Article

The persistence of benzimidazole-resistant cyathostomes on horse farms in Ontario over 10 years and the effectiveness of ivermectin and moxidectin against these resistant strains

J. Owen D. Slocombe, John F. Coté, Rolph V.G. de Gannes

Abstract — Three clinical trials with fecal egg count reduction tests and coproculture were conducted on 2 standardbred farms in Ontario. On Farm A, the treatment groups were mebendazole and ivermectin in trial 1, and fenbendazole and moxidectin in another. On Farm B, treatment groups were mebendazole and ivermectin. All horses treated with mebendazole or fenbendazole were subsequently treated with ivermectin or moxidectin. Strongyle eggs/g feces were estimated pre- and post-treatment using the Cornell-McMaster dilution and Cornell-Wisconsin centrifugal flotation techniques. After treatment, there was no change in the arithmetic mean eggs/g feces for horses given mebendazole, and a reduction of only 49.1% for those given fenbendazole. All horses receiving ivermectin or moxidectin had their egg counts reduced to 0. Only cyathostomes were found on culture. On both farms the benzimidazole resistant strains appeared to have persisted for at least 10 years. Development of and monitoring for anthelmintic resistance are briefly discussed.

Résumé — Persistance sur plus de 10 ans de cyathostomes résistants aux benzimidazoles dans des fermes équestres de l'Ontario et efficacité de l'ivermectine et de la moxidectine contre ces souches résistantes. Trois essais cliniques comportant des tests de réduction d'excrétion fécale d'œufs et de coproculture ont été menés dans 2 fermes de Standardbred de l'Ontario. Sur la ferme A, on retrouvait les groupes de traitement mébendazole et ivermectine dans l'essai 1 et les groupes fenbendazole et moxidectine dans l'essai 2. Sur la ferme B, les groupes de traitement étaient mébendazole et ivermectine. Tous les chevaux traités au mébendazole ou au fenbendazole ont été traités subséquemment à l'ivermectine ou à la moxidectine. Les œufs de strongles/g de fèces ont été estimés avant et après traitement par les techniques Cornell-McMaster de dilution et Cornell-Wisconsin de flottaison par centrifugation. Après traitement, il n'y avait pas de modification dans la moyenne arithmétique du nombre d'œufs/g de fèces chez les chevaux ayant reçu du mébendazole et une réduction d'à peine 49,1 % pour ceux ayant reçu du fenbendazole. Tous les chevaux ayant reçu de l'ivermectine ou de la moxidectine ont vu les comptes d'œufs réduits à 0. Seul des cyathostomes ont été retrouvés dans les cultures. Dans les 2 fermes, les souches résistantes aux benzimidazoles semblaient avoir persisté pendant au moins 10 ans. L'apparition et la surveillance de la résistance aux anthelminthiques sont brièvement présentées.

(Traduit par Docteur André Blouin)

Can Vet J 2007;48:56–60

Introduction

Anthelmintic resistant cyathostomes are increasingly being reported worldwide (1), but there are few reports for Canada (2–5). Two of these studies were in southwestern Ontario on 2 standardbred farms with cyathostomes resistant to mebendazole on one farm (2), and mebendazole and oxibendazole on the other (3). The aims of the present trials were to determine 1) if, on those farms, benzimidazole-resistant cyathostomes were present 10 y after they were first found, and 2) if the macrocyclic lactones, ivermectin and moxidectin, would be effective for the resistant nematodes. Some information from that study on the effectiveness of moxidectin has already been reported (6).

Materials and methods

Three clinical trials using fecal egg count reduction tests (FECRT) and coproculture were conducted on 2 farms with 54 standardbred horses: 49 mares, 3 stallions, and 2 geldings. In

Ontario Veterinary College, Department of Pathobiology (Slocombe), Department of Clinical Studies (Coté), University of Guelph, Guelph, Ontario N1G 2W1; Equine Veterinary Services, 191 Western Avenue, Schomberg, Ontario L0G 1T0 (de Gannes).

