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Short communication

Cyathostomes in horses in Canada resistant to pyrantel salts and effectively removed by moxidectin

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Abstract

Clinical trials using fecal egg count reduction tests and coproculture were conducted with yearlings and mares on a farm in 1997. Fecal samples were taken from each horse to estimate the number of strongyle eggs/g feces with Cornell–Wisconsin centrifugal flotation and Cornell–McMaster dilution techniques. Eleven of 15 yearlings, which had been on a daily feeding of grain with pyrantel tartrate for 66 d were found with strongyle eggs in feces. This was the first time the in-feed medication had been used on the farm. Nine yearlings were randomised into three groups; continuation of daily pyrantel tartrate or one treatment with pyrantel pamoate or moxidectin. Two of three yearlings given pyrantel tartrate or pamoate had no reduction in the eggs/g feces. These six yearlings were then given moxidectin and in all yearlings the eggs/g feces was reduced to zero. The 66 d of pyrantel tartrate use was an inadequate time for development of resistant cyathostomes and a hypothesis was the resistance was due to extensive use on the farm over many years of pyrantel pamoate at twice the label dose for control of tapeworms. That hypothesis was tested with 12 mares with strongyle eggs in the feces randomised into two treatment groups: pyrantel pamoate at label dose or moxidectin. Five of six mares given pyrantel had <80% reduction in egg/g feces. These mares were then given moxidectin and in all mares the eggs/g feces was reduced to zero. Only cyathostomes were found on culture and apparently there was side resistance among the pyrantel salts.

Keywords: Cyathostomes; Horses; Clinical trial; Pyrantel side resistance; Anthelmintic resistance; Moxidectin

1. Introduction

In June 1997, a Thoroughbred stud farm in Ontario, Canada instituted for the first time on the farm and with 15 yearlings a daily feeding of grain mixed with pyrantel tartrate/alfalfa/molasses pellets. After 60 d on the medicated feed 11 yearlings had strongyle eggs in the feces. This would have been inadequate time for development of strongyle resistance, but previously and for many years on that farm pyrantel pamoate paste was used frequently primarily for removal of tapeworms and may have caused development of that resistance. The study reported here was to determine if cyathostome resistance to pyrantel was present on the

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farm and if so to show that moxidectin gel could be effective in reducing the egg count. Cyathostome resistance to pyrantel occurs in Norway, Denmark, USA, United Kingdom (see review by Kaplan, 2002 and Kaplan et al., 2004) and some of the information from the present study has been reported for Canada (Slocombe and de Gannes, 1997).

2. Materials and methods

Two clinical trials were conducted on the farm one with yearlings and the other with mares and fecal samples were taken from the horses pre- and post-treatment for fecal egg count reduction tests (FECRT) and coproculture. For the FECRT, a 5-g fecal sample from each animal pre-treatment was examined by the Cornell–Wisconsin centrifugal flotation technique (Egwang and Slocombe, 1982) for parasite eggs which were identified and the number/g feces (epg) calculated. If in a sample there were more than 400 eggs (80+ epg) the counting was stopped at 400 and a 10-g fecal sample from the horse was processed by the Cornell–McMaster dilution technique (Georgi, 1985) and a second epg determined. The higher epg was used as the estimate for that animal. These pre-treatment strongyle epgs (PRT) for horses in a trial were ranked from highest to lowest and blocked for treatment. The first treatment block contained horses from the top of the rank order and the number of horses taken down the rank order was equal to the number of treatments in the trial. Horses within that block were randomised to treatment. Other treatment blocks were similarly constructed down the rank order and treatment assigned to each horse. On the day of the trial (Day 0) and prior to a single oral administration of an anthelmintic, the mouth of a horse was examined for food and if present removed. Post-treatment fecal samples were taken from each horse at the times described below and similarly assessed for strongyle epgs (POT). Efficacy of treatment for each horse was determined as follows: %efficacy = $100 \times [(\text{PRT} - \text{POT})/\text{PRT}]$. During the trials horses were maintained on pasture and brought in daily for their grain ration. For coproculture, a 20-g fecal sample from each horse in a treatment group was pooled and from the culture 100 infective larvae were retrieved randomly and identified. Samples for coproculture were taken at the times described below.

