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Monitoring of anthelmintic resistance in small strongyles in the Czech Republic in the years 2006–2009

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Abstract

The aim of the field study performed in 2006 was to investigate the occurrence and distribution of intestinal helminths in horses based on pre-treatment faecal egg counts. In total, 948 horses bred on 37 farms were tested. Thirty six (97.2%) farms tested were positive for cyathostomins; horses in 9 (24.3%), 6 (16.2%) and 1 (2.7%) different herds tested were positive for *Parascaris equorum, Anoplocephala perfoliata* and *Strongyloides westeri*, respectively. In 21 herds, 344 horses with values exceeding 100 eggs per gram were included in the trial for the presence of drug resistant cyathostomins by a faecal egg count reduction test. Horses were treated orally with recommended doses of fenbendazole and ivermectin. Resistance to fenbendazole was detected on 20 farms (95.24%) with values of faecal egg count reduction test ranging from 0 to 90%. Ivermectin remained effective in all tested herds with the value of faecal egg count reduction test 96–100%. In autumn 2008, 178 horses on 10 farms were examined. Of these, only seven horses tested were negative for cyathostomins. One farm was tested positively for *Anoplocephala perfoliata*, and one for *Parascaris equorum*. In spring 2009, six farms were examined, four of which were the same farms as in 2006. We found a decreased number of eggs per gram in all horses, but an increase in benzimidazole resistance, which was found in 5 farms out of 6 (faecal egg count reduction test 15.2–84.6%). This is the first wide survey in horses from the Czech Republic. Based on this study, we can conclude that benzimidazole resistant cyathostomins in horses are widespread but ivermectin is still fully effective.

Cyathostomins, faecal egg count reduction test, benzimidazoles, ivermectin, anthelmintic resistance

Cyathostomins or cyathostomes are known as "small strongyles" and are recently considered the most important helminth parasites of horses. Cyathostomins are associated with a syndrome known as larval cyathostominosis, in which there is synchronous emergence of the fourth stage larvae from the mucosa of the caecum and large colon. This can result in a protein-losing enteropathy, chronic diarrhoea, significant weight loss, oedema, and severe colitis, and can be fatal (Lyons et al. 2000).

Control of cyathostomins is complicated by the presence of larval stages that are refractory to many commonly used anthelmintics, as well as by their ability to develop resistance to anthelmintic agents. Resistance of cyathostomins to anthelmintics has been recognised for more than four decades and has been widely reported throughout the world.

In the Czech Republic, cyathostomin infestations in horses are also very common, with prevalence rates up to 100% (unpublished data). However, except for the studies demonstrating the presence of resistant cyathostomins on four small horse farms more than 10 years ago (Chroust 1998, 2000), and trials conducted on five small horse farms (Langrová et al. 2002), no evaluation of the presence and spread of benzimidazole resistant cyathostomins in the Czech horse population has been conducted.

The resistance to pyrantel, the only important member of the tetrahydropyrimidine anthelmintics, has been recently described in Norway, Denmark and the USA (Kaplan 2002). Moxidectin failed to fully control cyathostomins in two donkey herds at the Donkey Sanctuary in Devon (Trawford et al. 2005) and there is a suggestion that ivermectin

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resistance may be beginning to appear in Germany (Von Samson-Himmelstjerna et al. 2007). None of the macrocyclic lactons tested in Brazil (abamectin, ivermectin, moxidectin) provided adequate control of the cyathostomins (Molento 2008).

The development of anthelmintic resistance of cyathostomins has become a limiting factor in equine parasite control programs throughout the world and for the health management of horses (Lyons et al. 1999; Kaplan 2002, 2004). Therefore, the aim of the present study was to enhance our knowledge on the distribution and prevalence of drug resistant cyathostomins in horses bred in the Czech Republic.

Materials and Methods

Farms and animals

The trial was carried out between January 2006 and October 2006 on 37 farms, located in various parts of the Czech Republic. The farms and animals were selected at random from around the whole country. The criteria for inclusion were: willingness of the farm owners to fully cooperate with the study, presence of at least 20 animals on the property, and the condition that an anthelmintic drug had not been administered for at least 10 weeks prior to testing. The type of farms selected included breeding, training and pleasure establishments; with animals aged from 1 to 27 years.

The owners filled in a checklist about conditions of farm management (horse housing, time spent on pasture, way of feeding and water supply, removal of manure, and method and frequency of dehelmintization). We recommended some measures to improve management on farms based on observed results of eggs per gram (EPG) and faecal egg count reduction test (FECRT).

In autumn 2008, horses from 10 farms were examined for determination spectrum of parasites. In spring 2009, 10 farms with a minimum of 16 horses were examined. Farms were chosen based on distance and with a view to compare the same farms between 2006 and 2009, with the goal to find the effects of our recommendations on the occurrence of resistance.

