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Short Communication

Prevalence, Intensity and Risks Involved in Helminth Infections in Domestic Mountain Pony and Balkan Donkey in Nature Park Stara Planina, Serbia

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Received 10 Jan 2020
Accepted 12 Apr 2020

Keywords:

Equine;
Helminths;
Infection;
Prevalence;
Stara Planina

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Abstract

Background: This study aimed to investigate the prevalence, intensity and risk factors (age and gender) promoting the intestinal helminthic infections of the Domestic mountain ponies and Balkan donkeys in Serbia.

Methods: Prevalence, intensity and risk factors influencing helminth infection in horses (n=39) and donkeys (n=18) at the site of Nature Park Stara Planina, were studied from May to Sep 2015. The presence of one or several gastrointestinal helminth species was confirmed by faecal flotation in 97.43% of horses and 88.88% of donkeys included in the monitoring. The identified helminth species were *Trichostrongylus axei*, *Strongylus edentatus*, *S. vulgaris*, *Parascaris equorum*, *Dictyocaulus arnfieldi* and *Anoplocephala magna* in 84.61%, 46.15%, 5.13%, 58.97%, 94.87% and 38.46% of horses, respectively. The significant association of infection with *P. equorum* and sex of horses was established. ($\chi^2=13.33$, $P<0.01$).

Results: The prevalence of parasitic helminths identified in donkeys was the following: *D. arnfieldi*, *T. axei*, *Pa. equorum*, *S. vulgaris*, *A. magna* and *Strongyloides westeri* in 55.55%, 38.89%, 27.78%, 22.22% and 22.22% of donkeys, respectively. Moreover, the mean faecal egg count was higher in donkeys (369.9 EPG - egg per gram) than in horses (250.2 EPG). The association between the age and the mean EPG was significant ($P<0.05$) in both equine species.

Conclusion: The results of the investigation provided basic information that can be helpful for planning strategic control of nematode infection in equine population in Nature Reserves in Serbia.



Introduction

In recent decades, the populations of autochthonous livestock species and breeds have been rapidly decreasing. The devastating depopulation trend was very prominent in case of autochthonous equine and ruminant breeds so that these animals became endangered. During the last decades, the national program for Animal Genetic Resources (AnGR) protection and/or conservation included in-situ conservation of representative population samples of autochthonous equine and ruminant breeds. The animals included in the AnGR strategic conservation program are bred in a traditional free-range pasture system in the areas of protected nature, i.e. Nature Reserves and Parks. Domestic mountain pony is autochthonous horse breed of the Western Balkan. The breed descended from Tarpan (*Equus ferus*) and Przewalskii (*Equus przewalskii*) and that the original breed has developed through crossbreeding with Arab and other oriental horses. Mountain ponies of the former Yugoslavia, with exception of Bosnian pony, regardless of the geographic habitat and phenotypic differences were included into domestic mountain pony population, so that contemporary animals vary greatly both in morphology and in coat color.

Nowadays, domestic mountain pony in Serbia is bred at conservatory sites of Stara Planina, Stolovi, Prokletije, Shar Planina, Sjenica-Peshter (Reserve of Biosphere) and in Nature Reserves Zasavica and Kovilj. Trailović (1), Đermanović et al (2), Trailović et al (3,4) and Mitrović et al (5) previously described morphological, physiological and reproductive characteristics of this breed. As the other horse breeds developed on the Balkan, domestic mountain pony is sturdy, with characteristic good health, controlled and balanced temper, they are obedient and modest in demands. The breed adaptive characteristics are good; they easily endure the extreme climate

and are highly resistant to various pathogens and to some extent to parasitic infections. The estimated current population size is around 500 to 550 horses but active population is much smaller.

The Balkan donkey (*Equus asinus*), also included on AnGR conservation programme, is an indigenous breed with the traditional breeding area in the mountain region of Serbia and other Balkan countries (1). These animals are kept is carried in the traditional breeding area of several protected nature reserves: Special Nature Reserve Zasavica, National Park Stara Planina and in the village of Kovilj, near Novi Sad. Due to microevolutive adaptation to the habitat extending from the mountains of Central Balkan with characteristic harsh climate to the Mediterranean area of the Balkan Peninsula, Balkan donkey is sturdy, with modest demands in nutrition and care. Estimated population size is only 200-250 animals, and with only half of the population, reproducing the urgent conservation programme for this breed was implemented during the last couple of years. Morphological, physiological and reproductive characteristics of this breed was previously described (3, 4, 6, 7).

Grazing on pasture facilitates almost constant ingestion of eggs and/or developing infective larvae and intermediate hosts of the parasites, while the lack of prevention favors the expansion of the parasites (8). Therefore, faecal excretion allows the permanent contamination of the pasture where horses and donkeys graze and enables permanent parasitic infection of the resident animals (9). The permanently parasites infections continue to be a significant factor for the health of equines (10). In the host, parasites cause loss of nutrients and blood provoking serious health problems in horses and donkeys worldwide (11-16).

