DOI 10.24425/pjvs.2023.145046

Original article

Helminth infection in horses – a cross-sectional study from stables in Lower Silesia (Poland)

W. Hildebrand[1], P. Zielińska[2], J. Hildebrand[3], G. Zaleśny[4]

[1] Hirszfeld Institute of Immunology and Experimental Therapy, Polish Academy of Sciences, Rudolfa Weigla 12, 53-114 Wroclaw, Poland
[2] Department of Surgery, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Plac Grunwaldzki 51, 50-366 Wroclaw, Poland
[3] Department of Parasitology, Faculty of Biological Sciences, University of Wrocław, Przybyszewskiego 63, Wrocław, 51-148, Poland
[4] Department of Invertebrate Systematics and Ecology, Institute of Environmental Biology, Wroclaw University of Environmental and Life Sciences, Kożuchowska 5B, 51-631 Wroclaw, Poland

Abstract

Parasitosis in horses may be uncontrolled and expose breeders and owners to serious financial losses or, possibly, to the loss of animals. Therefore, the prevention and monitoring of the development of parasitic diseases should play an important role in the breeding process. The aim of this study was to confirm the influence of factors such as age, breed, herd size, deworming program, and type of anthelmintics, on the prevalence and intensity of parasites (helminths) in domestic horses in Lower Silesia. The study was carried out between August and November of 2020. The samples of horse feces were collected from 50 different stables in the area of Lower Silesia, Poland. A total of 286 individuals from various breeds were examined. Detailed analysis revealed that the mean age of infected horses was significantly lower than in uninfected horses. The mean time since the last deworming procedure was almost twice as low in uninfected horses than in infected ones. Additionally, the deworming agent affects the prevalence of infection. The analysis was also performed for the same factors in reference to quantitative data. The mean EPG was four-fold higher in juvenile horses than in adults and three-fold higher when the horses were dewormed with the use of fenbendazole instead of ivermectin or ivermectin with praziquantel combined.

Keywords: deworming scheme, helminths, horses, prevention

Correspondence to: G. Zaleśny, e-mail: grzegorz.zalesny@upwr.edu.pl

Introduction

In recent years, horse breeding has been treated as an exclusive activity that can only be carried out by professionals. Despite the clear decline in the number of purebred horses in Poland (the number of horses in Poland decreased from 329.5 thousand in 2002 to 185.5 thousand by 2016, i.e., by 43.7%), the interest in recreational horse riding seems to be growing (data taken from the Central Statistical Office). A natural consequence of such an approach is the increase in the number of small studs offering opportunities to spend "time in the saddle". However, regardless of the herd size, animal health and welfare are important to the breeder. Invariably, over the years, parasitic invasions caused by helminths constitute one of the major concerns for breeders. Among them, infection with nematodes of the Strongylidae family are considered the predominant and most pathogenic group of parasites (Gawor 1995, Love et al. 1999, Lyons et al. 1999, Gawor et al. 2006, Lichtenfels et al. 2008, Kaplan and Nielsen 2010, Kuzmina et al. 2016). In addition, this group of parasites shows a high tendency to drug resistance (Kaplan 2002, Kuzmina and Kharchenko 2008, Kaplan and Vidyashankar 2012), which is a serious problem for many horse breeders and owners. Effective strategies of horse helminth control must consider many factors (Osterman et al. 1999, Kornaś et al. 2004, Gawor et al. 2006, Francisco et al. 2009, Kornaś et al. 2010, Saeed et al. 2010, Kuzmina et al. 2016, Slivinska et al. 2016, Studzińska et al. 2017, Sallé et al. 2018). Among them, primarily is a properly selected deworming program or a properly selected active substance. In addition, the herd size, breed, and age of horses will also be potential factors influencing the dynamics of helminth infection.

The aim of the study was to confirm the influence of factors, such as age, breed, herd size, time since last deworming, and type of anthelmintics, on the prevalence and fecal egg count (FEC) of parasites (helminths) in domestic horses in Lower Silesia. We assumed two hypotheses: 1) in the group of horses infected with helminths the time between deworming and the active substance contained in the anthelmintic used are strictly correlated, and 2) the primitive breeds of horses are less predisposed to parasite infection.