Address all correspondence and reprint requests to Dr. Owen Slocombe; e-mail: oslocomb@uoguelph.ca

This study was funded by MSD AGVET (now Merial Canada), Montreal, Quebec and Ayerst Veterinary Laboratories (now Wyeth Animal Health), Guelph, Ontario.

Paper accepted for publication in February 2007.

Table 1. Pre- and post-treatment number of strongyle eggs/g feces in horses on Farm A treated with 1 dose orally of mebendazole or ivermectin on Day 0. Horses treated with mebendazole were subsequently treated with ivermectin

Horse ID	Day -1	Day 14	Day 35
Mebendazole on Day 0 and Ivermectin on Day 17			
2	1650	750	0
4	950	650	0
6	450	1300	0
7	400	300	0
9	250	300	0
11	150	200	0
14	100	29.8	0
16	50	350	0
18	16.6	100	0
20	7.8	6.4	0
23	4.6	24.6	0
24	2.8	1.8	0
25	1.2	1.4	0
28	0.6	3.4	0
Ivermectin on Day 0			
1	1700	0	ND[a]
3	1300	0	ND
5	600	0	ND
8	250	0	ND
10	150	0	ND
12	150	0	ND
13	100	0	ND
15	50	0	ND
17	50	0	ND
19	8.2	0	ND
21	7.6	0	ND
22	5.8	0	ND
26	1.0	0	ND
27	0.8	0	ND

[a] ND = Not Done

Table 2. Pre- and post-treatment number of strongyle eggs/g feces in horses on Farm A treated with 1 dose orally of fenbendazole or moxidectin on Day 0. Horses treated with fenbendazole were subsequently treated with ivermectin

Horse ID	Day -3	Day 10	Day 25
Fenbendazole on Day 0 and Moxidectin on Day 12			
29	1150	500	0
32	200	14	0
33	150	32	0
35	80	52.2	0
37	51.8	200	0
40	32.8	13.2	0
42	12.8	7.2	0
44	5.6	3	0
45	5	3.4	0
47	2.2	1.2	0
Moxidectin on Day 0			
30	850	0	ND[a]
31	500	0	ND
34	80	0	ND
36	63.2	0	ND
38	37.6	0	ND
39	37.2	0	ND
41	30.2	0	ND
43	12.2	0	ND
46	4.2	0	ND
48	1.2	0	ND

[a] ND = Not Done

each trial, fecal samples were collected from each horse prior to treatment (PRT) and post-treatment (POT) to assess the number of strongyle eggs in feces, as detailed below. The Cornell-McMaster dilution technique (7), which is more efficient when egg counts are high, and the Cornell-Wisconsin centrifugal flotation technique (8), which is more efficient when the counts are low, were used to recover and identify the parasite eggs, as described in the following text. Efficacy of treatment for each horse was determined as follows: % efficacy = 100 × PRT-POT/PRT. The weight of a horse was estimated with a weigh tape. Prior to treatment, the mouth of each horse was examined for food, which, if present, was removed. Horses were maintained on pasture but brought in daily for a period and fed a grain ration. The studies were approved by the University of Guelph Animal Care Committee in accordance with the guidelines of the Canadian Council of Animal Care.

Farm A

Benzimidazole resistance in December 1985/January 1986

There were 27 mares and 1 stallion, weighing 409–569 kg, in 2 treatment groups: mebendazole paste (Telmin; Pitman Moore, marketed by Moore, Thomson, Clinger, Mississauga, Ontario), 8.8 mg/kg body weight (BW), PO, or ivermectin paste (MSD AGVET, now Merial Canada, Montreal, Quebec), 0.2 mg/kg BW, PO. Fecal samples were taken on Day -1, Day 14, and Day 35, with Day 0 as the day of treatment. Procedures for estimation of strongyle eggs/g feces (epg) with the Cornell-McMaster technique, coproculture, and randomization of horses into treatment groups have been described previously (3). On Day 17, mebendazole-treated horses were given ivermectin. All horses had been in a previous study (3), where they had been given either 1 treatment with mebendazole paste and 2 with mebendazole suspension (3), or 1 treatment with mebendazole paste and 2 with oxibendazole suspension (Anthelcide; Norden Laboratories, Mississauga, Ontario). At the end of that trial, all horses had strongyle eggs in the feces and the epgs at the end of that study were the PRT count for the present study. Horses previously treated with only mebendazole were randomized in the present study into the 2 treatment groups separate from those treated with mebendazole and oxibendazole.

