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## Gastrointestinal parasites of the Polish primitive horses from the Biebrza National Park

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## Summary

Thirty-one Polish primitive horses (*Equus caballus*) from three herds (two from the reserve and one from the stable) were dewormed with ivermectin+praziquantel and examined for the gastrointestinal parasite fauna. A total of 21,231 parasites were collected from the faeces at 24, 36 and 48 hours posttreatment. There were 35 nematode species, one cestode and one botfly larva. *Strongyloides westeri* infection was confirmed pretreatment by faecal sample examination and no threadworm specimens were found after deworming. Large and small strongyle prevalence was 90 % – 100 % and represented by 31 species. Among a total of 25 cyathostome species recovered (from 19 to 24 in each group), five species (*C. catinatum*, *C. minutus*, *C. longibursatus*, *C. nassatus* and *C. ashworthi*) had a prevalence of 100 % in three groups of horses. Meanwhile 14 species were 100 % prevalent in one herd. A total of six large strongyle species were found in adult horses. *Oxyuris equi* was recorded in 60 – 100 % of the horses while *Parascaris equorum* was detected in 100 % of foals and 16.7 % – 30 % of adult mares. *Habronema muscae* was found in 30 % of the horses from one free-ranging herd. Tapeworms (*Anoplocephala perfoliata*) were found in 90 % of the horses from one free-ranging group, whereas botfly larvae (*Gasterophilus intestinalis*) were found in 50 – 80 % of all surveyed horses. The present results are compared with earlier studies of Polish primitive wild horses from similar reserves in Poland. A total of 36 gastrointestinal parasite species were recorded from wild and stabled horses from the Biebrza National Park. This is in comparison with 35 such species in free-ranging and stabled horses from the Roztocze National Park and with 28 such species of free-ranging horses from the Popielno forest reserve. Among parasites recovered, the highly prevalent *S. vulgaris*, tapeworms and botfly larvae pose a serious risk of serious abdominal disorders in horses.

**Keywords:** horse; parasite community; biodiversity; Strongylidae; Poland

## Introduction

The Polish primitive horses (*Equus caballus*) represent a breed of domestic horses that bear a strong resemblance to tarpans, their wild ancestors which had lived on the steppes of southeastern Europe and Asia (Western Siberia). The original breed was com-

pletely extinct by the end of the 19<sup>th</sup> century. In the mid-twentieth century, primitive horses exhibiting tarpans' exterior were found in small farms of eastern Poland and saved in the reserve as the Polish primitive horses, a subspecies of tarpans. Currently, there are four reserves in Poland in which these horses are being kept in natural conditions. The horses are perfectly adapted to the natu-

ral environment and climate where they live throughout the year. Polish primitive horses are kept in Biebrza National Park (BNP), situated in the valley of the Biebrza River in northeastern Poland since 2004. The habitat of horses embraces 200 hectares of forest, grassland and marsh places, currently occupied by two herds (10 and 11 horses each). For veterinary and zootechnical control, all free-ranging horses are captured in the reserve during November – December. No anthelmintic treatment has been applied to date to these horses.

Despite the fact that Polish primitive horses are well known for their strong disease resistance, parasitic infections may influence their health. Until now the investigations of parasitic fauna in Polish primitive horses were performed in the Popielno Forest Reserve in northeastern Poland (Slivinska *et al.*, 2009) and the Roztocze National Park in southeastern Poland (Slivinska *et al.*, 2013). There are no data available on the gastrointestinal parasite community of horses from the Biebrza National Park.

The aim of this study was to investigate the gastrointestinal parasite community structure in previously untreated Polish primitive horses in the Biebrza National Park, and compare these results with those previously obtained in free-ranging horses from two other natural reserves.

## Material and Methods

### Horses

In this study 31 horses were examined. Twenty-one from the Biebrza National Park free-ranging (Group I and II) and 10 stabled horses (Group III) from the area next to the BNP. Group I consisted of 10 horses (1 stallion 6 years old, 6 mares 4 – 6 years old, 3 offspring 6 months old). Group II was comprised of 11 horses (1 stallion 8 years old, 10 mares 2 – 6 years old). Group II was transported from the National Park “Seven Islands” and kept under quarantine in a paddock for 9 months in Biebrza National Park until the present study. No anthelmintic treatment had been applied to the wild horses from Group I and II previously. Group III has been kept under stabled management on a private farm in the area next to the BNP. This group consisted of 1 gelding (5 years old), 6 mares (4 – 6 years old) and 3 offspring (6 – 10 months old). The horses were kept in a stable at night and returned to fenced pastures during the day. They have been dewormed irregularly, once or twice a year.