Materials and Methods

Study design

The study was carried out between August and November of 2020. The samples of horse feces were collected from 50 different stables in the area of Lower Silesia, Poland. Horses between the ages of 1 to 25 years were examined, including mainly mares and geldings, and several stallions with a total of 286 individuals from various breeds. The study was performed in all types of stables (recreation, breeding, sports, etc.) having from one to more than 50 horses. Fecal samples were collected from 50% - 100% of the individuals living in the stables with 10 or less horses, 50% from stables with 10-20 individuals, and 25% - 30% from stables hosting more than 20 individuals to present a representative sample from each location. Feces were taken from specimens who had not changed stables for at least 6 months prior to the study. Collected samples were fresh (not older than 24 hours) and gathered from the stalls or paddocks immediately after each horse's defecation. Each sample was collected and stored separately, refrigerated, and examined within 48 hours. During the sampling, an interview with the owner or person responsible was carried out. Detailed information including age, gender, breed, health issues, and deworming procedures with the recent anthelmintic treatment were noted.

Each sample was examined separately, both macroscopically and microscopically. All of the fecal samples were tested by the same person to avoid any disparities within the results. The macroscopic examination consisted of a visual evaluation of the feces on a sieve and its goal was to find potential adult or larval forms of parasites and to examine the structure of the feces. All of the differences and abnormalities were noted. The microscopic examination consisted of two parts: quality evaluation and quantitative assessment (egg count). The fecal examination followed a modified McMaster centrifugation-enhanced method using a sugar-salt flotation solution with a specific gravity of 1.3 g/ml. In the first step, 4 g of feces were suspended in 56 ml of tap water, mixed, and sieved through a mesh with an aperture of 0.8 mm. The fluid was centrifuged for 5 min at 500 g, the supernatant was discarded, and the pellet mixed in the flotation solution. Two McMaster chambers were filled (0.5 ml/chamber) and the total number of eggs was counted (dividing into different groups or species) and the EPG (eggs per gram) was calculated (Saeed et al. 2010, Sallé et al. 2018, Selzer et al. 2021).

Database

Age: all of the individuals were assigned to one of the three groups: "young" which included horses from 1 to 4 years old (n=33); "adult" containing individuals between 5 and 11 (n=119) years old; and "old" including those over 11 years old (n= 134). Sex/gender: the horses were divided into two groups: "female" and "male". The group of stallions was combined with the geldings creating the "male" group as it was not numerous enough to separate it.

Breed*:* more than 25 breeds of horses participated. To make it statistically significant, the individuals were divided into three separate groups: "pony" (according to the Polish Equestrian Federation this includes: Hucul horse, Haflinger, Polish Konik), "warm-blooded", and "other". The last group, containing mainly cold-blooded horses, was excluded from the statistics as it was found to be non-significant due to a lack of representatives.

Herd size/breeding/rearing system: the animals examined included recreation, breeding, and sport horses. To standardize the results, they were divided into three groups based on the number of animals present in the herd: the "small herd", "medium herd", or "big herd" groups. The first group contained from one to 10 specimens, the second, from 11 up to 20, and the last group included herds with more than 20 individuals.

Anthelmintic treatment*:* all of the individuals taken for testing were dewormed before the study with a veterinary product. The owners or caretakers were asked about the frequency of deworming, the substance used, and the date of the last treatment. The horses were given the treatment one, two, three, or four times per year. Various anti-parasite products were used by the caretakers, although when divided by the active substance in the product it was possible to divide them into three different groups, which were: "ivermectin", "fenbendazole", and "ivermectin with praziquantel". The third parameter examined was the time between the last anti-parasite treatment and the time when the feces were collected. The time periods varied from 1 to 13 months.

Statistical analysis of qualitative (prevalence) and quantitative (EPG) data

In order to check if the presence of parasites in the sample is affected by quantitative and qualitative predictors, logistic regression was performed. The analysis was done using Statistica 13 (Tibco) software with application of the interactive model constructor. At the first step, all possible effects (age of horses [age], time since the last deworming procedure [deworm_t], size of the herds [herd_size], horse sex [sex], horse breed category [breed_cat], deworming substance [deworm_subst], and presence or absence of protists in the fecal samples [protista]) were added to the model. Next, based on the p value of Somers' D coefficient, significant variables were selected for the model construction. The EPG values are characterized by overdispersion and excessive zeros, thus the data were analyzed using zero-inflated negative binomial regression (zeroinfl function in the "pscl" package in R) (Zeileis et al. 2008). We used the same set of predictors as for the logistic regression [age, deworm_t, herd_size, sex, breed_cat, deworm_subst, and protista], while the dependent variable was the total number of EPG values (EPG_tot). Additionally, since the deworm_t has a very strong effect on the presence/absence of the parasites, we performed a second analysis where all horses, dewormed up to three months before fecal analysis, were removed from the dataset.