Benzimidazole resistance in November 1997

There were 19 mares and 1 gelding, none of which were in the ivermectin trial described previously. The horses weighed 466–659 kg and were in 2 treatment groups: fenbendazole paste (Panacur; Hoechst Roussel Vet, now Intervet Canada, Whitby, Ontario), 5 mg/kg BW, PO, or moxidectin gel (Quest; Ayerst Veterinary Laboratories, now Wyeth Animal Health, Guelph, Ontario), 0.4 mg/kg BW, PO. Procedures for estimation of strongyle epgs with the Cornell-Wisconsin technique, coproculture, and randomization of horses into treatment groups have been described previously (6).

Farm B

Benzimidazole resistance in February 1986

There were 3 mares, 2 stallions, and 1 gelding, weighing 250–568 kg, in 2 treatment groups: mebendazole paste,

Table 3. Pre- and post-treatment number of strongyle eggs/g feces in horses on Farm B treated with 1 dose orally of mebendazole or ivermectin on Day 0. Horses treated with mebendazole were subsequently treated with ivermectin

Horse ID	Day -1	Day 14	Day 35
Mebendazole on Day 0 and Ivermectin on Day 17			
	550	1050	0
	150	36	0
	0.6	0.4	0
Ivermectin on Day 0			
	550	0	ND[a]
	400	0	ND
	0.8	0	ND

[a] ND = Not Done

8.8 mg/kg BW, PO, or ivermectin paste, 0.2 mg/kg BW, PO. Fecal samples were taken on Day -1, Day 14, and Day 35. Procedures for estimation of strongyle epgs with the Cornell-McMaster technique, coproculture, and randomization of horses into treatment groups have been described previously (3).

Results

On both farms, all horses accepted the treatments readily and adverse reactions were not observed. On both farms, the primary parasite egg found was the strongyle egg, and all PRT counts and the POT counts from horses treated with mebendazole or fenbendazole had 100% cyathostomes. No strongyle larvae were recovered POT from horses treated with ivermectin or moxidectin. On Farm A in 1997, a number of horses were found also to have *Anoplocephala perfoliata* eggs and 1 had *Strongyloides westeri* eggs.

Farm A

The strongyle epgs for the 2 trials are shown in Tables 1 and 2. In the 1st trial, the arithmetic mean epgs on Days -1 and 14 for mebendazole-treated horses were 288.1 and 287.0, respectively, and for ivermectin-treated horses 312.4 and 0, respectively. On Day 35, all horses that had been treated with mebendazole and then given ivermectin had 0 epg. In the 2nd trial, the arithmetic mean epgs on Days -3 and 10 for fenbendazole-treated horses were 169.0 and 82.6, respectively, and for moxidectin-treated horses 161.6 and 0, respectively. On Day 25, all horses that had been treated with fenbendazole and then given moxidectin had their strongyle epg reduced to 0.

Farm B

The strongyle epgs are shown in Table 3. The arithmetic mean epgs on Days -1 and 14 for mebendazole-treated horses were 233.5 and 362.1, respectively, and for ivermectin-treated horses 316.9 and 0, respectively. On Day 35, all horses that had been treated with mebendazole and then given ivermectin had 0 epg.