### Faecal egg counting

Faecal egg counts were carried out on the day before treatment using the McMaster technique (Herd, 1992) with a sensitivity of 25 eggs per gram (EPG). EPG values were counted individually for each horse and calculated for each group (Table 1).

### Worm burden and species identification

All horses (n=31) were treated with ivermectin (18.7 mg) + praziquantel (140.3 mg) (Equimax paste, Virbac de Portugal Labora-

torios) in a dose of 1.07 g paste per 100 kg b.w. Faecal sampling for the expelled parasites (200g of faeces in each sample) was performed for each animal *per rectum* 24, 36 and 48 hours post-treatment. The samples were washed with tap water into a glass cylinder and the sediment preserved in 40 % alcohol.

The mean worm burden (total number of worm specimens per sample) was calculated for each parasite species. The total number for each individual parasite species (N) was calculated as

$$N = \left( \frac{Wf}{200} \right) \times N_s,$$

where  $W_i$  is the total faeces weight produced by the horse during the experiment and  $N_s$  is the mean number of worm individuals in all samples collected from the horse. Based on these data the mean worm burden was calculated for each parasite species (Kuzmina *et al.*, 2005).

In the laboratory the gastrointestinal parasites were isolated, fixed in 70 % alcohol, counted and identified to the species level using the key of Lichtenfels *et al.*, (2008).

### Statistical analyses

Data summaries and descriptive analyses were calculated with Microsoft™ Excel.

To characterize the species diversity of parasites, we used the Shannon-Wiener index:  $H' = -\sum \eta_i / N \log_2 \eta_i / N$ , where  $\eta_i$  is the population density of each of the species and N is the overall density of populations (Shannon & Weaver, 1949).

The similarities in the species composition of parasites in different groups of horses were estimated using the Chekanovsky-Sørensen index of similarity ( $I_{CS}$ ) (Pesenko, 1982).  $I_{CS} = 2a / (a + b) + (a + c)$ , where a is the number of common species and  $(a + b) + (a + c)$  is the arithmetic mean of the number of species in both lists under comparison.

## Results

### Overall findings

A total of 21.231 parasite specimens were recovered from 31 horses and identified to the species level. The expelled parasites represented 36 species, i.e. 25 small strongyle species, 6 large strongyle species and by one species of ascarid, oxyurid, habronematid, cestode and botfly larvae (Table 2 and 3). In addition, threadworm infection (*Strongyloides westeri*) was confirmed in four foals by faecal sample examination (Table 1).

The total number of parasites expelled per horse varied from 37 to 1.124 with an average of  $686.3 \pm 417.7$ . Positive correlation was observed between the EPG value of ascarids and the total number collected from faeces ( $r^2 = 0.92$ ,  $P < 0.01$ ). A negative correlation was observed between the EPG value of strongylides and the total number of strongylid worm collected ( $r^2 = -0.17$ ,  $P < 0.01$ ).

Table 1. Results of faecal egg counts in free-ranging and stabled Polish primitive horses (n=31) in the Biebrza National Park, Poland

Horses		Strongylidae			<i>Parascaris equorum</i>			<i>Strongyloides westeri</i>			Cestoda
		EI %	Mean EPG	SD	EI %	MeanEPG	SD	EI %	MeanEPG	SD	
Group I, n=10	Stallion, n=1	100	1425.0	0	0	0	0	0	0	0	0
	Mares, n=6	100	1087.5	412.5	16.7	50	0	0	0	0	0
	Foals n=3	100	1025.0	327.9	100	566.7	454.4	100	150	86.6	0
Group II, n=11	Stallion, n=1	100	75.0	0	0	0	0	0	0	0	0
	Mares, n=10	100	750.0	404.1	0	0	0	0	0	0	0
	Geld, n=1	100	575	0	0	0	0	0	0	0	0
Group III, n=10	Mares, n=6	100	529.2	389.0	16.7	25	0	0	0	0	0
	Foals, n=3	100	541.7	278.8	100	236.7	162.5	33.3	400	0	0

EPG - eggs per gram  
EI, % - prevalence

A total of six large strongyle species was found in adult horses from three groups. Regarding the horses from the reserves, *Strongylus vulgaris* and *S. edentatus* were the most prevalent (57.1 % – 72.7 % and 85.7 % – 90.9 %, respectively) and *Triodontophorus serratus*, *T. brevicauda*, *T. tenuicollis* and *Craterostomum acuticaudatum* were quite common (36.4 % – 54.6 %). In adult wild horses, 7.5 and 5.0 specimens of *Strongylus vulgaris* and *S. edentatus* were found, respectively. Only one specimen of *T. serratus* and *T. tenuicollis* was recorded in adult horses (Table 2).

