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Original article

Assessment of the antiparasitic effectiveness of pyrantel pamoate in treatment of Strongylidae invasions in young horses – preliminary studies

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Abstract

The resistance of strongyles to pyrantel pamoate has been reported in publications worldwide. There is no data on its efficacy in horses in Poland. Therefore, the aim of this study was to evaluate the effectiveness of pyrantel pamoate to control strongyle invasions in young horses. The study involved horses of both sexes, aged 1 to 2 years, and was conducted in two seasons (spring and the end of summer). Feces were collected 24 hours before and 14 days after deworming, and they were then examined using McMaster's and combined sedimentation-flotation methods. Results revealed that in spring, the average eggs per gram (EPG) before deworming was 1354.54, but after the treatment EPG was 485, and fecal egg count reduction test (FECRT) = 67.45%. Considering the age groups of the horses, the average EPG for one-year-olds was 1565.38, and 1050 for two-year-olds, respectively. The FECRT for strongyle invasions was 69.78% in one-year-old and 62.43% in two-year-old horses. At the end of summer, the average EPG was 1954.54 before deworming, and after the treatment, strongyle eggs were found in only two horses, and FECRT was 99.53%. Considering the age groups of horses before deworming, the average EPG in one-year-olds was 2103.85, but was 1738.89 in two-year-old horses. The FECRT values were 99.27 and 100%, respectively. The results revealed a quite limited efficacy of pyrantel pamoate in the deworming of Strongylidae invasions in young (one- and two-year-old horses) during the spring season. According to WAAVP recommendations, this was assumed to be the result of pyrantel pamoate resistance of Strongylidae nematodes in horses. In contrast, treatment using the same compound in the same age horses, but conducted at the end of summer, was found satisfactory. This was the first study concerning pyrantel pamoate efficacy in horses in Poland.

Keywords: horses, Poland, pyrantel pamoate, Strongylidae, treatment

Introduction

In recent years, a noticeable increase in the number of horses reared, particularly those used for hippotherapy and recreation in Poland, has been observed. This requires continuing parasite control to maintain the horses' health and welfare. However, anthelmintic therapy in horses is becoming increasingly difficult due to the increasing drug resistance of gastrointestinal worms to the most commonly used drugs. These include benzimidazoles (fenbendazole), tetrahydropyrimidine derivatives (pyrantel) and macrocyclic lactones (ivermectin and moxidectin). The most commonly used active substance in solipeds currently is ivermectin, which is available in many products. Due to its frequent use, studies have been conducted to evaluate its effectiveness in horses in different systems of maintenance. However, it was found that ivermectin has limited efficacy against *Parascaris* spp. infection (Studzińska et al. 2020). In addition, other researchers have documented the resistance of strongyles to benzimidazoles and macrocyclic lactones (Kaplan et al. 2004, Kuzmina and Kharchenko 2008, Cerňanská et al. 2009, Traversa et al. 2009, Traversa et al. 2012, Tomczuk et al. 2014, Nielsen et al. 2020). Other studies have also shown resistance to tetrahydropyrimidine, mainly in North America and Canada (Kaplan et al. 2004). In Europe, resistance to pyrantel has been described, although far less frequently than to benzimidazoles (Ihler 1995, Traversa et al. 2009, Traversa et al. 2012).

Pyrantel is not often used in horses, partly because of its limited efficacy on nematode developmental forms located outside the lumen of the digestive tract. Pyrantel as a cholinergic agonist causes an irreversible depolarization block in neuromuscular synapses, which leads to paralysis of the parasite. Resistance to cholinergic agonists may be produced by four general mechanisms: (1) changes in drug translocation (e.g. increased metabolism or excretion of the drug); (2) changes in receptor numbers (e.g. reduced number of receptors); (3) receptor modification (e.g. change in receptor binding sites due to amino acid substitution); or (4) post-receptor modification (e.g. change in the downstream pathway to contraction). All of these mechanisms could play a role in resistance to pyrantel and therefore it is anticipated that resistance to these drugs is polygenic (Sangster and Dobson 2002).