Discussion

Ivermectin and moxidectin were highly effective against the benzimidazole-resistant cyathostomes in these trials, as has been shown in several other studies (1). In Canada, there is only 1 other report on the effectiveness of ivermectin (4), and the present report is the 1st for moxidectin. The cyathostomes are the primary parasites in horses and anthelmintic resistant cyathostomes are increasing worldwide (1,9). In Canada, cyathostome resistance was 1st reported in 1977 (2), having been found on Farm B in 1976 with resistance to mebendazole and side resistance to cambendazole, anthelmintics which are no longer in the marketplace. The owners were advised on the benzimidazole resistance, and claimed not to have used benzimidazoles in their horses since that time. The herd on Farm B was relatively stable and closed, and all horses were born on the farm. One of the downsides for development of resistance on a farm is that the resistance may never go away (1). If the owners' claim is correct, these strains apparently can persist on a farm in Canada for at least 10 y. On Farm A, side resistance with mebendazole and oxibendazole was found in 1985 (3). After that time and despite warnings to the owner, benzimidazoles were used on the farm and allowed for the persistence of benzimidazole-resistant strains 12 y later. The herd was not closed, and the owner was also warned on the use of anthelmintics for horses entering the farm. The owner claims the recommendations were adhered to for horses entering as permanent members of the herd, but they were not followed for transients and those arriving for breeding and staying a few days to weeks.

The prevalence of benzimidazole-resistant cyathostomes in Canada is unknown, but in a small survey in 1982, 4 of 10 farms in Ontario had such resistance (Slocombe, unpublished observations). In a recent survey of 44 horse farms in southeastern USA, 97.7% of the farms were found with resistance to fenbendazole, 53.5% to oxibendazole, and 40.5% to pyrantel pamoate (9). The extent of cyathostome resistance to pyrantel in Canada is also unknown; there is only 1 report on this and it is for a thoroughbred farm in Ontario (5). However, with the considerable movement of horses throughout North America, the status for these resistant strains in parts of Canada may well be similar to that in southeastern USA. Where resistance to both benzimidazole and pyrantel is found on a farm, only 1 class of anthelmintics, the macrocyclic lactones ivermectin and moxidectin, can be used. There are no reports for cyathostome resistance to these compounds, but there are reports on *Parascaris equorum* in foals and weanlings with resistance to ivermectin and moxidectin (10–13).

Will cyathostomes develop resistance to macrocylic lactones? Prevalence of resistance to this class of drugs has increased in some parasites in sheep and cattle (14). Ivermectin for horses has been in the marketplace for over 20 y and moxidectin for close to 10 y, and many parasitologists consider development of cyathostome resistance to these drugs inevitable. There is also a debate on which macrocyclic lactone will be the first to stimulate resistance in the cyathostomes (15). Ivermectin is highly effective for the luminal stages, but ineffective for mucosal stages, which, numerically, are sometimes far in excess of the luminal stages. This large mucosal population could slow development to resistant strains. Moxidectin with its high efficacy for mucosal and luminal stages may stimulate resistance faster. However, moxidectin, because of its increased efficacy, has a long egg reappearance period. Moxidectin, therefore, can be used less frequently than ivermectin, and with its infrequent use, rapid

development of resistant strains may be prevented. No consensus has emerged from this debate. But obviously considerable effort must be directed to conserve and preserve these and all other anthelmintics.

Kaplan's (1) review on anthelmintic resistance in nematodes of horses has an extensive discussion on the reasons for the development of resistance and how to minimize and monitor it. Practitioners need to be aware of all these concerns when devising a control program. A few factors that influence development of resistance are briefly identified here. One is the suboptimal dose, so horses need to be weighed by using a weigh scale wherever possible and treated to weight. As in the present study, access to a weigh scale is not always possible, but the use of a weigh tape is reasonably effective. Carroll and Huntington (16) reported a correlation of 0.90 between weight and measurements of heart girth × length, and provide a nomogram to use those measurements to estimate body weight. A 2nd factor that influences development of resistance is frequent use of anthelmintics, so practitioners should medicate less often, using sound epidemiologic principles. Most horses are dewormed too frequently. A spring-summer strategy has been devised in Ontario for mares (maximum of 3 treatments annually) and yearlings (4 treatments annually) (17). A 3rd factor is the treatment of all the animals on a farm. In a grazing flock of sheep, few sheep harbor most of the nematodes and pass most of the parasite eggs (18), and in horses, there is some evidence for this over dispersion (19,20). Therefore, egg counts must be monitored and only those horses with high strongyle egg counts should be treated. Resistant strongyles passed by those fewer treated horses would be diluted out by susceptible strongyles passed by the larger number of untreated horses with low egg counts. Hamlen-Gomez and Georgi (19) selectively dewormed only horses with > 100 epg, and this resulted in low levels of contamination on the farm, with significant cost savings over routine deworming of all horses. What epg would signal a need to treat a horse is unknown, but 5 experts have recommended between 200–500 strongyle epg (21), and 1 expert documented that prevalence of colic markedly decreased when the counts were below 200 epg (21). Selective therapy was used in a 3-year study where only mature horses with over 200 epg were treated, and following treatment, egg counts that subsequently exceeded 200 were found only in treated horses (22). No comments were made about the health status of the horses. The 1st author in the present study has no "cut-off" because 1) there is no consistent body of evidence relating levels of epg with health status or performance in horses, and 2) a number of factors influence the epg level in horses (1), including annual seasonal fluctuations (23–26). In Canada, mean strongyle egg counts in untreated pony mares were highest in late spring to early summer, had a secondary peak in late summer to early fall, and were lowest over winter (26). The age of a horse will also be an influence; yearlings appear to require more treatment than mares (17,27). Practitioners need to consider all pertinent factors in determining that "cut-off."