Small strongyles occurred in all horses and were the most abundant among gastrointestinal parasites. Among a total of 25 cyathostome species found, eight (*Cyathostomum catinatum*, *Coronocyclus coronatus*, *Cylicostephanus calicatus*, *C. minutus*, *C. longibursatus*, *Cylicocyclus insigne*, *Cylicocyclus nassatus* and *C. ashworthi*) were 90 – 100 % prevalent in the three herds and constitute the bulk of the parasite community in surveyed horses (Table 2). The mean worm burden of cyathostomes per horse ranged from single specimens of a rare species (*Cylicocyclus radiatus* and *Gyalocephalus capitatus*) found in the prevalence of 10 % – 27.3 %, up to 21.4 – 170.2 specimens to the five 100 % prevalent species (*Cyathostomum catinatum*, *Cylicostephanus minutus*, *C. longibursatus*, *Cylicocyclus nassatus*, *C. ashworthi*). The prevalence of *Parascaris equorum* was 100 % in foals kept in the reserve and 16.7 % – 30 % in free-ranging and stabled mares. *Oxyuris equi* and botfly larvae (*Gasterophilus intestinalis*) were the most common non-strongylid parasites in horses, with prevalence 60 – 100 % and 50 – 80 %, respectively. The mean number of ascarids per horse was 9.0±4.2, oxyurids 6.1±4.8 and habronematids 1.0 ± 0.5 (Table 2).

#### Gastrointestinal parasite community in Group I (n=10)

Results of faecal egg counts are provided in Table 1. The mean epg for strongylids was 1,102.2 (ranging from 75 to 1,725). Three foals were found to be infected with ascarids. The mean epg was 566.7. *P. equorum* eggs also were excreted by one mare. The foals were infected with *Strongyloides westeri* (a mean epg 150).

Thirty-three species of gastrointestinal parasites were recovered after deworming. Among all 31 nematode species, 28 were Strongylidae and one species from Oxyuridae, Ascaridae and Habronematidae. One cestode (*Anoplocephala perfoliata*) and one species of botfly larvae (*Gasterophilus intestinalis*) also were found (Table 2). Nine to 25 parasite species were found per host (a mean 18.1) where four strongyle and 24 cyathostome species were recorded.

In adult horses, four large strongyle species (*S. vulgaris*, *S. edentatus*, *T. serratus* and *T. tenuicollis*) were found. *Strongylus vulgaris* and *S. edentatus* were of the highest prevalence (57.1 – 85.7 %). Small strongyles (Cyathostominae) were represented by 24 species. Among them nine species (*Cyathostomum catinatum*, *C. pateratum*, *Coronocyclus coronatus*, *Cylicostephanus calicatus*, *C. minutus*, *C. longibursatus*, *Cylicocyclus nassatus*, *C. ashworthi* and *C. insigne*) were 90 – 100 % prevalent.

*Oxyuris equi* and botfly larvae (*G. intestinalis*) were recorded in 70 – 80 % horses, while *Parascaris equorum* and *Habronema muscae* in 20 % – 30 %, respectively. Tapeworms (*Anoplocephala perfoliata*) were found in 90 % of the horses (Table 2).

Generally, the number of parasite specimens recovered from the surveyed horses was low. The mean number of large strongyle specimens varied from 1.0 – 7.5 per host, with the highest numbers for *S. vulgaris* and *S. edentatus* (a mean 5.0 – 7.5 per host). The nine cyathostome species recorded as 90 – 100 % prevalent. The mean number of specimens recovered ranged from 8.7 (*C. insigne*) to 72.0 (*C. nassatus*).

The mean number per horse for ascarids was 9.0, oxyurids 6.1, habronematids 1.0 and cestodes 4.1. One non-helminth gastrointestinal parasite, a botfly larvae (*G. intestinalis*), was recorded with the prevalence of 80 % and intensity of 2 – 12 instars per horse (a mean 4.4).

No threadworm specimens were recovered after deworming in foals despite detection of 150 *S. westeri* eggs per gram of faecal samples isolated from three animals.