The antiparasitic therapy in horses is often carried out without prior diagnosis, and the actions often do not take into account the specificity of individual invasions (e.g. life cycles of parasites), as well as environmental differences (e.g. location of the terrain and the maintenance of pastures, structure and hygienic conditions in stables, etc.). All these elements may influence the level of exposure and the risk of horses becoming infected with gastrointestinal parasites.

Previous studies (Gundłach et al. 2004, Gawor et al. 2006, Kornaś et al. 2010), as well as our own studies (Studzińska et al. 2008, 2017, 2020), presented the invasive situation in horses in the south-eastern region of Poland. The research took into account data on the origin of the animals and how they were kept. It was found that the highest percentage of infected horses occurred in the pasture system, with the most common infections caused by nematodes from the Strongylidae family (21.3-100%). In those studies, differences were found in the seasonality of infections and the efficacy of treatment using different antiparasitic drugs. Similarly, in other countries, authors point out that various difficulties related to the effectiveness of horse deworming are increasingly occurring (e.g.: Ihler 1995, Craven et al. 1998, Kaplan et al. 2004, Osterman-Lind et al. 2007, Traversa et al. 2007, Kuzmina and Kharchenko 2008, Molento et al. 2008, Cerňanská et al. 2009, Traversa et al. 2009, Nareaho et al. 2011, Traversa et al. 2012, Lassen and Peltola 2015, Nielsen et al. 2020, Dauparaitė et al. 2021).

The observations made were the basis for undertaking this study in order to determine the effectiveness and validity of the use of pyrantel pamoate to combat the invasion of nematodes from the Strongylidae family in young horses by comparing the effectiveness of this drug in the age groups of one-year-old and two-year-old horses.

Materials and Methods

The research was carried out in 2022 in south-eastern Poland, in a stable with over 100 horses used for recreational and sports purposes. The study included animals of both sexes aged 1 to 2 years. Both mares and stallions were kept in a pasture system and used separate pastures, but similar in terms of vegetation species composition and area. During previous antiparasitic treatments in young horses in this stable, unsatisfactory deworming results were recorded and at the same time a decrease in the animals' condition was observed. Horses were dewormed using ivermectin (in the form of oral paste) every four months, starting from the age of 3 months. Pyrantel pamoate had not been administered to animals at this stud farm in the previous 3 years.

In stage I, before spring deworming, all young horses (29 individuals) were subjected to coproscopic examination in order to select a group for further clinical examinations. Coproscopic tests were performed using macroscopic and McMaster methods (Zając et al. 2021). In order to identify other possible invasions of gastrointestinal parasites, the sedimentation – flotation method was additionally used (Tomczuk et al. 2014).

Ultimately, 22 horses with more than 200 strongyle eggs in 1 gram of feces (EPG > 200) were qualified for further tests. The study group included: 13 one-year-old and 9 two-year-old horses, including 14 mares and 8 stallions.

In stages II and III of the study, horses selected in stage I were subjected to routine deworming following the local schedule: in spring, in April (stage II), and at the end of summer, at the turn of August/September (stage III). Deworming was carried out by a veterinarian, a specialist in equine diseases. Before administering the drug, the horses' body weight was estimated using a standard method using a tape measure. Pyrantel pamoate was administered to the tested animals in the form of a paste, administered orally in a preparation containing 35g of active substance per 100g, at the manufacturer's recommended dose of 19 mg of active substance/kg body weight.

Feces for coproscopic examination were obtained from horses per rectum or immediately after defecation from the environment, 24 hours before and 14 days after drug administration. Fecal samples were transported in refrigerated conditions (+4°C) to the Department of Parasitology and Invasive Diseases of the University of Life Sciences in Lublin. In the laboratory, they were tested macro- and microscopically as described above.