Practitioners also need to determine if anthelmintic resistance is present on a farm before instituting a parasite control program. The FECRT, despite its deficiencies, is the most practical approach to assessing strongyle resistance in horses. Kaplan (1) discusses criteria for interpreting epgs and resistance to various anthelmintics. Further, practitioners should use the FECRT at least annually to monitor any program they institute on a farm. The broad spectrum paste formulation of anthelmintics in syringes with ready availability to horse owners was first released in the early 1970s, and at that time, unfortunately, practitioners "backed off" use of fecal analysis techniques. Horse owners continued to use these products for years without monitoring (2), only to recognize subsequently the loss of dollars spent as the products became ineffective due to development of resistance. That lack of monitoring of fecal egg counts continues today and this, together with the use of ineffective anthelmintics where there are resistant strains, may be a factor in the significant increase in the number of cases of larval cyathostomiois, some leading to death, recently reported for Ontario (28).

Where strongyle resistance to pyrantel pamoate exists and when the medication is used routinely primarily for control of tapeworms at 2× the label dose, there should be a reassessment on whether horses on the farm are infected with tapeworms. If a horse is negative for tapeworms, there could be less use of pyrantel pamoate; if it is positive, praziquantel would be the treatment of choice. However, identifying a horse with *Anoplocephala perfoliata* by fecal flotation techniques is difficult. Slocombe (29,30) and Slocombe et al (31) demonstrated how such infected horses can be identified with the Cornell-Wisconsin centrifugal flotation technique, and with fecal samples taken 18–24 h after horses had been treated with the cestocidal drugs pyrantel pamoate or praziquantel. At those times the sensitivity of the test is 94% to 100% and its specificity is 100%. CVJ

References

1. Kaplan RM. Anthelmintic resistance in nematodes of horses. Vet Res 2002;33:491–507.
2. Slocombe JOD, Cote JF. Small strongyles of horses with cross resistance to benzimidazole anthelmintics and susceptibility to unrelated compounds. Can Vet J 1977;18:212–217.
3. Slocombe JOD, Cote JF, McMillan I. Effectiveness of oxibendazole against benzimidazole-resistant strongyles in horses. Can Vet J 1989; 30:663–665.
4. Piché CA, Kennedy MJ, Buck S. Benzimidazole resistance in horses in western Canada. Can Vet J 1989;30:173–174.
5. Slocombe JOD, de Gannes RVG. Cyathostomes in horses in Canada resistant to pyrantel salts and effectively removed by moxidectin. Vet Parasitol 2006;140:181–184.
6. Slocombe JOD, de Gannes RVG. Effectiveness of moxidectin for benzimidazole and pyrantel resistant cyathostomes in horses. (Abstract) Proc 43rd Annu Meet Am Assoc Vet Parasitol 1998:53.
7. Georgi JR. Parasitology for Veterinarians 4th ed. Toronto: WB Saunders, 1985:258–276.
8. Egwang T, Slocombe JOD. Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. Can J Comp Med 1982;46:133–137.
9. Kaplan RM, Klei TK, Lyons ET, et al. Prevalence of anthelmintic resistant cyathostomes on horse farms. J Am Vet Med Assoc 2004;225: 903–910.
10. Boersema JH, Eysker M, Nas JWM. Apparent resistance of *Parascaris equorum* to macrocyclic lactones. Vet Rec 2002;150:279–281.
11. Hearn FPD, Peregrine AS. Identification of foals infected with *Parascaris equorum* apparently resistant to ivermectin. J Am Vet Med Assoc 2003;223:482–485.
12. Lyons ET, Tolliver SC, Collins SS. Field studies on endoparasites of Thoroughbred foals on seven farms in central Kentucky in 2004. Parasitol Res 2006;98:496–500.

