

A modified critical test for the efficacy of pyrantel pamoate for *Anoplocephala perfoliata* in equids

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Abstract

Aims of this study with 13 equids naturally infected with *Anoplocephala perfoliata* were to document (i) a critical test with a period of 48 h from treatment to necropsy to assess the efficacy of an anthelmintic against the tapeworm, (ii) the efficacy of pyrantel pamoate oral paste at 13.2 mg pyrantel base/kg body weight, and (iii) the time after treatment when fecal egg counts would best estimate the tapeworm's prevalence in a herd. Feces passed in successive 12-h periods after treatment were examined for tapeworms. At necropsy, tapeworms in equids were identified as attached to the mucosa or unattached and, with a stereoscope, as normal or abnormal. At the time of treatment and at 6-h intervals thereafter, fecal samples were taken for egg counts. The efficacy of pyrantel pamoate was 96.6%; in 1 equid the efficacy was 75.3%, and in 8 it was 100%. "Major fragments" (worms without a scolex) accounted for 10% of the tapeworms recovered; they were not included in the efficacy analysis but should be. In 3 untreated equids necropsied, tapeworms were in the cecum, and 21.3% were detached. This protocol, when compared with a 24-h one without examination of feces, was more efficient in the treatment of trial animals and reduced underestimation and overestimation of an anthelmintic's efficacy. However, a protocol similar to this 48-h critical test but with a 24- or 36-h post-treatment period should be investigated. The mean egg count peaked 18 to 24 h after treatment and the samples taken at that time would provide the best estimate of prevalence of tapeworms in a herd. The Cornell-Wisconsin centrifugal flotation technique had a sensitivity and specificity of 100% at 18 h and 92% and 100%, respectively, at 24 h.

Résumé

Treize chevaux infectés de façon naturelle par *Anoplocephala perfoliata* ont été suivis dans le but de documenter (i) les résultats d'un test décisionnel avec un délai de 48 heures entre le traitement et la nécropsie pour évaluer l'efficacité d'un anthelminthique contre les vers plats (ii) l'efficacité du pamoate de pyrantel en pâte administré oralement à la dose de 13,2 mg/kg de poids corporel et (iii) le moment après le traitement où le compte des œufs excrétés donnerait la meilleure évaluation de la prévalence du cestode dans le troupeau. Les matières fécales émises sur des périodes successives de 12 heures après le traitement furent examinées pour la présence des cestodes. À la nécropsie des chevaux, les cestodes furent identifiés comme adhérent ou non à la muqueuse et, à l'aide d'un stéréo-microscope, comme normaux ou anormaux. Au moment du traitement et à intervalles de 6 heures par la suite, des échantillons de matières fécales furent prélevés pour effectuer un compte d'œufs. L'efficacité du pamoate de pyrantel s'est située à 96,6 %; elle fut de 75,3 % chez un cheval et de 100 % chez huit autres. Des « gros débris » (vers sans scolex) représentaient 10 % des cestodes récupérés; ils n'ont toutefois pas été inclus dans l'analyse de l'efficacité, même s'ils auraient dû l'être. Chez trois chevaux non traités soumis à la nécropsie, les cestodes se situaient dans le caecum et 21,3 % étaient libres. Ce protocole, lorsqu'on le compare avec un protocole de 24 heures sans examen des matières fécales, présente une efficacité supérieure chez les animaux traités et minimise la sous-estimation ou la sur-estimation de l'efficacité d'un anthelminthique. Toutefois, un protocole similaire à celui présenté ici mais effectué sur des périodes de 24 ou 36 heures suivant le traitement mérite d'être étudié. Il serait utile d'estimer la prévalence 18 à 24 heures suivant le traitement, un moment où le nombre moyen d'œufs excrétés est à son maximum. La technique de flottation par centrifugation Cornell-Wisconsin présente une sensibilité et une spécificité de 100 % à 18 heures, et de 92 % et de 100 % respectivement, à 24 heures suivant le traitement.

(Traduit par Docteur Alain Villeneuve)

Introduction

Lyons and colleagues (1,2) proposed a modified critical test for determining the efficacy of an anthelmintic for tapeworms in equids.

Pyrantel pamoate, at a dose of 13.2 mg pyrantel base/kg body weight (BW), is effective for the removal of *Anoplocephala perfoliata* from equids (1,3). Hearn and Hearn (4) advised the use of a 24-h post-treatment fecal sample to determine the prevalence of tapeworms in

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Received April 17, 2003. Accepted September 29, 2003.