The evaluation of the clinical efficacy of pyrantel pamoate was performed according to the guidelines developed by the World Association for the Advancement of Veterinary Parasitology (Kaplan et al. 2023). The effectiveness of pyrantel pamoate was determined by analyzing the FECRT (Fecal Egg Count Reduction Test), using a web application (http://www.fecrt.com), which uses a hybrid Frequentist/Bayesian analysis method (Denwood et al. 2019, 2023).

The results of the study were statistically analyzed using the Dell Inc. Statistical Package (2016) Dell Statistica (data analysis software system), version 13. For the obtained results, the mean – x, standard deviation – SD and the confidence interval of the mean – CI ± 95% were calculated. Statistical differences between the extensiveness values of individual invasions were confirmed using Student's t-test for independent samples, with the assumed significance level of $p \leq 0.05$. Data distribution was tested using the Kolmogorov-Smirnov and Lilliefors test of normality.

No ethical committee permission was required as the samples were collected during routine examinations performed by veterinarian or from the environment.

Results

Stage I – preliminary examination

In all 29 tested horses, nematode eggs of the Strongylidae family were found, and the prevalence of the invasion was 100%. The average egg per gram of feces (EPG) level was 1291.38 and varied among individual animals. In 7 animals, the EPG level was < 200, and in the remaining 22 the EPG level was ≥ 250. Additionally, in 3 horses, roundworm eggs were found of the *Parascaris* spp. with levels of EPG from 150 to 350.

Stage II – spring deworming (April)

Before deworming, in 22 selected horses, the average EPG was 1354.54. In most individuals (18 horses), the EPG was > 500. After deworming, strongyle eggs were diagnosed in 20 horses (90.91%), with an average EPG of 485. In 5 of 22 horses the EPG was > 500 (Table 1).

The FECRT value was calculated and amounted to 67.45% (Table 1, Fig. 1). Using the hybrid Frequentist/Bayesian analysis method (http://www.fecrt.com), the automatically selected statistical method for the data set was the Delta method (90% CI = 42.8% - 85.9%). Based on the expected efficacy of 98% and the lower efficacy threshold of 88%, the final effect of pyrantel pamoate against strongyles was assessed as resistance.

In the yearlings, before deworming, the average EPG was 1565.38. In most individuals, EPG was in the following ranges: 550-1000 in 5 horses (38.46%); and 1050-2500 in 6 horses (46.15%). After deworming, the average level EPG was 512.50, including 7 horses with a low level EPG: 50-200.

However, in the group of two-year-old horses before deworming, the average EPG level was 1050. Higher EPG levels were found in 3 horses, in the range: 1050-1500 EPG (33.33%). After deworming, the average EPG was 443.75, with 5 of 9 horses having a low EPG < 200.

The calculated Fecal Egg Count Reduction Test (FECRT) value for strongyle invasion in one-year-old and two-year-old horses was 69.78% and 62.43%, respectively (Table 2). In order to compare the effectiveness of pyrantel pamoate in both age groups, the Delta method was automatically selected. Higher EPG and FECRT values were obtained in one-year-old horses, but in both age groups the effectiveness of pyrantel pamoate against strongyles was assessed as resistance. In the case of yearling horses, the following values were obtained: 90% CI = 40% - 90.3%; and in two-year-old horses: 90% CI = 9.2% - 93.8%.

Additionally, in 3 out of 13 one-year-old horses (23.08%), in a control fecal test after deworming, *Parascaris* spp. with EPG: 150 – 350 were detected.

able 1. Levels of strongyle egg per gram (EPG) and fecal egg count reduction test (FECRT) in 22 selected horses.