Parasitological techniques

At the initial farm visit, pre-treatment faecal egg counts (FEC) were performed to ensure that sufficiently high faecal eggs per gram were present in the animals to warrant inclusion in the trial. Individual faecal samples were collected from the rectum or as freshly expelled faeces off the ground of all animals bred on each farm. Faecal egg counts were performed by a modified McMaster technique with a minimum sensitivity of 15 EPG. During this examination other helminth eggs were also determined according to manual of veterinary parasitological laboratory techniques (MAAF 1986).

Animal treatment

One week after the first visit, the farm was visited again (day 0) and faecal samples were obtained only from those animals that had shown a sufficiently high FEC value on the first occasion. From the screened 37 farms, 21 were included in the study. On each farm, individuals with $\geq$ 100 EPG were allocated to two comparable treatment groups according to age and cyathostomins egg output. The groups, each comprising of 7–10 horses, were treated according to the manufacturers' recommendations, using commercially available anthelmintic pastes in the following way: group 1 with fenbendazole (FBZ, Panacur®, Intervet; 7.5 mg/kg bwt) and group 2 with ivermectin (IVM, Noromectin®, Norbrook Laboratories Ltd.; 0.2 mg/kg bwt). To calculate the dose of each drug, the body weight of each horse was estimated by calibrated tape measurement of the girth.

The farms were visited again within 10–14 days after treatment and faecal samples were collected from each horse and processed as described before, to obtain the post-treatment FEC value. The Faecal Egg Count Reduction Test was carried out according to the recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP), to determine anthelmintic resistance in horses (Coles et al. 1992). The efficacy of treatment for each horse was determined as follows:

$$\% \text{ efficacy} = [(\text{pre-treatment EPG} - \text{post-treatment EPG})/\text{pre-treatment EPG}] \times 100$$

In the case when FEC for an individual animal increased after treatment, the FECR percentage was considered to be zero. Mean values for percentage efficacy were calculated for each treatment group on each farm, and treatments were categorized as effective for FECR $\geq$ 90%, equivocal for FECR from 80 to 90% and ineffective for FECR $\leq$ 80% (Kaplan et al. 2004).

Results

A total of 948 horses bred on 37 farms were quantitatively analysed for helminth eggs in 2006. Our results showed that 36 (97.2%) herds were positive for intestinal helminths and cyathostomin infestations were found in all positive horse herds. Nine farms (24.3%) had at least one horse excreting eggs of *Parascaris equorum*, six farms had at least one horse excreting eggs of *Anoplocephala perfoliata* and *Strongyloides westeri* eggs were detected on one farm. Considerable individual variations in FEC were found (0–11 300) and the average number of EPG was higher in young horses (< 5 years) than in older horses (data not shown). Sixteen of the 37 farms examined were excluded, because too many horses showed too low faecal egg counts and thus a treatment group with a minimum of seven horses could not be established. Overall, 344 horses on 21 farms were treated with FBZ or IVM. The FECRT values following FBZ treatment ranged from 0 to 90.02% in 95.24% of farms (20/21 farms), revealing that resistance was widespread in the horse herds under study.

Mean percentage efficacy of the FBZ and IVM on each surveyed farm is reported in Table 1 together with the mean EPG value pre- and post- treatment for each horse group. In summary, FBZ resistance was found on 20 of the 21 farms examined (95.24%). No resistance was seen to IVM (Table 1). Fourteen days post-treatment, no farm met the criteria for IVM resistance as the mean FECR ranged from 96 to 100%.

In autumn 2008, 10 farms were examined and values of EPG were established for 178 horses (EPG range 0–4 200). In all of the positive FEC we found eggs of cyathostomins, in FEC from one farm we found eggs of *Parascaris equorum* (5/28 horses) and in one farm eggs of *Anoplocephala perfoliata* (9/28 horses). In spring 2009, following fenbendazol treatment, FECRT ranged from 15.2 to 95.1%, revealing resistance to fenbendazol in 5 out of 6 farms (Table 1). Four farms that were the same as the farms tested in 2006 all had lower FECRT (15.2–84.6%) than in 2006 (0–90.02%). Our study reports the first wide survey carried out in the Czech Republic to investigate the prevalence of anthelmintic resistance in equine cyathostomins.

Discussion

Initially in our study, the occurrence and distribution of intestinal helmith infections in horses were investigated in a field study based on faecal egg counts. The results of our study showed that cyathostomins were highly prevalent in the Czech horse population as 36 (97.2%) herds of a total of 948 horses were positive for cyathostomins.