Table 2. Results of the gastrointestinal survey in free-ranging and stabled Polish primitive horses (n=31) in the Biebrza National Park, Poland

Parasite species	Group I (n=10)				Group II (n=11)		Group III (n=10)			
	Prevalence %		MWB $\pm$ SD (Total number of specimens)		Prevalence %	MWB $\pm$ SD (Total number of specimens)	Prevalence %		MWB $\pm$ SD (Total number of specimens)	
	Adults horses, n=7	Foals, n=3	Adults horses, n=7	Foals, n=3	Adults horses, n=11		Adults horses, n=7	Foals, n=3	Adults horses, n=7	Foals, n=3
Strongylinae	60		6.3 (63)		100	17.2 (189)	30		6.3 (19)	
<i>Strongylus vulgaris</i>	57.1	0	7.5 $\pm$ 5.5	0	72.7	8.0 $\pm$ 5.6	28.6	0	3.0 $\pm$ 1.6	0
<i>S. edentatus</i>	85.7	0	5.0 $\pm$ 4.1	0	90.9	9.1 $\pm$ 5.5	28.6	0	5.5 $\pm$ 2.7	0
<i>Triodontophorus serratus</i>	28.6	0	1 $\pm$ 0.5	0	54.6	2.0 $\pm$ 1.3	0	0	0	0
<i>T. brevicauda</i>	0	0	0	0	45.5	2.0 $\pm$ 1.1	14.3	0	2.0 $\pm$ 0.8	0
<i>T. tenuicollis</i>	14.3	0	1 $\pm$ 0.4	0	36.6	1.5 $\pm$ 0.8	0	0	0	0
<i>Craterostomum acuticaudatum</i>	0	0	0	0	36.4	1.5 $\pm$ 0.8	0	0	0	0
Cyathostominae	100		312.2 (3122)		100	982.7 (10810)	100		63.9 (6385)	
<i>Cyathostomum catinatum</i>	100		57.4 $\pm$ 58.2		100	137.2 $\pm$ 26.9	100		115.6 $\pm$ 90.9	
<i>C. pateratum</i>	90		12.4 $\pm$ 9.3		100	22.9 $\pm$ 10.1	70		23.1 $\pm$ 60.3	
<i>Coronocyclus coronatus</i>	100		14.7 $\pm$ 10.5		100	47.0 $\pm$ 19.9	90		37.6 $\pm$ 28.1	
<i>C. labiatus</i>	50		2.4 $\pm$ 1.4		100	9.6 $\pm$ 4.2	60		10.3 $\pm$ 7.0	
<i>C. labratus</i>	50		3.8 $\pm$ 2.6		100	10.6 $\pm$ 3.4	60		7.2 $\pm$ 5.6	
<i>Cylicostephanus calicatus</i>	90		15.4 $\pm$ 12.2		100	50.7 $\pm$ 21.3	100		22.8 $\pm$ 18.4	
<i>C. minutus</i>	100		44.6 $\pm$ 42.1		100	158.5 $\pm$ 52.7	100		115.6 $\pm$ 88.0	
<i>C. hybridus</i>	30		1.0 $\pm$ 0.5		45.5	1.6 $\pm$ 0.9	0		0	
<i>C. longibursatus</i>	100		51.5 $\pm$ 57.3		100	166.7 $\pm$ 27.5	100		104.6 $\pm$ 81.3	
<i>C. goldi</i>	80		8.4 $\pm$ 6.4		100	30.0 $\pm$ 10.4	90		25.6 $\pm$ 19.7	
<i>C. bidentatus</i>	30		1.0 $\pm$ 0.5		72.7	2.1 $\pm$ 1.3	10		2.0 $\pm$ 0.6	
<i>Cylicocyclus radiatus</i>	10		1.0 $\pm$ 0.3		27.3	1.0 $\pm$ 0.5	0		0	
<i>C. elongatus</i>	70		2.4 $\pm$ 1.6		100	16.3 $\pm$ 4.8	90		16 $\pm$ 12.6	
<i>C. insigne</i>	90		8.7 $\pm$ 6.6		100	44.3 $\pm$ 19.9	100		20.3 $\pm$ 16.1	
<i>C. leptostomum</i>	80		4.8 $\pm$ 4.3		100	18.7 $\pm$ 6.3	60		13.8 $\pm$ 9.1	
<i>C. nassatus</i>	100		72.0 $\pm$ 60.5		100	170.2 $\pm$ 43.3	100		111.3 $\pm$ 85.9	
<i>C. ashworthi</i>	100		21.4 $\pm$ 17.9		100	86.6 $\pm$ 23.1	100		38.2 $\pm$ 30.6	
<i>C. ultrajectinus</i>	30		1.0 $\pm$ 0.5		72.7	4.5 $\pm$ 3.9	20		2.0 $\pm$ 0.8	
<i>Cylicodontophorus bicoronatus</i>	10		1.0 $\pm$ 0.3		63.6	3.1 $\pm$ 2.2	0		0	
<i>Parapoteriostomum euproctus</i>	40		2.0 $\pm$ 1.0		0	0	40		5.5 $\pm$ 3.3	
<i>P. mettami</i>	10		1.0 $\pm$ 0.3		72.7	2.5 $\pm$ 1.5	50		1.4 $\pm$ 0.8	
<i>Poteriostomum imparidentatum</i>	10		1.0 $\pm$ 0.3		63.6	1.9 $\pm$ 1.2	0		0	
<i>P. ratzii</i>	10		1.0 $\pm$ 0.3		54.6	2.0 $\pm$ 1.3	0		0	
<i>Petrovinema poculatum</i>	20		1.0 $\pm$ 0.4		81.8	1.6 $\pm$ 0.9	30		1.3 $\pm$ 0.7	
<i>G. capitatus</i>	0		0		27.3	1.0 $\pm$ 0.5	0		0	
Ascaridae										
<i>Parascaris equorum</i>	20		9.0 $\pm$ 4.2 (18)		0	0	30		2.0 $\pm$ 1.3 (6)	