2.1. Yearling trial

In August 1997, 11 of 15 yearlings had strongyle eggs in the feces at the end of the 60 d period on a daily feeding of grain mixed with pyrantel tartrate/alfalfa/molasses pellets (StrongidC: Pfizer Canada, Kirkland, Que., Canada). The fecal samples were taken on Day −6 and were intended to be the PRT for the trial, but in error the yearlings were treated that day with pyrantel pamoate paste (Strongid P: Pfizer Canada) at 6.6 mg pyrantel base/kg body weight (BW) and the PRT samples were taken 5 d later (Day-1). Six yearlings were eliminated from the trial; two which were positive and to be moved from the farm after a week and the four which were negative. The nine yearlings (five fillies and four colts) with strongyle eggs were placed in three treatment groups: (i) continuation of the daily in feed pellets with pyrantel tartrate at 2.6 mg/kg BW, (ii) discontinuation of daily pyrantel tartrate and a single treatment orally with pyrantel pamoate paste at 6.6 mg pyrantel base/kg BW, (iii) discontinuation of daily pyrantel tartrate and a single treatment orally with moxidectin gel (Quest: Wyeth Animal Health, Guelph, Ont., Canada) at 0.4 mg/kg BW. Weights of the yearlings were estimated as described by Hintz et al. (1979), and ranged from 410 to 462 kg. POT samples were taken on Day 11. On Day 12, pyrantel tartrate treated yearlings had their daily medication discontinued and these and pyrantel pamoate treated yearlings were treated with moxidectin. A further POT sample was taken on Day 24. Samples for coproculture were taken on Days −1, 11 and 24.

2.2. Mare trial

In 1997 and as per the usual practice on the farm, these mares had been each given in April and in July ivermectin, and in mid-September pyrantel pamoate paste at twice the label dose (the recommended treatment for tapeworms). In November 1997, 12 mares with PRT samples on Day −3 were allocated to two treatment groups, pyrantel pamoate at 6.6 mg pyrantel base/kg BW or moxidectin at 0.4 mg/kg BW. The weight of each mare was estimated using a weigh

tape and ranged from 440 to 650 kg. One mare treated with pyrantel pamoate had to leave the farm on Day 7 and POT samples were taken at that time. POT samples were taken from the remainder 11 mares again on Day 14. On Day 14, five mares, which had been treated with pyrantel pamoate were treated with moxidectin and POT samples taken on Day 25. Samples for coproculture were taken on Days −3 and 7.

3. Results

All horses accepted the treatments readily and adverse reactions were not observed. The primary parasite egg found was the strongyle egg and the epgs are shown in Tables 1 and 2. All PRT cultures and POT cultures from pyrantel tartrate or pyrantel pamoate treated horses had 100% cyathostomes. No larvae were recovered POT from horses treated with moxidectin.

3.1. Yearling trial

Arithmetic mean cyathostome epgs on Days −6, −1 and 10 for pyrantel tartrate treated yearlings were 6.5, 24.5 and 44.6, for pyrantel pamoate treated yearlings 14.4, 13.9 and 17.7 and for moxidectin 15.5, 21.7 and 0, respectively. Pyrantel tartrate and pyrantel pamoate treated yearlings when treated with moxidectin all had on Day 24, 0 epg.

Table 1
Pre- and post-treatment number of strongyle eggs/g feces in yearlings treated with an in feed pyrantel tartrate daily or one dose orally of pyrantel pamoate or moxidectin

Yearling ID	Day −6	Day −1	Day 10	Day 24
Pyrantel tartrate on Days −6 through 11 and moxidectin on Day 12				
1	18.2	66.8	122.2	0
5	1	5.2	11.4	0
7	0.4	1.6	0.2	0
Pyrantel pamoate on Day 0 and moxidectin on Day 12				
2	34.4	36.6	50.2	0
6	6.6	0.8	2.6	0
9	2.2	4.2	0.4	0
Moxidectin on Day 0				
3	34.4	51.2	0	ND[a]
4	5.6	12.8	0	ND
8	0.4	1	0	ND

[a] ND = not done.