In Serbia, parasitological studies of domestic equines were sporadically performed (9,17-19)

therefore, valid historical data concerning the prevalence of helminthes in equines are lacking. The lack of systematic data on parasitic load is even more apparent when domestic mountain ponies and Balkan donkeys are concerned. Therefore, the objective of this study was to investigate the prevalence, intensity and risk factors (age and gender) promoting the intestinal helminthic infections of the domestic mountain ponies and Balkan donkeys in Serbia.

Materials and Methods

The largest population of autochthonous domestic mountain pony and Balkan donkey kept in free-range grazing system in Serbia is located on Mountain Stara Planina

(42°43'00"N 24°55'04"E). This is the highest mountain in South-East Serbia. Stara Planina (Balkan mountain) is the Southern Karpathian fold stretching for 560 km from Vlashka Chuka Peak fist to the North on the border between Serbia and Bulgaria and then folding East through Central Bulgaria to Cape Emine on the Black Sea, thus framing the Pannonian Basin on the South of the Danube (Fig. 1). Stara Planina has a characteristic climate changing from moderate continental, mountain (Alpine) and on the high peaks subarctic. Going from low to higher slopes, the average temperature drops while humidity increases. With an average temperature of 0.5 °C, the coldest month is Jan, while July with 22 °C is the warmest. Average annual rainfall is 960.5 mm.



Fig. 1: Map of sampling area - Encircled part of the map

Nature Park Stara Planina in Serbia is the largest area of protected nature including both wildlife and autochthonous domesticated species. It is ancient grazing territory where pastoral rural culture was developed through centuries, so this territory became an important site for the development of in situ conservation of autochthonous animals in Serbia. Preservation of the animal breeding tradition and special cultural and ethnic tradition are promoted within the local community and local municipalities have declared the preservation of agro biodiversity as priority Serbia. It is also the traditional grazing area where herbivorous domestic animals were bred historically and animal production was developed through centuries.

Stara Planina with its huge grassland area is famous for its agro biodiversity being the traditional free-range breeding area for horses, donkeys and ruminants. Nowadays the rural settlements are depopulating, so the number of animals is decreasing and the herds and flocks are small. The extensive animal breeding with animals grazing on any natural grassland and after harvest, on wheat, oats and barley stubble was practiced through history by nomads and settled farmers in the area.

Methods

The research was conducted from May to Sep 2015, on several locations on Stara Planina. Fecal samples were collected from 39 horses (29 females and 10 males), aged 1 to 6 years. At the same time, the samples were obtained from 18 donkeys (12 females and 6 males).

All animals were free ranging on pasture. The animals included in the research were clinically healthy, in good condition and untreated with antiparasitic medications, upon the data obtained from the attending veterinarian.

The fresh fecal samples were either collected from the rectum of each animal examined, or,

if this was not possible, via identification and collection of fresh droppings. Each fecal sample was individually stored, labeled with the name, age, sex of the animal and the collection date and transported to the Laboratory of Parasitology in the Scientific Veterinary Institute of Serbia (Belgrade).

The presence of parasite eggs in feces was revealed by flotation method with supersaturated saline (NaCl) solution, while larvae of lungworms were discovered by Berman method (20). The intensity of infection (number of eggs per gram - EPG) was determined using the McMaster method. At same time, Clayton-Lane method, which is specific for examination of horse faecal samples, was applied (20). The identification of the parasite eggs was performed according to its morphological characteristics (20,21). The level of infection was defined according to Upjohn et al (22) as none, mild (<500 egg per gram (EPG)), moderate (500–1000 EPG) and high (>1000 EPG).

Data Analysis

Software: GraphPad Prism ver. 7.00 for Windows (GraphPad Software, San Diego, CA, USA) was used for statistical analyses of the data obtained. The association between the factors identified as important for parasitic infection and prevalence of helminths in equines was evaluated by χ^2 test. The significance of the differences, of faecal egg count was established by Student's *t*-test at levels 0.05 and 0.01.

Results

The overall prevalence of GIT nematode infection in horses and donkeys was 97.43% and 88.88%, respectively. The identified parasites were *Trichostrongylus axei* in 84.61% (33/39), *Strongylus edentatus* in 46.15% (18/39), *S. vulgaris* in 5.13% (2/39), *Parascaris equorum* in 58.97% (23/39), *Dictyocaulus arnfieldi* in 94.87% (37/39) and *Anoplocephala magna* in 38.46% (15/39) of

the horses. The result showed the significant association of age ($\chi^2=20.73$, $P<0.01$) and gender ($\chi^2=5.65$, $P<0.05$) of horses with the occurrence of *Anoplocephala magna* infection. In addition, gender was significantly associated ($\chi^2=13.33$, $P<0.01$) with the occurrence of *P.*

equorum infection in horses (Table 1). Significant differences in the infection intensity between young and adult, male and female animals was not detected ($P<0.05$) for other GIT nematode.

