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Monitoring the avermectin and pyrantel resistance status of nematode parasites of horses in the Czech Republic

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ABSTRACT: The avermectin and pyrantel resistance of equine parasites was monitored on four farms in the Czech Republic in 2014. The aim of the testing was to investigate the anthelmintic resistance of cyathostomins and ascarids. One hundred and eighty-six horses were included in the test, 54 of which were infected by ascarids and 174 by cyathostomins. Faecal egg count reduction tests were used to assess anthelmintic resistance. The faecal egg count reduction for ascarids was within the range of 87.9% to 100% for avermectins and 100% for pyrantel embonate. Faecal egg count reduction for cyathostomins ranged from 99% to 100% for avermectins and 89.3% to 98.2% for pyrantel embonate.

Keywords: equine; anthelmintic resistance; FECRT; ascarids; cyathostomins

Cyathostomins and ascarids (*Parascaris equorum*, *Parascaris univalens*) are the most common and ubiquitous equine parasites. The ascarid is most often found in the small intestine of foals and less commonly in weanlings and young horses. Slow growth, weight loss, cough and colic are all signs of potential massive gastrointestinal infection (Reinemeyer 2009; Nielsen et al. 2014). Cyathostomins are found in the large intestines of horses; aggregation of the developmental stages in the mucosa of the large intestine can lead to clinically apparent disease which manifests itself as diarrhoea, hypoproteinaemia, subcutaneous oedema and metabolic acidosis (Lyons et al. 2000).

Parasite population sizes on equine farms in the Czech Republic are normally regulated by anthelmintic therapy; an increase in anthelmintic resistance represents a risk to animal health. Anthelmintic resistance describes a scenario in which a higher number of individuals are resistant to the treatment dose in comparison to the total susceptible population of the same species (Prichard et al. 1980). The first case of ascarid resistance to macrocyclic lactones was confirmed in Utrecht, Netherlands in 2002 (Boersema et al. 2002). One year later it was identified in Canada (Hearn and Peregrine 2003), followed by Texas (Craig et al. 2007), Germany (von Samson-Himmelstjerna et al. 2007), Denmark (Schoogard and Nielsen 2007) and Italy (Veronesi et al. 2010). Ascarid resistance to pyrantel pamoate was noted in North America (Lyons et al. 2008). Ascarid resistance to benzimidazoles has not yet been described (Brady and Nichols 2009).

The occurrence of anthelmintic resistance in cyathostomins is a long-term observational problem. The presence of benzimidazole resistance has been proven in various countries all around the world including Central Europe (Chroust 1998; Varady et al. 2000). Pyrantel resistance has been observed in a number of European countries (Denmark, Norway, France) as well as in other countries (USA, Brazil); (Ihler 1995; Craven et al. 1998; Kaplan et al. 2004; Traversa et al. 2012; Canever et al. 2013). Macrocyclic lactones (avermectins, moxidectin) still show high efficacy in cyathostomin infection therapy, although nowadays the cyathostomin egg

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reappearance period for ivermectin and moxidectin is decreasing (von Samson-Himmelstjerna et al. 2007; Porr et al. 2017).

So far, comprehensive studies of avermectin and pyrantel resistance in ascarids and pyrantel resistance in cyathostomins have not been carried out in the Czech Republic. Avermectin resistance in cyathostomins has not yet been described in the Czech Republic (Bodecek and Vavrouchova 2013).

MATERIAL AND METHODS

In our trial, 302 horses were examined between April 2014 and November 2014. Of these, 52 were foals and 72 horses were under four years of age. The horses were sampled from four inland farms (Figure 1). The horses on these farms were used for sport, pleasure riding and breeding. Thoroughbreds, warmbloods and draft horses were represented. Only horses which had not received any anthelmintic drugs within the ten weeks prior to our testing were included in the study. Individual sampling immediately after defecation or rectal sampling was performed. During transport, samples were stored at a temperature of 4 °C and were examined in a laboratory within 24 hours after collection.

For the coprological assessment of avermectin and pyrantel resistance using faecal egg count reduction tests (FECRT), only horses with an eggs per gram (EPG) value of over 100 strongylid or ascarid eggs per gram of faeces were chosen. The EPG value was obtained using a modified McMaster method (Coles et al. 1992). One hundred and eighty-six horses were chosen for monitoring based on these criteria.