Ethics approval and consent to participate

All methods were carried out in accordance with the legal regulations of Poland. Our research only includes interactions involving the following: interview with the horse owners, collection of fecal samples (already defecated) and laboratory examinations of fecal samples. All these procedures were non-invasive, not harmful, and not stressful to the animals and therefore there was no necessity to apply for ethical approval (Polish Act of 15 January 2015). The participation of the horse owners in that study was voluntary. Consent to participate is not applicable pursuant to the Polish Act of 15 January 2015 "On the protection of animals used for scientific or educational purposes."

Results

In the present study 136 horses out of 286 (47.5%) were infected with helminths. The mean EPG was 323.96 and differed between study groups; for details see Table 1. The strongylids were the dominant group of parasites. Based on the McMaster method, the infection with other parasites (nematodes *Oxyuris equi* or *Parascaris* sp.) was found in three samples, and single egg of *Anoplocephala* spp. (Cestoda) was found in two samples. Since the total EPG for non-strongylid helminths was very low (1 600 vs 90 947 for Strongylidae) the statistical analyses were limited to strongylid infection only. The logistic regression (D Somers: 0.5806, KS statistics: 0.4835) revealed that infection with helminths is linked with the following variables: age of horses, time since the last deworming procedure, and deworming agent (for details see Table 2). Detailed analysis revealed that the mean age (in years) of infected horses was significantly lower (10.5 ± 0.5 S.E.M.) than in uninfected horses (12.4 ± 0.5 S.E.M.). The mean time (in months) since the last deworming procedure was almost twice as low in uninfected (2.7 ± 0.2 S.E.M) horses than the infected ones (4.3 ± 0.2 S.E.M). Additionally, the deworming agent affects the prevalence

Table 1. Basic parasitological parameters (prevalence and mean EPG) of analysed horses.

Age group	Sex	N	Prevalence [%]	Mean EPG overall	Mean EPG Strongylidae	Mean EPG non-Strongylidae
Juvenile	male	15	73.3%	927.40	915.40	12
	female	18	72.2%	801.12	758.89	42.23
Adult	male	55	40.0%	206.54	206.54	0
	felmale	64	48.4%	345.73	343.54	2.19
Old	male	76	38.2%	169.50	163.71	5.79
	female	58	51.7%	309.57	308.19	1.38
Overall		286	47.5%	318.37	323.69	5.59

Table 2. Factors affecting prevalence of helminths in horses.

Effect	Level	Estimate	Standard error	Wald Statistics	P value
Intercept		0.447	0.491	0.826	0.3631
age		-0.087	0.025	12.220	0.0004
deworm_t		0.469	0.075	39.582	0.0001
deworm_subst	iwerm_praz	-1.486	0.383	15.063	0.0001
deworm_subst	iwerm	-1.244	0.388	10.269	0.0013

Table 3. Factors affecting mean EPG of helminths in horses.

Effect	Estimate	Std. error	*z* value	*P* value
Intercept	-1.472	0.784	-1.876	0.0506
age	0.097	0.025	3.898	>0.001
breed	-0.351	0.403	-0.870	0.384
deworm_t	-0.427	0.072	-5.940	>0.001
deworm_subst (iverm)	2.433	0.692	3.514	>0.001
deworm_subst (iverm_praz)	2.505	0.678	3.694	>0.001

of infection. The analysis was also performed for the same factors in reference to quantitative data (Table 3), i.e., average value of EPG. The mean EPG is four-fold higher in juvenile horses than in adults (Fig. 1a) and three-fold higher when horses were dewormed with the use of fenbendazole instead of ivermectin or the combination of ivermectin with praziquantel (Fig. 1c). It is also worth emphasizing that the EPG values in horses of primitive breeds were lower by about 75% than in warm-blooded horses (Fig. 1b); however, according to multivariate statistical analysis, this result was not statistically significant. Moreover, according to the results, a longer time since the last administration of the anthelmintic results in a more intensive FEC (Fig. 2). We did not find a statistically significant relationship between the presence of helminths and the occurrence of intestinal microparasites; however, there was a ca. 50% higher OPG (Fig. 1d) value in those individuals infected with Protista.