Dates of examination Before and after deworming		I stage (April)		II stage (August/September)	
		24 h	14 d	24 h	14 d
Mean EPG		1354.54	485	1954.54	100
% of horses in particular EPG area	0 EPG	0 (%)	9.09	0 (%)	90.91
	50 - 200	0	54.54	0	9.09
	250 - 500	18.18	13.64	4.55	0
	> 500	81.82	22.73	95.45	0
> 500 :	550-1000	27.27	9.09	13.64	0
	1050-1500	22.73	4.55	22.73	0
	1550-2000	13.64	0	13.64	0
	2050-2500	13.64	4.55	18.18	0
	2550-3000	0	4.55	9.09	0
	3050-3500	0	0	4.55	0
	3550-4000	0	0	9.09	0
	4050-4500	0	0	4.55	0
	4550-5000	0	0	0	0
	5050- 5500	4.55	0	0	0
FECRT		67.45%		99.53%	

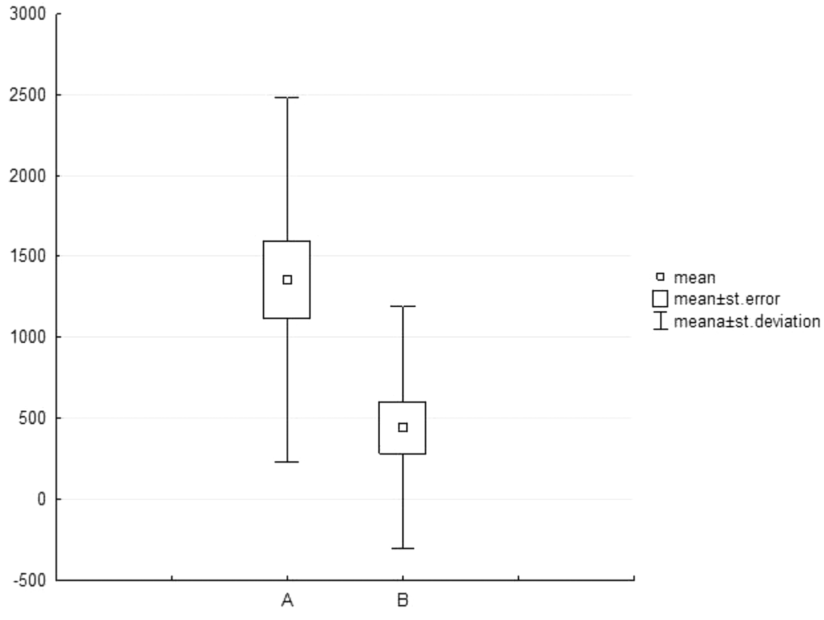

Fig. 1. Comparison of levels of strongyle EPG before (A) and after (B) deworming in the spring.

But *Parascaris* spp. in the group of two-year-old horses was not detected.

Stage III – deworming at the end of summer (August/September)

Before deworming, all selected horses had strongyle eggs and the average EPG level was 1954.54. In 18 of 22 animals EPG was >1000 (Table 1). After deworming only 2 horses had strongyle eggs at EPG=100.

Consequently, to assess the effectiveness of pyrantel pamoate against strongyles, the FECRT value was calculated and was 99.53% (Table 1, Fig. 2). Using the hybrid Frequentist/Bayesian analysis method (http://www.fecrt.com), the automatically selected statistical method was the Beta Negative Binomial (BNB) method (version B). For the different variants of the BNB methods, including version B, only p-values are provided (test for resistance $p = 1.000$; test for susceptibility: $p<0.001$). Based on an expected efficacy of 98% and a lower efficacy threshold of 88%, the final effect of