Resistance to benzimidazol (BZ) caused by using only this class of anthelmintics for many years was reported. Chroust (1998, 2000) reported FECRT for fenbendazol to be 76–84.1% and for mebendazol 61.5–75.4% in four small horse farms. Langrová et al. (2002) reported BZ resistance in five farms (FECRT for mebendazol and fenbendazol was 20.6–83.9% and 6.1–78.8%, respectively). The results of our study showed that benzimidazole resistance was present on 95.24% of the farms investigated, thus the prevalence of benzimidazole resistant cyathostomins in Czech horses appears to be higher than recently described in other European countries (Várady et al. 2000, 2004; Wirtherle et al. 2004; Osterman-Lind et al. 2007; Traversa et al. 2007).

Our results from 2009 confirmed high prevalence of small strongyles and increased occurrence of FBZ resistance. Eggs per gram value seemed to be higher in horses housed on deep bedding compared to horses housed in individual stabling with removal of manure done twice daily. In 2009, the values of EPG were lower in horses compared to results from 2006 because of using ivermectin instead of BZ that was previously used. At the majority of farms, FECRT decreased due to the lack of adherence to the recommendations given. The only exception was farm No. 11, where a decrease in FECRT was not significant (from 90.02 to 84.6%). In this farm, they implemented a yearly rotation of anthelmintic preparations and separated parts of pasture that were harrowed during the dry season. Farm No. 2 changed frequency of using anthelmintics from once to twice a year, which led to a

Table 1. Arithmetic mean cyathostomin eggs per gram value pre- and post- treatment for each horse group and mean percentage (%) of the efficacy of fenbendazole in years 2006 and 2009.

Farm No.	MP	Fenbendazole (7.5 mg·kg^{-1}), 2006			Ivermectin (0.2 mg·kg^{-1}), 2006			Fenbendazole (7.5 mg·kg^{-1}), 2009		
		TH	PrT/PoT	%	TH	PrT/PoT	%	TH	PrT/PoT	%
1	B/P/T	7	850/250	70.5	7	485.7/0	100	11	905/767	15.2
2	B	10	2161/760	70.8	10	1430/0	100	14	507/411	18.9
3	B/T	8	1675/200	88.5	10	890/0	100			
4	T	9	1622/322	80.1	7	457/0	100			
5	B/T	8	500/462.5	7.5	7	714.3/0	100			
6	B	10	1920/370	79.5	8	2012.5/0	100	9	500/172	65.6
7	B/T	7	1428/628.6	56.2	7	371.4/0	100			
8	B/T	7	914/514	43.8	7	557.1/0	100			
9	B/T	7	785.7/400	49.08	7	585.7/0	100			
10	B/P	8	2012.5/735.5	63.37	9	855.5/0	100			
11	B/T	10	510/50	90.02	10	386.5/0	100	10	680/105	84.6
12	T/T	7	714.3/357.1	50	7	342.9/0	100			
13	B/P/T	7	1728.6/500	71.1	7	985.7/0	100			
14	B/P/T	7	1028.6/1085.7	0	7	1085.7/0	100			
15	P/T	7	1414.3/271.4	80.8	7	457.1/0	100			
16	B/T	7	1457.1/357.1	65.7	7	1342.9/0	100			
17	T	10	630/90	86	10	330/0	100			
18	B/T	10	1360/260	8	10	740/30	96			
19	B/T	7	160/120	25	7	210/0	100			
20	T	10	1250/980	22	10	930/20	98			
21	B/T	10	1530/1500	2	10	1090/0	100			
22	B/T/P							7	586/29	95.1
23	T/P							7	393/143	63.6

MP - management practice, B - breeding, T - training, P - pleasure, TH - treated horses in each treatment group in each farm, PrT/PoT - pre-/post- treatment for each horse group decrease of EPG. In spring 2009, mebendazol was administered in the unsuitable form of granulate mixed with feed; using this administration there is no guarantee that the horses receive a sufficient dose. This is probably the most likely reason for athe enormous FECRT decrease (from 70.8 to 18.9%) at this farm. The reason for the FECRT decrease (from 70.5 to 15.2%) at farm No. 1 was most likely due to the use of the same class of drugs (BZ), as they were alternating fenbendazol and mebendazol. It is generally accepted that the frequent use of the same class of anthelmintics is responsible for the development of resistance. Historically, for more than 30 years Czech horse owners and veterinarians have almost exclusively treated horses with benzimidazole anthelmintics.

In conclusion, the results of this study suggest that benzimidazole resistant cyathostomins are widespread in horses in the Czech Republic, but that IVM is still fully effective. Reports from other European countries (Wirtherle et al. 2004; Osterman-Lind et al. 2007; Travesa et al. 2007) do not indicate that IVM resistance has developed in the investigated horse population. Nevertheless, a recent study in Germany showed a shorter egg-reappearance period (von Samson-Himmelstjerna et al. 2007), which may be a first evidence of IVM resistance in cyathostomins. One recent study from Kentucky showed reduced activity of moxidectin and ivermectin on small strongyles in young horses (Lyons et al. 2008). Annual monitoring of anthelmintic efficacy should therefore be part of all health programs in horses.

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