Discussion

This study focused on the influence of different factors, such as horse age, breed, herd size, breeding system, and deworming programs on helminth infections in stables located in Lower Silesia (Southwest Poland). In recent years, an increase in the number of stables, of various sizes and with different types of maintenance, has been observed in Poland. Simultaneously, due to the problem of deworming effectiveness being reported by owners and breeders, there is a growing need to develop an appropriate parasite control program, perhaps individual for a given stable because the interaction of various variables in different horse breeding conditions cannot be ruled out. Since the structure of intestinal parasites infecting equids, mainly strongyle nematodes, has been well characterized recently (Lichtenfels et al. 2008, Kornaś et al. 2010, Kuzmina et al. 2011, Kuzmina et al. 2016), we focused on the overall hel-

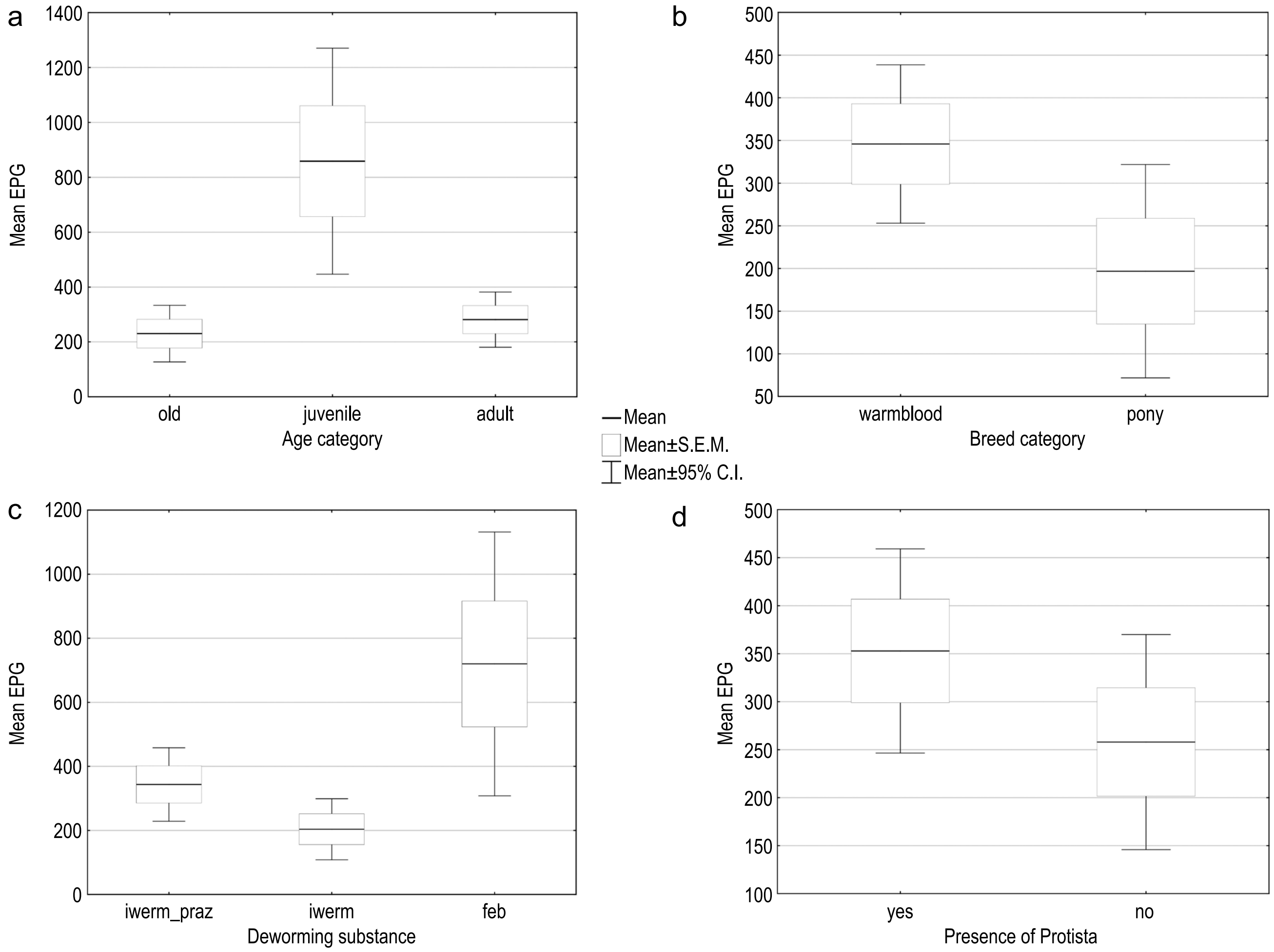

Fig. 1. The relationship between biotic and abiotic factors and mean EPG of helminths; (a) the effect of horse age; (b) the effect of horse breed; (c) the effect of anthelminthic active substance and (d) the relationship between mean EPG and the presence of the representatives of Protista.

minth infection as well as strongyle infection, and the intensity (EPG) of infection was also taken into consideration.

Our research shows that the major factor which significantly affects prevalence and FEC of helminths in horses is the active substance used in the deworming procedure. The analysis of three deworming programs, using ivermectin, fenbendazole, and ivermectin with praziquantel, indicated ivermectin as the most effective anthelmintic, although particular studies show or discuss a resistance of some nematodes to ivermectin (Martin et al. 2021, Selzer and Epe 2021). Thus, the current conclusion is in line with recent research conducted on nine stud farms from the same region, i.e., Southwest Poland, which indicated that strongyle resistance to ivermectin in Poland is not a serious problem (Zak et al. 2017). Additionally, according to the results, a longer time since the last administration of the anthelmintic results in a more intensive FEC. The application of the above statistical methods to qualitative and quantitative data made it possible to observe that younger horses are more susceptible to infections, and this relationship also applies to warm-blooded breeds. Although the latter relationship is not statistically significant, which is likely due to the number of horses studied, but nevertheless, the trend is visible in the analyses. These insights are partially consistent with the results of Kuzmina et al. (2016). This study, conducted on 197 horses of various ages and breeds and derived from 15 farms throughout different regions of Ukraine, revealed that the frequency of anthelmintic treatments and management on the farm have a strong influence on infections with strongylids, more significant than the age and breed of the horses. Interestingly, the analysis provided by Kuzmina et al. (2016) of the relationship between horse breeds and EPG values revealed that brood horses had much higher EPG values as compared to other breeds; for example, the Hucul horse, a primitive breed, shed the lowest number of strongylid eggs. The herd size and breeding system were not significant factors influencing helminth infection according to this current analysis. Similar conclusions were drawn in studies conducted 20 years ago in several regions of Poland, where the decisive factor in the level of infection with