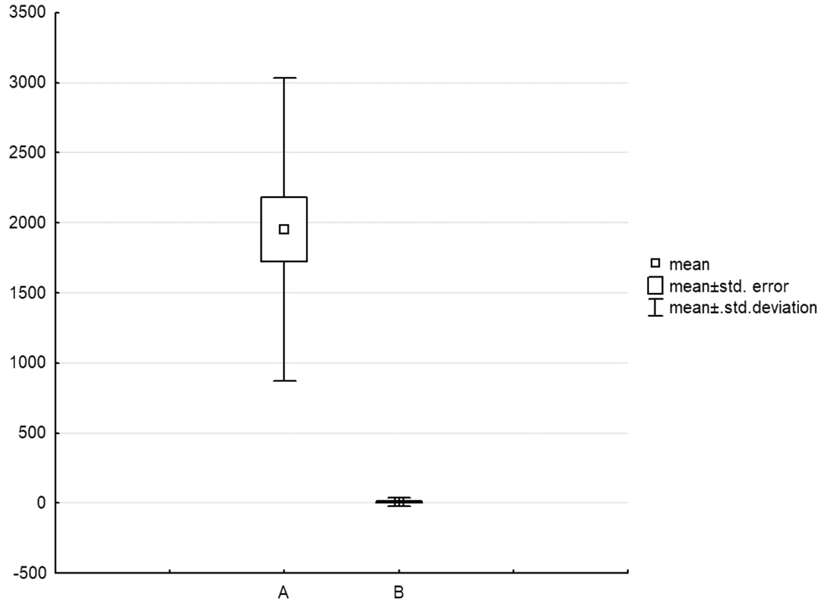

Fig. 2. Comparison of level of strongyle EPG before (A) and after (B) deworming at the end of summer.

Table 2. Comparison of mean levels of strongyle EPG and FECRT for two age groups of horses.

Dates of examination Before and after deworming		I stage (April)		II stage (August/September)	
		24 h	14 d	24 h	14 d
yearlings (13 horses)	mean EPG	1565.38	512.50	2103.85	100
	FECRT	69.78%		99.27%	
two-year-olds (9 horses)	mean EPG	1050	443.75	1738.89	0
	FECRT	62.43%		100%	

pyrantel pamoate against strongyles was assessed as positive.

Moreover, in 7 horses before and 2 horses after deworming, in addition to strongyle eggs, *Parascaris* spp. eggs were found (the EPG level ranged from 50-1150 and 450–550, respectively).

Analyzing the results in the group of one-year-old horses, it was found that before deworming

the average EPG level was 2103.85. In 6 out of 13 horses (69.23%), the EPG was from 550 to 2500. After deworming, strongyle eggs were found in only 2 horses, with EPG =100.

In the group of two-year-old horses, the average EPG level was 1738.89. In 5 out of 9 horses (55.56%), EPG was from 1050 to 2500. On the 14th day after deworming, no strongyle eggs were found in the feces of the examined horses.

The FECRT in yearlings and two-year-old horses was 99.27% and 100%, respectively (Table 2). The Beta Negative Binomial (BNB) method (version B) was automatically selected to assess the efficacy of pyrantel pamoate in both age groups. Based on an expected efficacy of 98% and a lower efficacy threshold of 88%, the efficacy of pyrantel pamoate against strongyles was assessed as positive in both age groups.

Additionally, *Parascaris* spp**.** eggs were found in 5 yearlings and 2 two-year-old horses before deworming (EPG: 100–1150 and 50-100, respectively). After deworming, *Parascaris* spp. eggs were still present in a single yearling (EPG = 550) and a single two-year-old horse (EPG = 440).

Discussion

It is generally accepted that one of the reasons for the unsatisfactory effectiveness of anthelmintic therapy in horses may be the problem of increasing drug resistance of nematodes from the Strongylidae family. Due to the limited number of active substances in antiparasitic preparations available for horses, the same drugs are often used for a long time.

In the world, as in Poland, antiparasitic/nematocidal drugs for horses are available, which belong to three main chemical groups: benzimidazoles (albendazole, febantel /pro-drug/, fenbendazole, mebendazole); macrocyclic lactones (abamectin, doramectin, ivermectin, moxidectin) and tetrahydropyrimidines (pyrantel pamoate).

Guidelines on the effectiveness of active substances are provided in the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Kaplan et al. 2023). Using the hybrid Frequentis/Bayesian analysis method (http://www.fecrt.com) (Denwood et al. 2019, 2023), their effect was calculated. A testing protocol was used whereby resistance to pyrantel pamoate was determined if the upper 90% confidence level (UCL) was lower than the expected efficacy of 98%. Complete susceptibility to pyrantel pamoate was concluded if the lower 90% confidence level (LCL) was ≥ 88% and the UCL was ≥ 98%. However, if the results indicated UCL ≥ 98% but LCL < 88%, it was considered inconclusive (Kaplan et al. 2023).

