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Short communication

Macrocyclic lactone-resistant *Parascaris equorum* on stud farms in Canada and effectiveness of fenbendazole and pyrantel pamoate

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Abstract

The aims of studies in 2002 and 2003 on three farms with 76 foals naturally infected with *Parascaris equorum* were to (i) identify if the nematode was resistant to ivermectin and moxidectin, and (ii) confirm the effectiveness of fenbendazole and pyrantel pamoate for the parasite. Twelve clinical trials, each with a Fecal Egg Count Reduction Test, were conducted on two Thoroughbred and one Standardbred farms in southwestern Ontario, Canada. In each trial, *Parascaris* eggs/g feces were estimated for each foal pre- and post-treatment using the Cornell-Wisconsin double flotation and Cornell-McMaster dilution techniques. On each farm and for each trial, foals were randomized into treatment groups. Treatments were ivermectin, moxidectin, fenbendazole, pyrantel pamoate administered at the manufacturers' recommended dosages, and some foals were untreated. The overall efficacy for ivermectin was 33.5% (19 foals) and for moxidectin 47.2% (28 foals). Fenbendazole (16 foals) and pyrantel pamoate (21 foals) were highly effective for *P. equorum* each at 97.6%. For fenbendazole, 15 foals had 100% and for pyrantel pamoate 17 foals had >97% with 14 at 100%.
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1. Introduction

In October 2001, a practitioner (the second author, de Gannes) was alerted to an apparent failure of ivermectin to remove *Parascaris equorum* from foals and weanlings on a Thoroughbred farm in southwestern Ontario, Canada. In a preliminary trial, two weanlings on that farm were then treated with ivermectin and two were untreated and from a comparison of their pre- and post-treatment egg counts there appeared to be some resistance by *Parascaris* to the anthelmintic. The present report documents further trials to determine (1) if there was resistance to macrocyclic lactones (specifically ivermectin and moxidectin) by *P. equorum* on that farm and on two others in the area, and (2) the effectiveness of fenbendazole and pyrantel pamoate to remove the nematode. Resistance of *P. equorum* to ivermectin and moxidectin has been found in the Netherlands (Boersema et al., 2002) and to ivermectin in Canada (Hearn and Peregrine, 2003), and some of the information from the trials being reported here has been presented (Slocombe et al., 2003, 2004).

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2. Materials and methods

Twelve clinical trials were conducted with foals during August–November 2002 and August–September 2003. The foals were naturally infected with *P. equorum* and on three stud farms (designated Thoroughbred A, Thoroughbred B and Standardbred) in southwestern Ontario. There were five clinical trials on Thoroughbred A, four on Thoroughbred B and three on the Standardbred.

2.1. Foals

On each farm in each year, foals were entered into a trial as they were made available by the management of the farm and when they were positive for *P. equorum* on fecal analysis. A total of 76 foals were used in the trials; 43 in 2002 and 33 in 2003. In 2002 and 2003 and prior to a trial, foals had been given their 2-month anthelmintic treatment as customary for the farm. In addition in 2002 on the Standardbred, many foals had also been given their 4-month treatment. After a foal entered a trial, the customary anthelmintic treatment for the farm ceased. Ages of foals in the trials ranged from 3 to 8 months. In 2002 and 2003 on Thoroughbred A and B, foals entered the trial with mean ages ranging from 4.5 to 5.25 months. In 2002 on the Standardbred, the mean age of foals entering the trial was 6.25 months and in 2003, 4 months. On all farms, a foal's weight was measured with a weigh band and its weight was used to calculate the dosage for an anthelmintic. Weights of foals in these trials ranged from 150 to 285 kg. On the day of a trial (Day 0) and prior to a single oral administration of an anthelmintic, the mouth of a foal was examined for food and if present removed.

2.2. Fecal sampling and analysis

Fecal samples were taken from each foal 2–3 days pre-treatment (PRT) and 12–18 days post-treatment (POT). A fecal sample from each foal was examined first with the Cornell-Wisconsin centrifugal flotation technique (Egwang and Slocombe, 1982) and if required another sample was examined with the Cornell-McMaster dilution technique (Georgi, 1985). *Parascaris* eggs were counted and the eggs/g feces (epg) were calculated as described previously (Slocombe and de Gannes, 2006). Other parasite eggs observed in PRT and POT fecal samples were also identified and counted.

2.3. Treatments

Only the PRT *Parascaris* epgs were used to randomize foals to treatment groups and the procedure for the randomization has been described (Slocombe and de Gannes, 2006). In four of the 12 trials, the POT epg was used as the PRT epg for the next trial. The treatment groups were as follows: ivermectin paste at 0.2 mg/kg body weight (BW), moxidectin gel at 0.4 mg/kg BW, fenbendazole paste at 10 mg/kg BW, pyrantel pamoate paste at 6.6 mg pyrantel base/kg BW and no treatment. The Fecal Egg Count Reduction Test was used in each trial and the efficacy of a treatment for each foal was determined as follows: efficacy (%) = $100 \times ((\text{PRT} - \text{POT})/\text{PRT})$.