Oxyuridae						
<b><i>Oxyuris equi</i></b>	70	6.1 ± 4.8 (43)	100	39.6 ± 12.5 (435)	60	2.0 ± 1.6 (12)
Habronematidae						
<b><i>Habronema muscae</i></b>	30	1.0 ± 0.5 (3)	0	0	0	0
Anoplocephalidae						
<b><i>Anoplocephala perfoliata</i></b>	90	4.1 ± 2.0 (37)	0	0	0	0
Gasterophilidae						
<b><i>Gasterophilus intestinalis</i></b>	80	4.4 ± 3.4 (35)	72.7	5.8 ± 3.3 (46)	50	2.4 ± 1.5 (12)

MWB – mean worm burden; SD – standard deviation

#### Gastrointestinal parasite community in Group II (n=11)

This herd consisted of adult horses only. Based on faecal egg count results (Table 1), all horses were infected with strongylids and no other parasite eggs were found. Among all 32 species recovered, 31 were nematode species (30 strongyle and one oxyurid) and one species of botfly larvae (Table 2). Twenty to 28 species were found per host (a mean 24.9) where six strongyle and 24 cyathostome species were recorded. Among large strongyles found, *S. vulgaris* and *S. edentatus* were the most prevalent (72.7 % and 90.9 %, respectively) and the three species of *Trichostrongylus* were quite common (36.6 – 54.6 %). Fourteen small strongyle species (*C. catinatum*, *C. pateratum*, *C. coronatus*, *C. labiatus*, *C. labratus*, *C. calicatus*, *C. minutus*, *C. longibursatus*, *C. goldi*, *C. elongatus*, *C. insigne*, *C. leptostomus*, *C. nassatus* and *C. ashworthi*) were 90 – 100 % prevalent (Table 2).

The mean number of large strongyles was 1.5 – 9.1 and of the most common small strongyle species ranged from 9.6 (*C. labiatus*) to 170.2 (*C. nassatus*) per horse. *Oxyuris equi* was found to have a mean of 39.6 specimens per horse. Botfly larvae (*G. intestinalis*) were recorded in 72.7 % of horses with 2 – 9 specimens found per horse (a mean 5.8).

#### Gastrointestinal parasite community in Group III (n=10)

Based on faecal egg counts, all horses were infected with strongylids (529 – 575 epg) and one mare and three foals were infected with ascarids (Table 1). One foal was infected with *Strongyloides westeri* (a mean 400 epg); however no threadworm specimens were recovered after deworming this foal.

In total, 26 gastrointestinal parasite species were found in stabled horses, i.e. 25 were nematodes and one species was a botfly larva (Table 2 and 3). Ten to 21 species with a mean of 15.6 per host were found. Three large strongyle species (*S. vulgaris*, *S. edentatus* and *T. brevicauda*) were 14.3 – 28.6 % prevalent. This was similar to Group I from the reserve where large strongyles were detected only in adult horses. Among a total 19 cyathostome species recorded, ten were 90 – 100 % prevalent (*C. catinatum*, *C. coronatus*, *C. calicatus*, *C. minutus*, *C. longibursatus*, *C. goldi*, *C. elongatus*, *C. insigne*, *C. nassatus* and *C. ashworthi*). *Oxyuris equi*, *Parascaris equorum* and botfly larvae (*G. intestinalis*) were found in 60 %,

30 % and 50 % of horses, respectively (Table 2).