Analyzing the results of our study, significant differences were found in the effectiveness of the drug after deworming in spring and at the end of summer. In the spring, despite smaller differences in the EPG level before deworming, the antiparasitic effectiveness of pyrantel pamoate was significantly lower (67.45%), which, based on the research protocol, indicated the drug resistance of strongyles. However, at the end of summer, although before deworming the percentage of horses with EPG > 500 was high (95.45%), more than 25% of the animals (26.09%) had EPG of 2500-4500, the calculated effectiveness of pyrantel pamoate was definitely higher (99.53%).

Therefore, based on the results obtained, it should be concluded that resistance of strongyles to the action of pyrantel pamoate was demonstrated in spring – in contrast to the effects of deworming at the end of summer, when the drug turned out to be fully effective in the treatment of this invasion.

Comparing the results in particular age groups, significantly higher EPG levels (above > 500) were found in one-year-old horses compared to two-year-olds, in which they remained at a comparable level in both periods of the study. Some researchers noted higher EPG levels in younger horses, which decreased in older ones (Kornaś et al. 2010, Kuzmina et al. 2016). However, other researchers observed different relationships – e.g. in a study on mares, the highest average EPG was found in horses aged 6-10 years (Lyons et al. 2012). The different intensity of strongyle invasions found in horses in different age groups, found in our own study, is an indication for systematic monitoring of EPG levels in order to control the effectiveness of combating parasitic invasions.

Resistance of strongyles to wormers containing pyrantel pamoate in horses has been reported in many European countries. In Italy, horses on two out of six stud farms (12.5%) had FECR values ranging from 43% to 85.4% (Traversa et al. 2007). In other studies conducted in Germany, Great Britain and Italy, the percentage of stud farms in which strongyle resistance to pyrantel salts was found in horses was 25% and no significant differences between countries were found (Traversa et al. 2009).

In France, suspected resistance of strongyle worms against pyrantel pamoate was described on every fifth of the stud farms tested, with an average reduction in the effectiveness of this drug of 94.7% (95% CI: 88.9-98.5%), and resistance of these worms was confirmed on 3 of the 30 farms (10%) (Traversa et. al. 2012). Similar data were obtained in countries geographically neighboring Poland, including Lithuania, and further north in Estonia and Finland, where the percentage of farms where strongyle resistance to pyrantel pamoate was found in horses was: 25; 75 (i.e. in 25% of the tested horses) and 43% (Nareaho et al. 2011, Lassen and Peltola 2015, Dauparaitė et al. 2021).

Drug resistance of horse strongyles to pyrantel pamoate has also been reported in North America. Research conducted in the southern USA showed resistance to this drug in over 40% of cultures. It was considered that the main reason for the emergence of drug resistance in nematodes in the USA is the daily addition of pyrantel pamoate in low doses to food (Kaplan et al. 2004).

Other factors leading to the development of drug resistance in nematodes may be incorrect estimation of the weight of horses and, consequently, the administration of underestimated doses of drugs during routine deworming of animals. Therefore, in order to limit or counteract the phenomenon of increasing drug resistance, it is important to provide appropriate management, select appropriate parasite control programs and adjust the frequency of their use to the climatic zone in which the animals live (Nielsen et al. 2006, Kaplan and Nielsen 2010, Nielsen et al. 2019).

In the study by Nielsen et al. (2019) showed that, especially in highly seasonal climates, treating all horses twice a year markedly reduces the rate of resistance development, compared to treating horses four or six times a year. The authors suggest using mixed treatment regimens in which horses receive a combination of different active substances (antiparasitic drugs) administered to all horses in the treated herd.