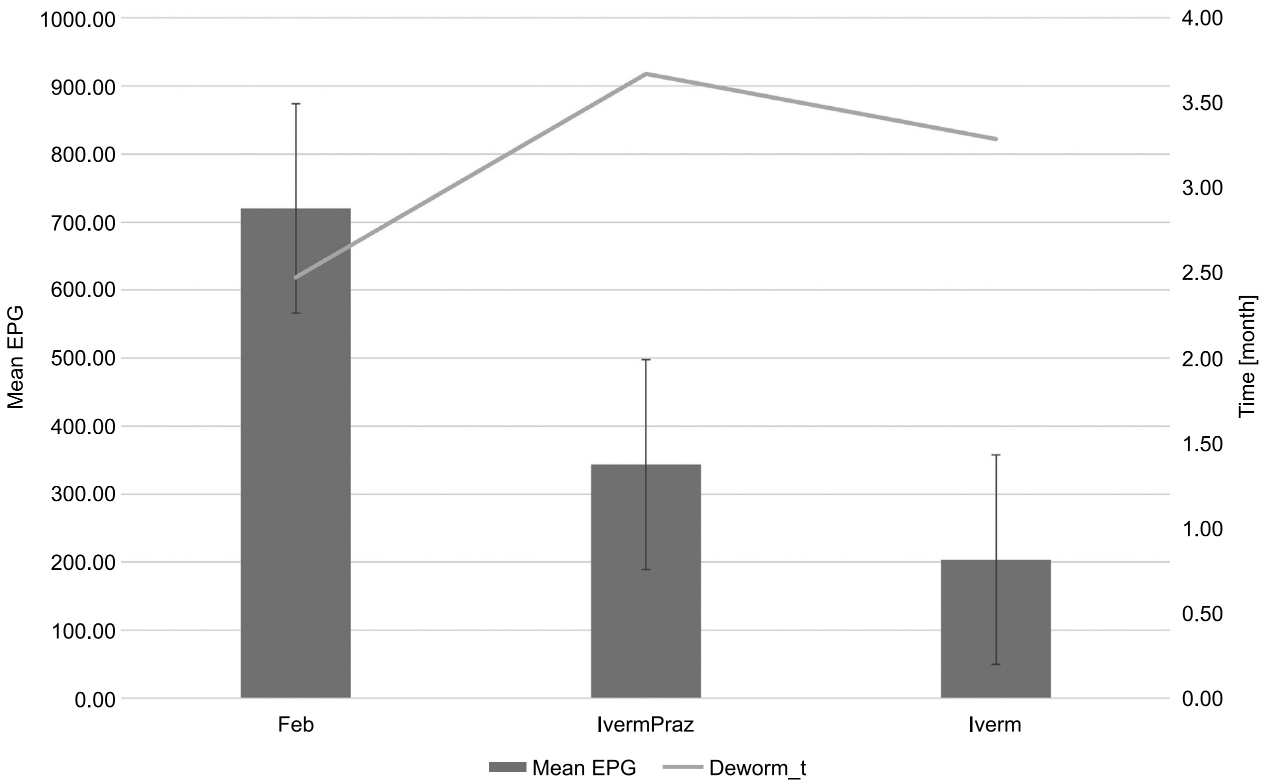

Fig. 2. The relationship between active substance and time of the deworming in the group of horses infected with strongylid nematodes. The use of ivermectin or ivermectin and praziquantel has a positive effect on the extension of time in individual deworming cycles.

intestinal nematodes turned out to be the deworming program (Kornaś et al. 2004). During the microscopic examination, each fecal sample was also checked for the presence of Protista, which was mostly represented by the Eimeriida order. Samples containing these microparasites were taken for further and more specific examination (with the use of a molecular approach). However, individuals with coccidia present in their feces were marked as a "Protista positive" group, while the others constituted the "Protista negative" group. In this study, we did not find a statistically significant relationship between the presence of helminths and the occurrence of intestinal microparasites; however, observed values (i.e. higher OPG in individuals infected with Protista) can be a contribution and a starting point for research on the role of Protista as a possible factor increasing helminthiasis.

Further long-term studies, carried out on appropriate groups of horses, taking into account various factors as well as biodiversity of helminths (e.g., Petney and Andrews 1998), an increase of species richness and biodiversity of parasites is known to reduce their pathogenicity and overall infection) are needed to understand the complexity of the interactions of intrinsic and extrinsic factors and to develop effective deworming programs, preceded by coproscopic analysis, that will take into account the possibility of parasite resistance, related to, among others, too high a concentration of anthelmintics in the environment.

References

Francisco I, Arias M, Cortinas FJ, Francisco R, Mochales E, Dacal V, Suárez JL, Uriarte J, Morrondo P, Sánchez-Andrade R, Díez-Banos P, Paz-Silva A (**2009**) Intrinsic factors influencing the infection by helminth parasites in horses under an oceanic climate area (NW Spain). J Parasitol Res 2009: 616173.

Gawor J (**1995**) The prevalence and abundance of internal parasites in working horses autopsied in Poland. Vet Parasitol 58: 99-108.

Gawor J, Kornaś S, Kharchenko V, Nowosad B, Skalska M (**2006**) Intestinal parasites and health problems in horses in different breeding systems (in Polish). Med Weter 62: 331-334.

Kaplan RM (**2002**) Anthelmintic resistance in nematodes of horses. Vet Res 33: 491-507.

Kaplan RM, Nielsen MK (**2010**) An evidence-based approach to equine parasite control: it ain't the 60s anymore. Equine Vet Educ 22: 306-316.

Kaplan RM, Vidyashankar AN (**2012**) An inconvenient truth: global worming and anthelmintic resistance. Vet Parasitol 186: 70-78.