In general, the number of gastrointestinal parasites recovered from the stabled horses was low. Means of 3.0 to 5.5 specimens of *S. vulgaris* and *S. edentatus* were found and 20.3 (*C. insigne*) to 115.6 (*C. minutus* and *C. catinatum*) of the ten most common cyathostome species recovered (Table 2). The prevalence of ascarids and oxyurids was 30 % and 60 %, respectively. Mean of 2.0 specimens was found per horse after deworming. *Gasterophilus intestinalis* larvae were recorded in 50.0 % of the horses for which 1 – 4 specimens were found (a mean 2.4).

#### Biodiversity approach

Species richness of the parasite community was much higher in free-ranging horses kept under natural conditions (Group I and II) when compared with stabled horses (Group III). The results of the multivariate analyses of the parasite community showed the significant differences of the Shannon-Wiener diversity indices for the parasite community in wild horses from Group II and I ( $H' = 2.499$  and  $2.381$ , respectively), when compared with communities of stabled horses from Group III ( $H' = 2.285$ ).

Biodiversity of the parasite communities of wild horses from Group I and II are rather similar. The Chekanovsky-Sørensen Index ( $I_{cs}$ ) is 0.89. Species richness of the parasite communities in horses from Group I and III is more similar ( $I_{cs} = 0.83$ ) than in horses from Group II and III ( $I_{cs} = 0.81$ ).

#### Discussion

The results presented herein are the first *in vivo* comparative investigations of parasite communities in free-ranging and stabled horses in Poland. The effectiveness of intravital collection of intestinal helminthes was shown to provide data comparable with those based on postmortem methods (Osterman Lind *et al.*, 2003; Kuzmina *et al.*, 2005).

Only one infection (*Strongyloides westeri*) was not confirmed with diagnostic deworming but was defined by presence of eggs in faeces of three foals from one free-ranging herd and one foal from the stable. Despite a mean of 150 and 400 eggs per gram in faecal samples, no threadworm specimens were recovered af-

Table 3. The gastrointestinal parasite species found in Polish primitive horses from Biebrza National Park (present study), Roztocze National Park (Slivinska *et al.*, 2013) and Popielno forest reserve (Slivinska *et al.*, 2009)

Parasite species	Present study			Roztocze	Roztocze	Popielno
	Wild horses, n=21		Stabled horses, n=10	Wild horses, n = 16 (Slivinska <i>et al.</i> 2013)	Stabled horses, n=13 (Slivinska <i>et al.</i> 2013)	Wild horses, n=11 (Slivinska <i>et al.</i> 2009)
	Group I	Group II				
<i>Strongylus vulgaris</i>	+	+	+	+	+	+
<i>S. edentatus</i>	+	+	+	+	+	-
<i>Triodontophorus serratus</i>	+	+	-	+	+	+
<i>T. brevicauda</i>	-	+	+	+	+	-
<i>T. tenuicolis</i>	+	+	-	+	+	-
<i>Craterostomum acuticaudatum</i>	-	+	-	+	+	-
<i>Cyathostomum catinatum</i>	+	+	+	+	+	+
<i>C. pateratum</i>	+	+	+	+	+	+
<i>Coronocyclus coronatus</i>	+	+	+	+	+	+
<i>C. labiatus</i>	+	+	+	+	+	+
<i>C. labratus</i>	+	+	+	+	+	+
<i>Cylicostephanus calicatus</i>	+	+	+	+	+	+
<i>C. minutus</i>	+	+	+	+	+	+
<i>C. hybridus</i>	+	+	-	+	-	-
<i>C. longibursatus</i>	+	+	+	+	+	+
<i>C. goldi</i>	+	+	+	+	+	+
<i>C. bidentatus</i>	+	+	+	+	-	+
<i>Cylicocyclus radiatus</i>	+	+	-	+	-	-
<i>C. elongatus</i>	+	+	+	+	+	+
<i>C. insigne</i>	+	+	+	+	+	+
<i>C. leptostomus</i>	+	+	+	+	+	+
<i>C. nassatus</i>	+	+	+	+	+	+
<i>C. ashworthi</i>	+	+	+	+	+	+
<i>C. ultrajectinus</i>	+	+	+	+	-	+
<i>C. bicoronatus</i>	+	+	-	+	-	+
<i>C. euproctus</i>	+	-	+	+	+	+
<i>C. mettami</i>	+	+	+	+	+	+
<i>Poteriostomum imparidentatum</i>	+	+	-	+	+	+
<i>P. ratzii</i>	+	+	-	+	-	-
<i>Petrovinema poculatum</i>	+	+	+	+	+	-
<i>Gyalocephalus capitatus</i>	-	+	-	+	-	+
<i>Parascaris equorum</i>	+	-	+	+	+	+
<i>Habronema muscae</i>	+	-	-	+	+	+
<i>Oxyuris equi</i>	+	+	+	-	-	+
<i>Anoplocephala perfoliata</i>	+	-	-	+	+	+
<i>Gasterophilus intestinalis</i>	+	+	+	+	+	+
Number of cyathostome species	24	24	19	25	18	21
Number of parasite species	33	32	25	35	28	28

ter deworming. Possibly it was the result of low infection intensity or the worms may have been overlooked during examination for expelled parasites. These nematodes have a very thin cuticle and thus probably specimens passed out disintegrated. Moreover, they could have been digested during their transfer from the intestine to the rectum and passed in the faeces.