Table 1: Overall prevalence of major gastrointestinal helminthes in horses vs. risk factors

Helminthes	Age		Gender	
	Young (1-3 yr) (n=12)	Adult (3-6 yr) (n=27)	Male (n=10)	Female (n=29)
	Prevalence n (%)			
<i>Trichostrongylus axei</i>	11 (91.67)	22 (81.48)	7 (70.00)	26 (89.66)
<i>Strongylus edentatus</i>	3 (25.00)	15 (55.56)	4 (40.00)	14 (48.28)
<i>Strongylus vulgaris</i>	-	2 (7.41)	1 (10.00)	1 (3.45)
<i>Dictyocaulus arnfieldi</i>	11 (91.67)	26 (96.30)	10 (100.00)	27 (93.10)
<i>Parascaris equorum</i>	6 (50.00)	17 (62.96)	1 (10.00) ^A	22 (75.86) ^A
<i>Anoplocephala magna</i>	11 (91.67) ^A	4 (14.81) ^A	7 (46.67) ^a	8 (53.33) ^a

Same letters in the same row indicate statistically significant difference (^a $P<0.05$; ^A $P<0.01$) at each factor

The identified helminths in donkeys were *D. arnfieldi* in 55.55% (10/18), *T. axei* in 38.89% (7/18), *P. equorum* in 27.77% (5/18), *S. vulgaris* in 33.33% (6/18), *A. magna* in 22.22% (4/18), and *S. westeri* in 22.22% (4/18) of animals included in the monitoring. Significant differences in the intensity of infection between

young and adult animals were not detected ($P>0.05$) (Table 2). GIT helminth infection in donkeys was significantly associated with the gender ($\chi^2=4.08$, $P=0.04$) of the animals included in the study.

Table 2: Overall prevalence of major gastrointestinal helminthes of donkey vs. risk factors

Helminthes	Age		Gender	
	Young (1-3 yr) (n=8)	Adult (3-6 yr) (n=10)	Male (n=6)	Female (n=12)
	Prevalence n (%)			
<i>Trichostrongylus axei</i>	1 (12.50)	6 (60.00)	2 (33.33)	5 (41.67)
<i>Strongylus vulgaris</i>	-	6 (60.00)	1 (16.67)	5 (41.67)
<i>Strongyloides westeri</i>	4 (50.00)	-	3 (50.00) ^a	1 (8.33) ^a
<i>Dictyocaulus arnfieldi</i>	3 (37.50)	7 (70.00)	3 (50.00)	7 (58.33)
<i>Parascaris equorum</i>	3 (37.50)	2 (20.00)	1 (16.67)	4 (33.33)
<i>Anoplocephala magna</i>	2 (25.00)	2 (20.00)	1 (16.67)	3 (25.00)

Same letters in the same row indicate statistically significant difference (^a $P < 0.05$; ^A $P < 0.01$) of each factor

The mean EPG-s for donkeys and horses were 369.9 and 250.2, respectively (Table 3). Out of 38 infected horses, 13.15% were mildly infected, 50.00% moderately and 36% highly (Fig. 2). The levels of infection for donkeys and horses were summarized in Fig. 2. Mean

EPG recorded in adult animals was significantly higher ($P < 0.05$) compared to young donkeys. However, species and gender were not established as significant risk factors influencing mean helminth EPG in each equine species.

Table 3: Analysis of EPG variation in relation to risk factors

<i>Risk factors</i>	<i>No. of infected animals</i>	<i>Mean of EPG</i>	<i>Standard error</i>	<i>95 % CI</i>	<i>P-value</i>
Species					
Horse	38	250.2	19.66	211.2-289.1	0.009
Donkey	16	369.9	49.42	269.4-470.3	
Gender					
Male	15	366.5	83.7	184.2-548.9	0.943
Female	39	359.4	56.43	242.9-475.9	
Age					
Young	17	220.1 ^a	30.88	158.1-282.1	0.0435
Adult	37	302.1 ^a	23.44	255.6-348.5	

Same letters in the same column indicate statistically significant differences ($P < 0.05$); 95% CI 95% confidence interval of mean EPG