Kornaś S, Nowosad B, Skalska M (**2004**) Intestinal parasite infections in horses from different types of environments (in Polish). Med Weter 60: 853-856.

Kornaś S, Cabaret J, Skalska M, Nowosad B (**2010**). Horse infection with intestinal helminths in relation to age, sex, access to grass and farm system. Vet Parasitol 174: 285-291.

Kuzmina T, Kornaś S, Basiaga M, Kharchenko V, Vyniarska A (**2011**) Biodiversity of strongylids (Nematoda: Strongylidae) communities in domestic horses from Poland and Ukraine. Helminthologia 48: 77-84.

Kuzmina TA. Dzeverin I, Kharchenko VA (**2016**) Strongylids in domestic horses: Influence of horse age, breed and deworming programs on the strongyle parasite community. Vet Parasitol 227: 56-63.

Kuzmina TA, Kharchenko VO (**2008**) Anthelmintic resistance in cyathostomins of brood horses in Ukraine and influence of anthelmintic treatments on strongylid community structure. Vet Parasitol 154: 277-288.

Lichtenfels JR, Kharchenko VA, Dvojnos GM. (**2008**) Illustrated identification keys to strongylid parasites (Strongylidae: Nematoda) of horses, zebras and asses (Equidae). Vet Parasitol 156: 4-161.

Love S, Murphy D, Mellor D (**1999**) Pathogenicity of cyathostome infection. Vet Parasitol 85: 113-121.

Lyons ET, Tolliver SC, Drudge JH (**1999**) Historical perspective of cyathostomes: prevalence, treatment and control programs. Vet Parasitol 85: 97-112.

Martin F, Svansson V, Eydal M, Oddsdóttir C, Ernback M, Persson I, Tydén E (**2021**) First report of resistance to ivermectin in *Parascaris univalens* in Iceland. J Parasitol 107: 16-22.

Osterman Lind E, Hoglund J, Ljungstrom BL, Nilson O, Uggla A (**1999**) A field survey on the distribution of strongyle infections of horses in Sweden and factors affecting faecal egg counts. Equine Vet J 31: 68-72.

Petney TN, Andrews RM (**1998**) Multiparasite communities in animals and humans: frequency: structure and pathogenic significance. Int J Parasitol 28: 377-393.

Roepstorff A, Nansen P (**1998**) Epidemiology, diagnosis and control of helminth parasites of swine. FAO Animal health manual. Food and Agriculture Organization of the United Nations, Rome, p 161.

Saeed K, Qadir Z, Ashraf K, Ahmad N (**2010**) Role of intrinsic and extrinsic epidemiological factors on strongylosis in horses. J Anim Plant Sci 20: 277-280.

Sallé G, Kornaś S, Basiaga M (**2018**) Equine strongyle communities are constrained by horse sex and species dipersal-fecundity trade-off. Parasit Vectors 11: 279.

Selzer PM, Epe C (**2021**) Antiparasitics in animal health: Quo Vadis? Trends Parasitol 37(1): 77-89.

Slivinska K, Kharchenko V, Wroblewski Z, Gawor J, Kuzmina T (**2016**) Parasitological survey of Polish primitive horses (*Equus caballus gmelini* Ant.): influence of age, sex and management strategies on the parasite community. Helminthologia 53: 233-242.

Studzińska MB, Demkowska-Kutrzepa M, Bogucki J, Roczeń--Karczmarz M, Tomczuk K (**2017**) Influence of horse management systems in south-western Poland on the prevalence and intensity of gastrointestinal parasites (in Polish). Med Weter 73: 721-725.

Vadlejch J, Petrtýl M, Zaichenko I, Čadková Z, Jankovská I, Langrová I, Moravec M (**2011**) Which McMaster egg counting technique is the most reliable. Parasitol Res 109: 1387-1394.

Zak A, Siwinska N, Slowikowska M, Borowicz H, Kubiak K, Hildebrand J, Popiolek M, Niedzwiedz A (**2017**) Searching for ivermectin resistance in a Strongylidae population of horses stabled in Poland. BMC Vet Res 13: 210.

Zeileis A, Kleiber C, Jackman S (**2008**) Regression Models for Count Data in R. J Stat Soft 27: 1-25.