared to 7.9% of adults. A *post-mortem* study of 134 horses in Victoria, Australia, reported a prevalence of 26% of *T. serratus* in juvenile horses (less than two years old). This was the age group with the highest prevalence for this parasite in the study, and the difference was also statistically significant (Bucknell *et al.*, 1995). In the same way, *Poteriostomum* spp. had a higher prevalence in juveniles than in adults. Kuzmina *et al.* (2016) reported that *Poteriostomum imparidentatum* was more prevalent in animals less than four years old: the group 1.5–4 years old had the highest value (15.7%), followed by the group of equids less than one year old (6.9%). Feasibly, the association of these two species of parasites can be their common propensity to infect younger individuals.

Surprisingly, there was no statically significant difference in *Parascaris* sp. prevalences between age groups, since this parasite is usually associated with younger horses. In some regions, the prevalence of this parasite can be 100% in foals aged less than one year old (Leathwick *et al.*, 2016; Jota Baptista *et al.*, 2021). However, Liu *et al.* (2018) reported a case of a six-year-old Przewalski's horse with a heavy *Parascaris* sp. infection associated with volvulus and sudden death. As mentioned, the collection was made on a convenience basis. To better evaluate this correlation in Przewalski's horses, a more extensive and proportional sample collection would be needed.

To date, this represents the most detailed parasitological survey performed in the Pentezug population, with a higher number of samples when compared to similar studies conducted in other European populations of Przewalski's horses.

This is a populational study from a single period of sample collection. We believe it represents a non invasive way of evaluating the parasite community of this population. However, similar continuous monitoring during different seasons can characterize possible seasonal trends in strongyles infection, with consequences for local management and possible reintroductions. Regular parasitological surveillance would also reveal the true influence of parasitism on body condition, reproduction status or immune responses, and consequent repercussions at the population level, at both Pentezug and in future reintroduction sites.

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**Conflicts of interest.** None.

**Ethical standards.** Not applicable

**Author contributions.** All the authors contributed equally to this work.

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