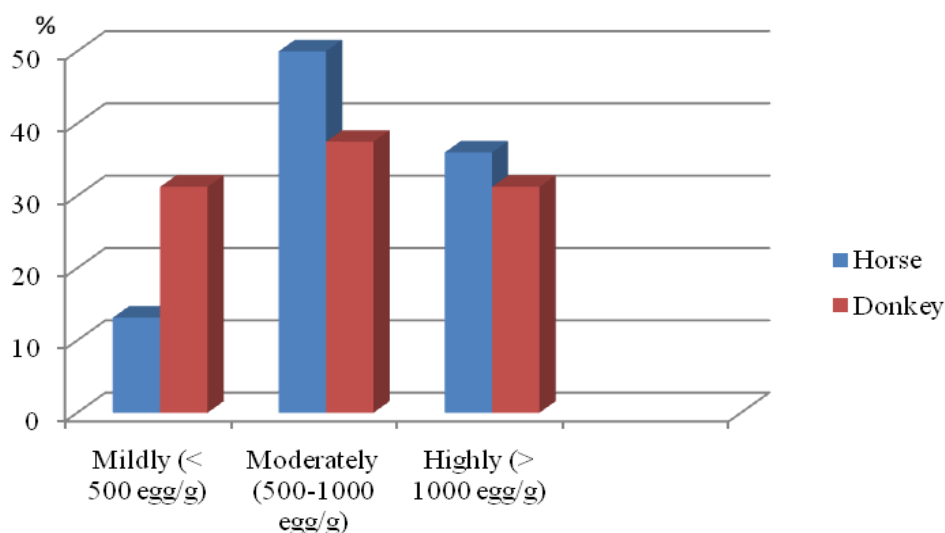


Fig. 2: Level of infection upon the total egg count in horses and donkeys in Nature Reserve Stara Planina

Discussion

D. arnfieldi is a common parasite of horses (23, 24). This was confirmed during our research i.e. establishing high prevalence of the

parasite in horses and donkeys (94.87% and 55.55%, respectively). The disease is common in the pastures where donkeys and horses graze together. Donkeys are a lot more susceptible to these parasites than horses (14,25). However, the simultaneous grazing of horses and donkeys, observed in our monitoring allowed constant parasitic loading of the pasture through elimination by both horses and donkeys allowed constant infection of both equines regardless of the species susceptibility.

T. axei is a common parasite of ruminants in Serbia and the world (26). It was found in horses in Europe, in the Middle East and in the United States (27-29).

P. equorum, is a widespread disease in both young and older horses. The disease is prevalent in the world and has a great importance in the pathology of the horse (30-32). The data obtained in our research also established infection with *P. equorum* as a frequent disease in both horses and donkeys on Stara Planina, which is in accord with the data from literature concerning the stabled horses breed in Serbia (17, 18).

Of the species of the genus *Strongylus* dominate *S. vulgaris*, followed by *S. edentatus* and *S. equinus* (33, 34). Exogenous development of these parasites is similar. The eggs via faeces reach the external environment where they embryonate under favorable conditions. Horses are infected by ingestion of infective larvae through contaminated food and water.

S. edentatus is a common parasite of horses widespread in the world. The *S. edentatus* is pathogenic helminth species for equines (34). Their presence is associated with very severe disease and intestinal colic and the outcome of the infection in foals can be lethal (9, 32, 33, 35).

S. vulgaris is a helminth whose larvae penetrate the wall of the cecum and nest after infection. After about a week, they break through the arterioles, venules and lymphatic vessels and migrate upstream to larger arteries (9, 34). *S. westeri* was found only in donkeys and are common parasites in this equine.

A. magna is often present in horses in Serbia, in areas where there are conditions for the persistence of an intermediate host of parasites-nonparasitic mites from the family *Oribatidae*. This disease is also widespread around the world (35-38).

The mean EPG was significantly higher in donkeys than in horses stationed on Stara Planina. This finding could be the result of poor management, overwork stress and immune-compromised responses in donkeys and in other equines with poor body condition score (39). Similarly, the differences among species in concern to susceptibility to nematodes could be controlled by better management practices and inherent immune capability (40). Therefore, poor management and inadequate plan of nutrition could decompensate the immune status of equines, which in return, could create a favorable condition to heavy parasitic infections (41). Even though there was no significant variation, higher mean EPG was recorded in older, compared to adult and young equines. The observed higher mean EPG in older equines in our report may be attributed to the compromised immune response related to ageing animals.

Conclusion

The helminth infection established in horses was high, with an overall prevalence of 97.43%. Higher overall prevalence of helminth infection was recorded in all horses followed by *T. axei*, *A. magna* and *D. arnfieldi*, while *P. equorum* and *D. arnfieldi* was more prevalent problems in young horses. There were equines suffering from high levels of infection with major helminths. Therefore, this information might help in designing strategies to control endoparasites infection in nature reserve Stara Planina, in Serbia. More emphasis should be given to planning of management that would include deworming strategy

Competing interests

The authors declare that they have no competing interest.

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