Table 1. Number of *Anoplocephala perfoliata* recovered from the feces or at necropsy from equids treated with pyrantel pamoate, 13.2 mg pyrantel base/kg body weight, and percent efficacy of the anthelmintic

Equid ID	Number of abnormal and normal tapeworms; where recovered					Percent efficacy
	Feces	Abnormal		Normal		
		Animal	Total	Animal	Total	
11C	12	—	12	1	13	92.3
30C	13	1	14	1	15	93.3
35C	8	1	9	—	9	100.0
33	1	3	4	—	4	100.0
10C	170	12	182	3	185	98.4
5C	45	16	61	20	81	75.3
19C	8	—	8	—	8	100.0
18C	24	6	30	1	31	96.8
17C	7	—	7	—	7	100.0
746	513	30	543	—	543	100.0
185	4	—	4	—	4	100.0
597	1	—	1	—	1	100.0
753	53	2	55	—	55	100.0
Mean	—	—	—	—	—	96.6
Total	859	71	930	26	956	—

a herd. The purpose of this report is to (i) document another modification of the critical test, (ii) provide more data on the efficacy of pyrantel pamoate at this dose, and (iii) determine when feces should be examined after treatment to best estimate the prevalence of *A. perfoliata* in a herd. Some of the information from this study has been reported elsewhere (5), and the modification to the critical test described here is alluded to in the World Association for the Advancement of Veterinary Parasitology guidelines for evaluating the efficacy of equine anthelmintics (6).

Materials and methods

The trial was performed in Ontario from May to August 1994. It was a modified critical test with 16 equids: 5 standardbred horses and 11 crossbred ponies of different ages and sexes, weighing between 119 and 554 kg. All were found to have *A. perfoliata*, presumably naturally acquired, by examination of fecal samples for eggs with the Cornell–Wisconsin centrifugal flotation technique (7) when the equids were acquired and a few times before the trial commenced. Each equid, when acquired, was placed in quarantine at the Ontario Veterinary Teaching Hospital for at least 7 d and then moved to a stall outside the quarantine area for at least 5 d for acclimatization for the trial. All experiments were conducted after approval by the Animal Care Committee, University of Guelph, Guelph, Ontario.

Immediately before treatment, each animal was weighed and its mouth examined for food, which was removed. Thirteen equids were administered orally pyrantel pamoate paste (Strongid-P; Pfizer Canada, Kirkland, Quebec), 13.2 mg pyrantel base/kg BW. When an equid's weight did not coincide with a notch on the plunger of the Strongid-P syringe, the amount of paste administered was to the notch above the weight for the equid. All feces passed by each animal after treatment were pooled in 12-h batches up to 48 h and examined; the number of tapeworms in each batch was determined. At the time of

treatment and every 6 h until necropsy, the number of *A. perfoliata* eggs in a 5-g fecal sample was determined with the Cornell–Wisconsin centrifugal flotation technique. The sensitivity and specificity of the technique were determined by conventional methods.

The equids were euthanized for necropsy 48 h after treatment. Immediately after death, the ileum, cecum, ventral colon, dorsal colon, and small colon and rectum were ligated, and each section was examined for worms. The contents of each section were removed gently, so as not to dislodge attached tapeworms. When tapeworms were recovered, they were noted as attached to the intestine or detached and as normal or abnormal. Tapeworms were characterized as abnormal if on examination with a stereoscope they were fragmenting or disintegrating or had a brownish discoloration of the scolex or strobila or both. The mucosa of the ileocecal junction and about a 5-cm area around it was examined with a stereoscope for attached scolices that might have lost their segments and not been visible by eye.

Three equids were untreated and examined at necropsy as described for the treated animals.

Only worms with scolices were counted as tapeworms. A tapeworm without a scolex but with numerous segments and judged to be nearly a complete worm was identified as a "major fragment." Major fragments were counted but not included in the analysis for efficacy of pyrantel pamoate. That efficacy for each equid was determined as follows: % efficacy = $100 \times (\text{number of tapeworms in feces} + \text{number of abnormal worms in equid}) / (\text{number of worms in feces} + \text{number of abnormal worms in equid} + \text{number of normal worms in equid})$.

Results

There were 694 (36, 156, and 502) *A. perfoliata* recovered from the 3 untreated equids. All tapeworms were in the cecum: 546 were attached, some to the ileocecal junction, and the other 148 were

Table II. Number of *Anoplocephala perfoliata* recovered from 12-h batches of feces from the treated equids

Equid ID	Number of tapeworms				Total	Percent in 1st 24 h
	Period after treatment (h)					
	0–12	12–24	24–36	36–48		
11C	—	4	8	—	12	50.0
30C	—	1	7	5	13	7.7
35C	—	5	2	1	8	62.5
33	—	—	1	—	1	0
10C	1	12	81	76	170	7.6
5C	—	4	38	3	45	8.8
19C	—	1	6	1	8	12.5
18C	1	—	16	7	24	4.2
17C	—	7	—	—	7	100.0
746	1	236	223	53	513	46.2
185	1	1	2	—	4	50.0
597	—	1	—	—	1	100.0
753	—	17	29	7	53	32.1
Mean	—	—	—	—	—	34.1
Total	4	289	413	153	859	—

Table III. Number of “major fragments” of *Anoplocephala perfoliata* and number of equids from which they were recovered after the equids were treated

12-h batch of feces or position in equid at necropsy	Number of major fragments	Number of equids
Period after treatment (h)		
12–24	22	2
24–36	42	6
36–48	12	6
Position in colon		
Ventral	4	2
Dorsal	7	2
Small	6	1
Total	93	7

detached. The proportion of detached worms for each equid was 0%, 8.3%, and 26.8%, respectively. All worms, attached and detached, appeared normal.