2.4. Farm management

The established practices for mares and foals on the farms were as follows.

2.4.1. Thoroughbred A and B

Mares foaled indoors or outdoors and foals were maintained with their dams outdoors as much as possible in paddocks or pastures and brought in daily to be fed a grain ration. Foals were weaned at 17–18 weeks of age and, thereafter, were always outdoors on pasture and brought in daily to be fed a grain ration. Foals were treated at 2 and 4 months of age with ivermectin paste (Eqvalan; Merial Canada Inc., Montreal, Quebec, Canada), and at 8 months ivermectin paste or moxidectin gel (Quest; Wyeth Animal Health, Guelph, Ontario, Canada). Foals were treated at 6 months of age with twice the label dose of pyrantel pamoate paste (Strongid P; Pfizer Canada, Montreal, Quebec, Canada). Mares were treated with ivermectin paste in April, July and in late November to early December, and with twice the label dose of pyrantel pamoate paste in mid-September.

2.4.2. Standardbred

Mares foaled indoors and when foals were a few days old they and their dams were allowed outdoors in a paddock daily for a short period that gradually increased as foals aged. When the foals were about 2 months of age they and their dams were mostly outdoors with grain fed on pasture. Foals, regardless of age, were weaned during October and weanlings remained outdoors and were fed grain on pasture. Foals were treated at 2 and 4 months of age with pyrantel pamoate paste at twice the label dose, at 6 months fenbendazole paste (Panacur; Intervet Canada, Whitby, Ontario, Canada) and at 8 months ivermectin paste. Mares were treated with moxidectin gel in April, ivermectin paste in August and in late November to early December, and pyrantel pamoate paste at twice the label dose in mid-September.

3. Results

The epgs for *P. equorum* in five clinical trials on Thoroughbred A, four on Thoroughbred B and three on the Standardbred are shown in Tables 1–6. Other parasites found were *Strongyloides westeri* (six foals), *Eimeria leuckarti* (nine foals) and *Anoplocephala perfoliata* (two foals). Strongyle eggs were found in some foals on all farms. In 2002 on the Standardbred and 2003 on Thoroughbred A, the majority of foals had strongyle eggs. Strongyles were not found in POT samples from foals treated with ivermectin or moxidectin. But strongyle eggs were found in POT samples from foals on all farms treated with pyrantel pamoate, and from foals on the Standardbred farm treated with fenbendazole. The strongyle POT epgs for these pyrantel pamoate and fenbendazole treated foals were similar to or higher than their PRT epgs.

Table 1
Pre- and post-treatment number of *Parascaris* eggs/g feces in three trials in 2002 in foals on Thoroughbred A farm treated orally with ivermectin, moxidectin, fenbendazole or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Ivermectin	2	5.4	96.6	0
Untreated	1	7.2	39.8	0
Trial 2				
Ivermectin	3	400	38.2	90.4
	4	53.6	300	0
	6	17	35.6	0
Moxidectin	2	61.2	21.6	64.7
	5	21.4	47.2	0
	7	12.2	0	100
Untreated[a]	8	0	1100	0
	9	0	400	0
	10	0	400	0
	11	0	150	0
	12	0	68.5	0
	13	0	1.2	0
Trial 3				
Moxidectin	8	1100	3050	0
	4	300	600	0
	12	68.5	1100	0
	3	38.2	0	100
	2	21.6	0	100
	13	1.2	27.8	0
Fenbendazole	9	400	0	100
	10	400	0	100
	11	150	0	100
	5	47.2	0	100
	6	35.6	0	100
	1	3.2	0	100

[a] In Trial 2, foals 8–13 were not used in the assignment of foals to treatment blocks or for randomization to treatment groups.

4. Discussion and conclusion

Resistance of *P. equorum* to ivermectin and moxidectin appeared to be present on Thoroughbred A farm in 2002 and 2003 and Thoroughbred B farm in 2002, and to moxidectin on Thoroughbred B farm in 2003. On the Standardbred farm in 2002, four of seven foals had a significant decrease in epg following treatment with moxidectin. But there was also a marked reduction in epgs in three of five untreated foals. On that farm in 2002, the foals were generally older than foals on the other two farms and the reduction in the POT epgs could have resulted from natural age-related