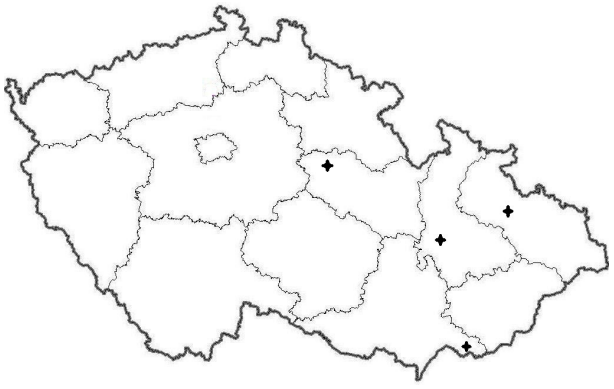

Figure 1. Map presenting approximate locations of the monitored farms in the Czech Republic

During a subsequent visit to the farms, additional samples were collected from chosen horses to determine the EPG value on day 0, and, concurrently, each horse was administered a dose of anthelmintics corresponding to its weight.

Two anthelmintic pastes containing avermectin, from two European producers (Equiverm® Bioveta, Czech Republic; Equalan Duo®, Merial, France), were used to evaluate avermectin resistance. Both pastes contain a combination of ivermectin and praziquantel and were used in 92 horses in dosages of 0.2 mg/kg, administered orally. Two anthelmintic pastes containing pyrantel embonate (Equistrong®, Bioveta, Czech Republic; Pyratape P®, Elanco, United Kingdom) were used in 94 horses in dosages of 6.6 mg/kg, administered orally.

The third visit to the farms took place 14 days after anthelmintic therapy (day 14). Faecal samples were collected, and the EPG value was obtained to calculate faecal egg count reduction (FECR). The calculation of FECR is well described by the World Association for the Advancement of Veterinary Parasitology (Coles et al. 1992). FECR is the percentage egg reduction in 1 gram of faeces determined by the difference of EPG values before (day 0) and after (day 14) deworming. FECR values lower than 90% are considered to indicate resistance (Coles et al. 1992).

RESULTS

A total number of 302 horses from four farms in the Czech Republic were coprologically examined in 2014. Of those 302 horses, 116 were eliminated from the study based on the determined parameters. From 186 monitored horses, 12 foals were infected by ascarids only, 42 foals and young horses were infected by ascarids and cyathostomins and 132 horses were infected by cyathostomins only. The numbers of examined and monitored horses on each farm, the numbers of horses with positive findings and the numbers of ascarid and cyathostomin eggs are reported in Table 1. Table 1 also shows the distribution of anthelmintic paste treatments.

Samples from the monitored individuals were collected on day 0 and showed the following range of EPG values: 100–4700 for ascarids (mean EPG value 525) and 100–4800 in cyathostomins (mean EPG value 938). EPG values on day 0 on each farm

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Table 1. Numbers of horses (screened/enrolled) by farm, and numbers of enrolled horses infected with cyathostomins and/or ascarids by farm and treated with avermectin (AVM) or pyrantel embonate (PYR)

	Anthelmintic	Total 302/186	Farm 1 206/122	Farm 2 35/26	Farm 3 30/25	Farm 4 31/13
Positive for ascarids	total	54	39	6	8	1
	AVM	37	22	6	8	1
	PYR	17	17	0	0	0
Positive for cyathostomins	total	174	111	26	24	13
	AVM	82	55	6	8	13
	PYR	92	56	20	16	0

and measured mean EPG values are reported in Table 2.

The FECR values for avermectin on the individual farms were the following: 87.9–100% in ascarids and 99–100% in cyathostomins. Avermectin resistance of ascarids was suspected on farm No. 2, where two individuals out of six did not show a FECR value of 100% (mean 87.9%). FECR values for pyrantel embonate were 100% in ascarids and 89.3–98.2% in cyathostomins. A decreased effect of pyrantel against cyathostomins was recorded on farm No. 2. The FECR values in individual farms are reported in Table 3.

DISCUSSION

Avermectin resistance monitoring in ascarids is one of the most current topics in equine parasitology. Ascarids are one of the most pathogenic equine parasites and can cause serious health problems in foals. Driven by fear of these complications, avermectin products are repeatedly administered to foals. Frequent exposure of a parasite population to an anthelmintic drug is one of the main causes of resistance development (Herd 1990). The aim of this anthelmintic program is the restriction of ascarid egg contamination of the pasture soil. Ascarid eggs are very resistant to climatic conditions in the external environment. Our results show a decreased efficacy of ivermectin on farm No. 2 (87.9%).

On farm No. 2, anthelmintics were administered three times per year, while on the other farms administration of the anthelmintics was performed twice a year. Farm No. 2 had a higher intake of new horses compared to the other monitored farms, because the main focus of this farm was natural thoroughbred breeding and rearing of foals. Some of these horses were not treated with anthelmintics prior to coming to the farm. There is a high risk that these horses, especially mares with older suckling foals, may introduce new resistant ascarid populations to a farm. New foals can be infected by horizontal transmission of resistant ascarid eggs from contaminated premises. These infected foals will have coprologically detectable ascarid eggs only after three months. Because application of avermectin or moxidectin is usually chosen as a preventive therapy in these foals, pastures and paddocks on the new farm can be contaminated by a resistant ascarid population (Craig et al. 2007).