13. Slocombe JOD, de Gannes RVG, Lake MC. Macrocyclic lactone-resistant *Parascaris equorum* on stud farms in Canada and effectiveness of fenbendazole and pyrantel pamoate. Vet Parasitol 2007 (In Press — Accepted).
14. Waller R. Anthelmintic resistance. Vet Parasitol 1997;72:391–412.
15. Sangster NC. Pharmacology of anthelmintic resistance in cyathostomes: Will it occur with the avermectins/milbemycins? Vet Parasitol 1999;85:189–204.
16. Carroll CL, Huntington PJ. Body condition scoring and weight estimation of horses. Equine Vet J 1988;20:41–45.
17. Slocombe JOD. Anthelmintic resistance in strongyles of equids. In: Plowright W, Rossdale PD, Wade JF, eds. Equine Infect Dis, 6th Int Conf, Cambridge Univ Pr, UK. 1991:137–143.
18. Sreter T, Molnar V, Kassai T. The distribution of nematode egg counts and larval counts in grazing sheep and their implications for parasite control. Int J Parasitol 1994;24:103–108.
19. Hamlen-Gomez H, Georgi JR. Equine helminth infections: Control by selective chemotherapy. Equine Vet J 1991;23:198–200.
20. Doopfer D, Kerssens CM, Meijer YGM, Boersema JH, Eyseker M. Shedding consistency of strongyle-type eggs in Dutch boarding horses. Vet Parasitol 2004;124:249–258.
21. Uhlinger CA. Uses of fecal egg count data in equine practice. Compend Contin Educ Pract Vet 1993;15:742–748.
22. Nielsen MK, Haaning N, Olsen SN. Strongyle egg shedding consistency in horses on farms using selective therapy in Denmark. Vet Parasitol 2006;135:333–335.
23. Poynter D. Seasonal fluctuations in the number of strongyle eggs passed by horses. Vet Rec 1954;66:74–78.
24. Duncan JL. Field studies on the epidemiology of mixed strongyle infection in the horse. Vet Rec 1974;94:337–345.
25. Herd RP, Willardson KL, Gabel AA. Epidemiological approach in the control of horse strongyles. Equine Vet J 1985;17:202–207.
26. Slocombe JOD, Valenzuela J, Lake MC. Epidemiology of strongyles in ponies in Ontario. Can J Vet Res 1989;51:470–474.
27. Herd RP, Gabel AA. Reduced efficacy of anthelmintics in young compared with adult horses. Equine Vet J 1990;22:164–169.
28. Peregrine AS, McEwen B, Weese S. Larval cyathostomiasis: An emerging disease in Ontario horses. Proc Vet Med Assoc Conf 2003:281–283.
29. Slocombe JOD. A modified critical test for the efficacy of pyrantel pamoate for *Anoplocephala perfoliata* in equids. Can J Vet Res 2004;68:112–117.
30. Slocombe JOD. A modified critical test and its use in two dose titration trials to assess efficacy of praziquantel for *Anoplocephala perfoliata* in equids. Vet Parasitol 2006;136:127–135.
31. Slocombe JOD, Heine J, Barutzki D, Slacek B. Clinical trials of efficacy of praziquantel horse paste 9% against tapeworms and its safety in horses. Vet Parasitol 2007;144:366–370.