The present results are similar to those obtained in the earlier surveys in the Polish primitive horses living in the free-ranging conditions (Slivinska *et al.*, 2009, 2013). A total of 37 gastro-intestinal parasite species were recorded in wild and stabled horses from the BNP in comparison with 35 such species in horses from the Roztocze National Park (Slivinska *et al.*, 2013) and 28 species

in wild yearlings from the Popielno Forest Reserve (Slivinska *et al.*, 2009) – Table 3. Among a total of 25 cyathostome species recovered in the current study (from 19 to 24 in one group), five species (*C. catinatum*, *C. minutus*, *C. longibursatus*, *C. nassatus* and *C. ashworthi*) were of 100 % prevalence in three herds, while 14 species were 100 % prevalent in at least one group. We found the same 25 small strongyle species in horses from two wild herds as in the Roztocze National Park reserve (Slivinska *et al.*, 2013). In the present study we examined horses that were not dewormed previously, which suggested that there might exist a rich parasite fauna. Kuzmina *et al.*, (2008). The study aimed to evaluate the influence of anthelmintic treatments on strongylid community struc-

ture based on the hypothesis that regular anthelmintic treatments cause a reduction of species richness, diversity and concentration in strongylid communities. These parameters were significantly higher in cyathostome communities of horses from nontreated and occasionally treated horses than in regularly treated horses. The strongylid communities of wild equids or equids from natural reserves are characterized by multimodal distribution of species, with dominant, subdominant, background and rare species (Kuzmina *et al.*, 2008). Some cyathostome species found dominant or subdominant in the wild Polish horses in the present study and in the previous surveys, have been recorded as background or rare/very rare species in brood horses in Poland. *Cylicostephanus bidentatus*, fairly prevalent in this study in horses from the reserve (30 % and 72.7 %), also was found in one horse from the stabled group (10 %). This cyathostome species recorded in 27.3 % of yearlings in the Popielno Forest Reserve, was the first occurrence in the horse in Poland (Slivinska *et al.*, 2009). Cyathostome species, with 9.1 % prevalence, were rare in yearlings from Popielno (*Poteriostomum imparidentatum* and *Parapoteriostomum mettami*), while *Poteriostomum ratzii* and *Petrovinema poculatum* were not recorded in these horses. The present study showed these four species being subdominant or dominant (54.6 – 81.8 %) in horses from one free-ranging herd (Group II). For comparison, the prevalence of these species in working horses examined after slaughter in central Poland was 2 %, 8 %, 10 % and 20 % for *P. ratzii*, *P. poculatum*, *P. mettami* and *P. imparidentatum*, respectively (Gawor 1995). More recent survey in irregularly dewormed domestic horses from southern Poland (Kuzmina *et al.*, 2011) revealed *P. poculatum*, *P. mettami* and *P. imparidentatum*. Regarding four cyathostome species, namely *Cylicostephanus hybridus*, *Cylicocyclus radiatus*, *Poteriostomum ratzii* and *Gyaloccephalus capitatus* found in the group I and/or the group II (10 – 45.5 %) and also recorded in the wild horses from Roztocze Park, neither of them were revealed in stabled horses in the current study, and nor in such horses from Roztocze Park (Table 2 and 3). These horses occasionally were dewormed, which possibly eliminated the presence of these rare/background species. However, even under frequent anthelmintic treatments, dominant species are still present in the strongylid community (Kuzmina *et al.*, 2008). The present findings that a few cyathostome species constitute the bulk among strongylids in the wild horses are similar to results of surveys conducted in various breeds of horses in different countries, i.e. Poland and Ukraine (Gawor 1995; Kuzmina *et al.*, 2011), Spain (Meana *et al.*, 2004), United States (Reinemeyer *et al.*, 1984; Lyons *et al.*, 1997), South Africa (Scialdo-Krecek *et al.*, 1983) and Australia (Mfitilodze & Hutchinson 1990). In the present study, 9 cyathostome species found in horses from Group I, 14 species from Group II and 10 species from Group III were 90 – 100 % prevalent and comprised 92.5 – 96.9 % of the strongyle population representing 89.8 – 93.2 % of the total parasites recovered in each group.