Many researchers (including: Molento 2008, Kaplan and Nielsen 2010, and Osterman Lind et al. 2023) indicate that an effective method of parasitic infection control in horses is their selective deworming. In the case of horses undergoing selective antiparasitic therapy, treatment of the infection should be based on the number of parasite eggs excreted in the feces, i.e. the detected EPG level. Based on general guidelines for deworming horses, it is assumed that if the number of parasite eggs excreted in the faeces is less than < 200-250, the administration of drugs may be discontinued, as this is the level of infection intensity that is considered "neutral" for the health of horses. The above-mentioned authors indicate that on stud farms intended for riding, high levels of EPG are usually found in a minority of horses, and only these animals should be subjected to antiparasitic treatment.

Based on the results obtained, we may conclude that the invasive situation in individual horses on a specific stud farm may be different. High levels of EPG were found in most animals from the stud farm (76%) where the tested horses came from, which makes it difficult to use the selective deworming method in such cases. In the examined stud farm, the percentage of horses with EPG > 200 was significantly higher both in spring and at the end of summer.

Therefore, it is generally recognized that it is necessary to develop local deworming plans for horses, taking into account the invasive situation and environmental contamination with dispersal forms of parasites. This applies especially to large stud farms, as both the density of horses and the failure to remove faeces from pastures contribute to the constant exposure of horses to invasions with high levels of EPG (Gawor et al. 2006, Kuzmina and Kharachenko 2008, Kornaś et al. 2010, Studzińska et al. 2017, Dauparaitė et al. 2021).

The observations made and the results obtained justify the conclusion that when developing plans to treat strongyle infection in spring in horses, the use of pyrantel pamoate should not be recommended. Unsatisfactory, limited effects of treatment of strongyle infection during this period do not have to result from the "acquired" drug resistance of these nematodes, but may be a consequence of the in which pyrantel pamoate affects the development of strongyles. This substance does not affect migrating larval forms and does not eliminate larvae present in worm nodules. The time of year when horses are treated with pyrantel pamoate is important – it has been shown that climate, especially with significant seasonality, may be an important factor in the development of drug resistance in parasites (Nielson et al. 2019).

In spring, following the low air temperatures of winter, the development of small strongyle larvae in the intestinal wall may be further inhibited. During this period, fourth-stage larvae (L4) can undergo developmental arrest and remain within the nodules for an extended time. As ambient temperatures rise, these larvae become reactivated, migrate into the intestinal lumen, and mature, resulting in increased egg shedding into the environment despite prior deworming (Corning 2009). It is likely that, during this time, changes occur in drug translocation, modifications to receptor binding sites, or in signal transduction pathways involved in muscle contraction (Sangster and Dobson 2002).

On the other hand, our results indicate that at the end of summer, when the air temperature is higher and there are no large fluctuations in its amplitude, pyrantel pamoate may to be effective in the control of strongyle infection. To the best of our knowledge, the study conducted and the clinical data obtained are the first analysis of this type in Poland regarding the limited effect of pyrantel pamoate against strongyle infection in horses. If appears that the lack (or significant reduction) of the effectiveness of pyrantel pamoate in horses in spring, it does not necessarily indicate drug resistance of strongyles, but may result from the mechanism of action of the drug on these parasites. Therefore, further research is required on a larger population of horses of different utility levels, kept in different environmental conditions, and at different times of the calendar year.

According to, among others, Vidyashankar et al. (2007), there are many animal- and farm-related variables that may influence the interpretation of results, especially when many different horse housing locations are examined. Another issue to be considered is whether the procedures for testing drug resistance described in the guidelines of the World Association for the Advancement of Veterinary Parasitology (WAAVP) should also take into account the aspect of seasons.

In conclusion, both the time of year and the type of parasitic infection play important roles in selecting the appropriate active substance for deworming horses. Under climatic conditions in Poland, late summer appears to be a suitable period for the use of pyrantel pamoate in the treatment of Strongylidae infections. In contrast, administration during the spring may be ineffective and therefore not recommended.

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