Table 2
Pre- and post-treatment number of *Parascaris* eggs/g feces in three trials in 2002 in foals on Thoroughbred B farm treated orally with ivermectin, moxidectin, fenbendazole or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Ivermectin	4	36	47.2	0
	5	4.2	11	0
Untreated	2	45.8	46.8	0
	3	30	27	10
Trial 2				
Ivermectin	6	3350	3300	1.5
	8	300	25.8	91.4
	10	72.8	14.8	79.7
	2	46.2	25.6	44.6
	5	3.2	1.4	56.2
Moxidectin	9	750	300	60
	4	57.2	59.8	0
	12	32.4	0	100
	13	0.6	0	100
Untreated	7	2400	3300	0
	11	59.6	44.6	25
Trial 3				
Moxidectin	6	3300	2750	16.7
	3	400	25.2	93.7
	11	44.6	1150	0
	2	25.6	29.4	0
	9	23.6	0	100
Fenbendazole	7	3300	0	100
	4	59.8	23.4	60.9
	1	50	0	100
	8	25.8	0	100
	10	14.8	0	100
	5	1.4	0	100

Table 3
Pre- and post-treatment number of *Parascaris* eggs/g feces in two trials in 2002 in foals on Standardbred farm treated orally with moxidectin, fenbendazole, pyrantel pamoate or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Moxidectin	2	2500	78.8	96.8
	6	500	3.4	99.3
	11	12.2	1	91.8
	14	0.8	1.6	0
Fenbendazole	1	3100	0	100
	8	150	0	100
	12	10.8	0	100
	13	1	0	100
Pyrantel pamoate	4	1400	31.2	97.8
	5	850	0	100
	9	17.2	0	100
	15	0.8	0	100
Untreated	3	1950	1300	33.3
	7	400	68.8	82.8
	10	14.2	5.2	63.4
Trial 2				
Moxidectin	3	1300	2000	0
	6	3.4	0	100
	11	1	29.2	0
Pyrantel pamoate	7	68.8	0	100
	10	5.2	0	100
	14	1.8	0	100
Untreated	16	98.8	0	100
	17	31.2	19.4	37.8

elimination of the parasites. In 2003, younger foals were used on the Standardbred farm and there appeared to be resistance to ivermectin. Efficacy over all farms for the 2 years of trials for ivermectin (19 foals) was 33.5% and moxidectin (21 foals) 47.2%. Fenbendazole (16 foals) and pyrantel pamoate (21 foals) were highly effective against *P. equorum* each with an efficacy of 97.6%. For fenbendazole, 15 foals had 100% efficacy and for pyrantel pamoate 17 foals had >97% with 14 at 100%. No adverse affects were seen in any foal with any anthelmintic.

All farms had used ivermectin in foals, weanlings, yearlings, mares and stallions almost since its inception in the marketplace some 20 years earlier. In the trials, 12 of 47 foals treated with a macrocyclic lactone had a *Parascaris* POT epg of or close to zero and with excellent efficacy for the anthelmintic. There was, therefore, a great range in efficacy for ivermectin and moxidectin against *P. equorum* and why this occurred we were unable to determine.

Table 4
Pre- and post-treatment number of *Parascaris* eggs/g feces in two trials in 2003 in foals on Thoroughbred A farm treated orally with ivermectin, moxidectin, pyrantel pamoate or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Ivermectin	1	1400	350	75
	6	200	0	100
	7	26.8	1050	0
	10	11.8	37.6	0
Pyrantel pamoate	2	1150	0	100
	4	350	0	100
	9	12.2	0	100
	11	2.8	0	100
Untreated	3	350	950	0
	5	250	33.6	86.4
	8	15	250	0
	12	2.4	13.2	0
Trial 2				
Moxidectin	3	950	0	100
	4	29.4	150	0
	15	8.6	4000	0
Pyrantel pamoate	7	1050	5.8	99.4
	8	250	0	100
	12	13.2	0	100
Untreated	13	550	1550	0
	1	350	500	0
	19	37.6	0	100

Our findings that *P. equorum* appeared to be resistant to ivermectin have now been supported (Kaplan et al., 2006). For that study, and from Thoroughbred A farm in 2004 we collected *P. equorum* eggs from the feces of

Table 5
Pre- and post-treatment number of *Parascaris* eggs/g feces in one trial in 2003 in foals on Thoroughbred B farm treated orally with pyrantel pamoate or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Pyrantel pamoate	1	150	7.8	94.8
	4	31.8	8.2	74.2
	6	20.2	2.6	87.1
Untreated	2	150	58.4	61.1
	3	35.6	95.4	0
	5	29.6	16.4	44.6

Table 6
Pre- and post-treatment number of *Parascaris* eggs/g feces in one trial in 2003 in foals on Standardbred farm treated orally with ivermectin, pyrantel pamoate or untreated and percentage efficacy of the treatment