In general, surveyed horses from BNP, Roztocze and Popielno

with the high helminth diversity were associated with low intensity of infection. The mean number of cyathostome specimens in the current study was 1.0 – 170.2 and 1.0 – 134.7 and 1.3 – 166.1 in Roztocze and Popielno, respectively. Concerning large strongyle species, the mean number in the present study was 1.0 – 9.1 and 1.5 – 11.0 in Roztocze or 1.9 – 2.5 in Popielno (Slivinska *et al.*, 2009, 2013).

We found the same six large strongyle species in two groups from the BNP reserve, similar to the wild horses from the Roztocze National Park. In the current study no large strongyle infection was found in the six foals examined. These results differ from the previous results found in 11 yearlings captured in the Popielno Reserve in which *S. vulgaris* and *Triodontophorus serratus* were found; the prevalence was 81.8 % and 90.9 %, respectively (Slivinska *et al.*, 2009). These differences may be due to the younger foals' age in the current study (6 – 10 months) when compared to those examined in the Popielno Reserve (12 – 14 months). On the whole, in wild horses from the recently examined reserves, Roztocze and Popielno, *S. vulgaris* and *S. edentatus* were highly prevalent, 57.1 % – 93.8 % and 81.3 % – 85.7 %, respectively. Two recent surveys in rarely dewormed horses from individual farms in southeastern Poland revealed these species had prevalences of 4 % for each species (Kuzmina *et al.*, 2011) and 22.8 % and 18.3 %, respectively (Studzińska *et al.*, 2012). Recent studies from different countries have shown that in well managed horse populations, *S. vulgaris* have become uncommon among equine parasites. Increased occurrence of this most pathogenic parasite infecting horses, has recently been associated with the widely recommended selective therapy approach for parasite control (Nielsen *et al.*, 2012). Reduced treatment intensities, with the general approach to leave untreated horses with a low egg count value (selective therapy), potentially delays development of ivermectin and moxidectin resistance in cyathostomes and ascarids. This approach to leave horses with low egg counts (selective therapy) untreated, may be a possible reason of an increased prevalence of *S. vulgaris* in stud farms and training stables (Nielsen *et al.*, 2012).

The present and previous surveys in the wild Polish primitive horses from the reserves in Poland, confirmed that these horses represent populations from the pretreatment era with parasite fauna not influenced by deworming practices. Large strongyle species richness with the high prevalence of *S. vulgaris* and *S. edentatus*, seems to be proof of this assumption.

The prevalence of pinworms (*Oxyuris equi*) in horses in three examined herds in the current study (60 – 100 %) was higher than in yearlings from the Popielno Forest Reserve (23.1 %) (Slivinska *et al.*, 2009). No oxyurids were recovered in horses from the Roztocze National Park (Slivinska *et al.*, 2013). The prevalence of *Parascaris equorum* in adult mares was 16.7 % – 30 % which is similar to that found in mares from Roztocze (15.4 % – 25.0 %). *Habronema muscae* specimens with 30 % prevalence were recorded in one group of wild horses which is similar to results from Roztocze and Popielno (55.2 % and 18.2 %, respectively). *Ano-*

*plocephala perfoliata* with prevalence of 90 %, was found in one wild herd. This was similar to a level found in Roztocze (72.2 %), meanwhile in Popielno, 24.2 % of yearlings were found infected with tapeworms. The prevalence of the botfly larvae (*Gasterophilus intestinalis*) was 50 – 80 %, which is different from their occurrence in wild horses in Popielno (90.9 %) and Roztocze (41.4 %) (Slivinska *et al.*, 2009, 2013).

## Conclusions

Among the major parasitic pathogens in horses, cyathostomins, *P. equorum*, *A. perfoliata* and *S. vulgaris* are mentioned. Along with *G. intestinalis* having the potential for serious abdominal disorders, all these parasites were found at high prevalences in wild horses examined in the present study. Reliable detection and differentiation of these parasites is an important requirement for diagnostics in equine parasitology. Diagnostic deworming, used with collection and identification of expelled parasites to the species level, is laborious method. However, it provides detailed information on prevalence and intensity of gastro-intestinal parasite infection, including tapeworms whose eggs are specifically difficult to detect by fecal examination. The wild horses living freely in the reserve and not treated previously with anthelmintics, represent exceptional hosts for investigation of the horse primeval, indigenous parasite fauna. Performing comparative studies between differently managed and not managed herds of horses helps to address proper recommendations for control programs and predict the consequences of inadequate treatment strategies used.

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