Our detected FECR value of 87.9% suggests resistance to ivermectin on farm No. 2. Previous studies have described an even more serious drop in efficacy. In Texas, a decrease in the EPG to 75% of the original EPG value or even an increase of the value was detected (Craig et al. 2007). In Canada, where the monitoring was carried out on three farms, the FECR after avermectin administration was found to be only 33.5% (Slocombe et al. 2007) and in Kentucky a 0% reduction was detected in a group of 18 foals (Lyons et al. 2007). Nevertheless,

Table 2. Ranges and arithmetic mean of faecal egg counts prior to treatment, by parasite and farm

	Parasite	Farm 1	Farm 2	Farm 3	Farm 4
EPG range	*Parascaris* spp.	100–1300	100–4700	100–1150	900
	Cyathostomins	100–4800	200–3900	100–2550	200–2200
EPG average	*Parascaris* spp.	319	1033	567	900
	Cyathostomins	1351	931	1067	638

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Table 3. Faecal egg count reduction test results for ascarid and cyathostomin infections, by farm. The table shows the faecal egg count reduction test results on individual farms and for the tested anthelmintics

Anthelmintic	Infection	Farm 1	Farm 2	Farm 3	Farm 4
Avermectin	*Parascaris* spp.	100%	87.9%	100%	100%
	Cyathostomins	100%	99%	100%	100%
Pyrantel embonate	*Parascaris* spp.	100%	N/A	N/A	N/A
	Cyathostomins	97.5%	89.3%	98.2%	N/A

N/A = not applicable

the FECRT result obtained from farm No. 2 was taken from a statistically insignificant group of six foals, where only one individual showed no reduction of the EPG value and one individual did not show a 100% reduction (FECR value 97.9%). The individual with the FECR value of 0% concurrently showed a 90% EPG reduction of cyathostomins (day 0 – 500 EPG, day 14 – 50 EPG). In all other horses, a 100% efficacy of avermectins against cyathostomins was observed. Thus, it is feasible that unintentional underdosing caused therapeutic failure in this one individual. To confirm the presence of avermectin resistance on farm No. 2, it would be desirable to repeat the FECRT at a future date.

The development of macrocyclic lactone resistance in ascarids is conditional on several factors. One of them is administration of anthelmintics to foals within their first month of life, and subsequently at an interval shorter than three months in order to reduce environmental contamination with eggs. Administering the anthelmintic at an interval shorter than the prepatent period (11–12 weeks) combined with the ability of macrocyclic lactones to maintain persistent and effective plasma concentrations exposes the parasites to a permanent anthelmintic effect. This leads to refugium reduction, both inside the host and in the environment. Thus, repeated macrocyclic lactone application to foals at intervals shorter than three months allows the development of resistant ascarid populations (Sangster 1999; Kaplan 2004; Craig et al. 2007).

Dealing with the increase and spread of avermectin resistance in ascarids can present a significant future problem in the Czech Republic. It is known that adult parasites are more susceptible to anthelmintic effects than the developmental stages. The efficacy of oxibendazole (benzimidazole group) is 94–100% against ascarid adults but only 44.5% in 28-day-old larvae (Austin et al 1991; Lyons et al 2008). Thus, anthelmintic therapy 60–75 days post infection would be ideal to prevent pasture contamination and to reduce the ascarid population size. Parasites of this age are susceptible to the anthelmintic effect but do not yet produce eggs. This solution is not realistic, though, because exposure of foals occurs gradually, the foals are simultaneously infected by various developmental stages of the parasites and younger, less susceptible developmental stages can survive the therapy (Reinemeyer 2012). Some authors recommend starting anthelmintic therapy around 60–70 days after birth on farms where ascarid infection in foals poses a problem in order to reduce the pasture contamination (Reinemeyer et al. 2010).

Benzimidazoles (BZD) have a good therapeutic effect against macrocyclic lactone-resistant adult ascarids, and fenbendazole dosed for five consecutive days is very effective, even against the developing larval stages of ascarids. Repeated administration may be applied as an individual therapy rather than in preventive anthelmintic management (Reinemeyer et al. 2010). Benzimidazole anthelmintic resistance of roundworms has never been described (Brady and Nichols 2009). It is possible that BZD resistance will develop among ascarids in the future due to the use of dosages of 5 mg/kg against cyathostomins and pinworms, which is one-half of the dosage required for efficacy against ascarids (Reinemeyer 2012).