Table 2
Pre- and post-treatment number of strongyle eggs/g feces in mares treated orally with pyrantel pamoate or moxidectin

Horse ID	Day −3	Day 7	Day 14	Day 25
Pyrantel pamoate on Day 0 and moxidectin on Day 14				
10	89.8	46.8	ND[a]	ND
12	39.4	9.6	34.2	0
15	4.5	0.2	0.6	0
17	1.6	0.4	0.2	0
18	1.6	1	1	0
21	0.2	0.4	0.6	0
Moxidectin on Day 0				
11	62	0	ND	ND
13	33.6	0	ND	ND
14	12.2	0	ND	ND
16	4	0	ND	ND
19	0.6	0	ND	ND
20	0.2	0	ND	ND

[a] ND = not done.

3.2. Mare trial

Arithmetic mean cyathostome epgs on Days −3, 7 and 14 for mares treated with pyrantel pamoate were 22.9, 9.7 and 7.3, respectively and for moxidectin treated mares on Days −3 and 7 were 17.1 and 0, respectively. Pyrantel pamoate treated mares when treated with moxidectin all had on Day 25 0 epg.

4. Discussion and conclusion

Two of three yearlings given pyrantel tartrate and two of three given pyrantel pamoate had no reduction in epgs after treatment and we infer from these results cyathostome resistance to pyrantel. The daily in feed pyrantel tartrate was first used on the farm in 1997 and with these yearlings. The 66 d of its use was an inadequate time for development of a pyrantel tartrate resistant cyathostome population on the entire farm. Resistance on the farm probably originated with the extensive use over several years of pyrantel pamoate for removal primarily of tapeworms but also nematodes in horses. That hypothesis appeared to be validated when there was less than 80% epg reduction in five of six pyrantel pamoate treated mares on Day 7 and three of five mares on Day 14 (one mare had left the farm before Day 14). As had been reported in 1997 (Slocombe and de Gannes, 1997), this is the

first evidence for cyathostome resistance to pyrantel in Canada. Moxidectin reduced the epgs to 0 in all 9 yearlings and all 13 mares; not a surprising finding but it is the first record for moxidectin's high efficacy against pyrantel resistant cyathostomes.

The lack of reduction in epgs in the yearlings following pyrantel pamoate treatment was also not surprising. All had been on pyrantel tartrate pellets daily for 66 d and in error had received an additional treatment of pyrantel pamoate paste 6 d prior to the start of the trial. The strongyle egg counts actually increased following that treatment error and the cyathostomes in those yearlings going into the trial were likely all resistant nematodes. Also not surprising was the similarity in POT fecal egg counts in yearlings treated with pyrantel tartrate or pyrantel pamoate. Development of resistance to one of the pyrantel salts, which on this farm was pyrantel pamoate, would likely induce side resistance to the other. We infer from the data in the trial that side resistance among pyrantel salts apparently does occur and this would be the first record for such resistance.

Since 1990 the farm had closely monitored and constantly made adjustments to its parasite control program and fecal egg counts had been generally low. Pyrantel resistance was probably not recognized earlier because pyrantel pamoate, used primarily for control of tapeworms on the farm, was administered for many years at twice the label dose of the anthelmintic and the strongyle egg counts recorded for most horses following those treatments had been zero. The second author (de Gannes) was alerted when most of the yearlings with almost 2 m of daily treatment with the label dose of pyrantel tartrate were found with strongyle eggs in feces. Had the feces of these yearlings not been checked the potential presence of the resistance might not have been detected. Fecal examination techniques are not useful as an aid in diagnosing resistant nematodes until the level of resistance is relatively high and this can take several years from onset of the resistance (Kaplan, 2002). The early recognition of resistance on the farm due to the close monitoring was timely and allowed for defining strategies to minimize development of more significant populations of resistant worms.

Until recently, pyrantel pamoate was the only medication available for treatment of horses with tapeworms, but diagnosing a horse infected with tapeworms with the aid of fecal flotation techniques is difficult. Slocombe (2004, 2006) recorded a high level of success identifying horses infected with *Anoplocephala perfoliata* using the Cornell–Wisconsin double flotation technique and fecal samples collected 18–24 h after treatment. At those times the specificity of the test is 100% and its sensitivity high ninety percent. If such a test is negative pyrantel pamoate could be used less frequently on a farm or if positive praziquantel may be a treatment of choice.

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