Treatment	Foal ID	Pre-treatment epg	Post-treatment epg	Efficacy (%)
Trial 1				
Ivermectin	2	2150	65.8	96.9
	4	900	1400	0
	8	75.6	2350	0
	11	0.4	56	0
Pyrantel pamoate	1	2350	96	95.9
	6	178.8	0	100
	7	135.2	0.4	99.7
	12	0.2	0	100
Untreated	3	900	400	55.6
	5	600	750	0
	9	50	1000	0
	10	19.8	2300	0

naturally infected foals. In a critical test, Kaplan et al. (2006) fed these eggs to uninfected foals and some of these foals were subsequently treated with ivermectin at 200 μg/kg BW. At necropsy, the *Parascaris* in the small intestine of the treated foals were similar in number to those in untreated controls confirming that *P. equorum* at least in foals on Thoroughbred A farm was resistant to ivermectin.

In the present studies 76 foals were used, but for any one trial only a small group of foals were available. The constraint of having only small groups of animals for clinical trials is generally recognized for the strongyles in horses, but that constraint is more acute with the roundworms. Each year there is a short span of time for scheduling trials. Foals may have a useful number of roundworm eggs in feces from only the fourth to eight month of age. Since foals are born over a period of several months, this further reduces the number of foals available at any one time for a trial. Owners of farms required all foals to be treated with an anthelmintic at 2 months of age. They were concerned that untreated 2-month old foals could develop subsequently large roundworm burdens and predispose them to reduced growth rates and impaction colic. After treatment there was the obvious delay until foals were positive for *Parascaris* on fecal analysis and could be used in a trial. For similar reasons, when the foals were positive owners were reluctant also to allow them to remain untreated to await a few more foals to become positive for a larger group for a trial. Because there were small numbers of foals for any one trial, we repeated essentially similar trials several times over the 2-year period to gain some insight into the problem on the farms.

Early reports of ivermectin's high efficacy for *P. equorum* (Egerton et al., 1981; Craig and Kunde, 1981) were challenged in the mid 1980s by many practitioners who found the anthelmintic less than effective in the field. This prompted further studies (DiPietro et al., 1988; Daurio and Leaning, 1989; Austin et al., 1991 and see earlier studies listed in those citations) that confirmed the high efficacy of ivermectin for adult and migrating larval stages. One of us (de Gannes) had been monitoring fecal egg counts in horses on several farms in Ontario consistently since the late 1970s and found no lack of efficacy for ivermectin until his observation in 2001. Moxidectin is also highly effective for *P. equorum* (Bauer et al., 1998 and see earlier studies listed in that citation).

There are several classes of anthelmintics that are effective for *P. equorum*, but anthelmintic resistance for this nematode had not been reported until recently. There are two reports on resistance to macrocyclic lactones; one in The Netherlands on a Trotter farm with resistance to ivermectin and moxidectin (Boersema et al., 2002), the other in southwestern Ontario, Canada on a Thoroughbred farm with resistance to ivermectin (Hearn and Peregrine, 2003). Boersema et al. (2002) found pyrantel pamoate effective for the macrocyclic lactone-resistant *P. equorum* and Hearn and Peregrine (2003) reported fenbendazole "eliminated" it. In both The Netherlands (Boersema et al., 2002) and Canadian studies (Hearn and Peregrine, 2003), ivermectin had been used more frequently in the foals than in the present study, but the egg counts observed in The Netherlands were much higher and in Canada much lower than in the present study. Hearn and Peregrine (2003) reported that one foal probably acquired the ivermectin-resistant *Parascaris* prior to its arrival on the farm. The authors suggested: "In light of the large number of foals shipped onto and off the farm, it is likely that *P. equorum* with decreased susceptibility to ivermectin also occur on other farms in North America". This may refer to our findings of apparent macrocyclic lactone-resistant *Parascaris* on other farms in southwestern Ontario. Hearn and Peregrine (2003) reported on results of fecal samples taken in September 2002. Prior to that time, both authors had been informed of our findings for the fall of 2001 and August 2002, and in January 2003 one of them had read a report prepared on our findings for 2002.

In the present trials, eggs for other parasites including the strongyles were found in fecal samples. Strongyle eggs were not found in the POT fecal samples

for foals treated with ivermectin or moxidectin, but were found in POT fecal samples from all foals treated with pyrantel pamoate and on the Standardbred farm from foals treated with fenbendazole. In Canada, strongyle resistance to pyrantel pamoate has been reported for Thoroughbred B farm (Slocombe and de Gannes, 2006) and to benzimidazoles for the Standardbred farm (Slocombe et al., 1989, in press). There are no other reports in Canada for pyrantel pamoate resistance to strogyles in horses, and only one other for benzimidazoles (Piche et al., 1989). In southeastern USA, strongyle resistance to pyrantel and benzimidazoles is prevalent (Kaplan et al., 2004) and the extent of this resistance in parts of Canada may well be similar.

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