Pyrantel resistance monitoring is not yet as up-to-date in Europe as in other regions. A product containing pyrantel was registered in 2013 in the Czech Republic. In our monitoring in 2014, drugs containing pyrantel showed 100% efficiency against ascarids in all foals. The monitoring was performed on one farm only; other farms did not have a sufficient number of positive foals for monitoring with anthelmintics. Similar results were described for instance in Sweden (Osterman-Lind and Christennsson 2009), where a 95% efficacy of pyrantel against ascarids was demonstrated. Ascarid resistance against pyrantel has so far only been described in North America (Craig et al. 2007; Lyons et al. 2008). It has been hypothesized that pyrantel resistance on some farms may have been selected for as a result of daily administration of pyrantel tartrate as a prophylactic agent (Reinemeyer 2009). This is a so-called continuous antiparasitic program, which consists in daily administration of pyrantel tartrate to horses at a dosage of 2.6 mg/kg in a pelleted product incorporated into

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feeding rations. This programme is not being used in Europe and was only available in North America (Kaplan and Nielsen 2010).

The efficacy of pyrantel products is often described in the context of their use against ivermectin-resistant ascarid populations. Such studies were conducted in the USA and Europe and all of them confirmed a good effect of tetrahydropyrimidines on resistant ascarid populations (Slocombe et al. 2007; von Samson-Himmelstjerna et al. 2007; Reinemeyer 2009; Reinemeyer et al. 2010).

Along with monitoring of resistant ascarid populations, monitoring of cyathostomin resistance against avermectins was conducted on all four monitored farms and cyathostomin resistance against pyrantel was monitored on three farms. Only individuals with values exceeding 100 strongylid eggs per gram were included in the study. No attempts were made to characterise strongylid populations by coproculture because large strongyles have not been reported in the Czech Republic for a protracted period (Langrova 1998).

Our results show a moderately good efficacy of pyrantel anthelmintics when compared with foreign studies. Smith et al. (2015) reported only 68.58% FECR using pyrantel in the USA. Nareaho et al. (2011) detected an FECR of 43% and proved cyathostomin resistance against pyrantel in 30 out of 38 foals in Finland. The lowest value of FECRT in our study was 89.2% on farm No. 2. On other farms, the pyrantel anthelmintics were highly efficacious.

The lowest value of FECRT for cyathostomin eggs was found on the same farm (No. 2) with the lowest value of FECRT for roundworms. This result might highlight the effectiveness of antiparasitic management on this farm, as stated above. It corresponds with the results of antiparasitic efficacy monitoring in studs in the UK (Relf et al. 2014). Reduced pyrantel efficacy was observed in five groups of yearlings, while a very good efficacy was found in groups of mares. Shorter egg reappearance periods for pyrantel were also observed, implying a possible development of resistance. Very good efficacy on the farms where deworming was carried out twice a year is probably due to the fact that pyrantel anthelmintics have only recently been reregistered after a long period of time in the Czech Republic. As for the efficacy of pyrantel anthelmintics, one study showed that using a combination of pyrantel and benzimidazole gives the additional benefit of treating cyathostominosis (Kaplan et al. 2004). This might pose a good option where there is a higher demand for antiparasitic efficacy.

Cyathostomin resistance against avermectins was not proven in our study, similar to previous monitoring trials in the Czech Republic (Bodecek and Vavrouchova 2013).

Reports from other European countries (Wirtherle et al. 2004; Osterman-Lind et al. 2007; Travesa et al. 2007) do not indicate that avermectin resistance has developed in the investigated horse populations. Nevertheless, a recent study in Germany showed a shorter egg reappearance period (von Samson-Himmelstjerna et al. 2007), which may be the first evidence of avermectin resistance in cyathostomins. A recent study from Kentucky showed reduced activity of moxidectin and ivermectin on cyathostomins in young horses (Lyons et al. 2008).

Potential environmental infection together with the development of resistance can be controlled by correct anthelmintic administration, strict pasture management and consistent zoohygiene. Regular manure removal from the pastures and systematic rotation of horses in paddocks and pens is recommended (Lyons et al. 1976). Yearly coprological examination is recommended to assess the efficacy of the management procedures (Kaplan 2002).

In our study, which was carried out on four farms in the Czech Republic in the year 2014, resistance to ivermectin was demonstrated on farm No. 2. (87.9%). On the same farm, which is focused on Thoroughbred breeding, cyathostomin resistance against pyrantel embonate (89.3%) was also shown. Ascarid resistance against pyrantel embonate and cyathostomin resistance against avermectin was not found. Despite the fact that resistance was found on only one farm and in a small number of horses, an increase in resistance could be expected in the future. This will require regular parasite and resistance monitoring and also the implementation of measures which will delay further resistance development on horse farms.

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