The other 13 equids received at least 13.2 mg pyrantel base/kg BW; 9 received 2.2% to 6.4% more pyrantel base/kg BW, and 4 received 12.6% to 15.9% more. The efficacy of pyrantel pamoate for *A. perfoliata* in 1 equid was 75.3%; for all others it was greater than 92%, and for 8 it was 100% (Table I). Six of the 8 with 100% efficacy received an overdose in the lower range; the other 2 received an overdose in the upper range. One equid with 100% efficacy had the lowest overdose (2.2%). The equid with 75.3% efficacy had a 14.3% overdose. Adverse reactions were not seen in any treated horse.

There were 956 *A. perfoliata* recovered from the 13 treated equids, 859 from the feces (Tables I and II). There were 97 tapeworms in the animals: 27 in the cecum, 11 in the ventral colon, and 59 in the dorsal and small colon. There were 24 worms attached in the cecum, and 1 worm was attached in the ventral colon; all were normal. There

were 3 detached worms in the cecum: 1 was normal, and 2 were abnormal. All detached worms in the ventral, dorsal, and small colon were abnormal. Scolices were not found when the ileocecal-junction mucosa was examined with a stereoscope. Fewer than 1% of the 859 tapeworms recovered from the feces were in the first 12-h batch; 33.6% were in the 12- to 24-h batch (Table II). Tapeworms recovered from 4 treated equids were only in the feces, and for 2 they were passed within 24 h. For the other 11 equids, 0% to 62.5% of the tapeworms in feces were passed within 24 h.

There were 93 major fragments recovered (Table III): 17 from 4 equids at necropsy and 76 from 7 equids in the feces, all more than 12 h after treatment. These major fragments accounted for 6% to 15% of the total number of tapeworms in 6 horses and for 75% of the total number in the 7th equid. About 85% of the major fragments were in feces within 24 h after treatment.

The mean number of *A. perfoliata* eggs in a 5-g fecal sample from the 13 treated equids was 4.2 at the time of treatment, was 43.2 and 40.6 at 18 and 24 h, respectively, after treatment, and then declined (Table IV).

Discussion

In this critical test, pyrantel pamoate oral paste (Strongid-P) at twice the label dose was highly effective against *A. perfoliata*. Its mean efficacy in 13 equids was 96.6%; for 8 it was 100% efficacious, and for 1 equid its efficacy was 75.3%. No explanation was found for the low efficacy in the 1 equid. Each animal was administered the paste with use of the calibrations for weight on the plunger of the Strongid-P syringe, and each equid received 2.2% to 15.9% more pyrantel base/kg BW. More animals receiving an overdose in the lower end of the range had 100% efficacy compared with those receiving an overdose in the higher end.

In a standard critical test, animals are treated, feces are collected and examined for up to 7 d before necropsy, and numbers of parasites

Table IV. Number of *Anoplocephala perfoliata* eggs recovered from a 5-g fecal sample from the equids at the time of treatment (time 0) and at 6-h intervals thereafter and total number of tapeworms and major fragments recovered from each equid

Equid ID	Interval after treatment (h)									Total count
	0	6	12	18	24	30	36	42	48	
11C	0	0	0	4	9	25	3	0	4	15
30C	0	0	2	3	1	1	1	0	0	15
35C	6	0	5	2	13	13	1	0	0	9
33	2	1	1	4	35	29	15	5	3	7
10C	20	13	11	23	142	NS	40	12	1	195
5C	0	11	9	15	4	8	7	0	5	90
19C	11	20	58	42	62	15	5	2	0	8
18C	2	1	1	4	35	29	15	5	3	34
17C	7	3	5	358	116	155	35	3	4	7
746	5	6	8	50	28	100	25	19	2	601
185	0	0	0	4	0	0	1	0	0	4
597	0	13	10	21	3	3	0	0	0	1
753	2	4	5	32	82	57	296	3	1	61
Mean	4.2	5.5	8.8	43.2	40.8	36.3	34.2	3.8	1.8	—
Sensitivity ^a	62	69	85	100	92	92	92	54	62	—

NS — no sample

^a Specificity 100%

expelled in feces are compared with those remaining in the animal. A major difficulty with this test is that the longer the period between treatment and necropsy, the more the dead and dying parasites fragment and disintegrate, and the more difficult it is to find intact worms in the feces, which are required for calculation of efficacy. Lyons and colleagues (1,2) proposed a 24-h period between treatment and necropsy to overcome this problem and to recover tapeworms before they were evacuated by defecation. The authors did not collect feces from treated horses and admitted that some parasites could have been lost by defecation with the 24-h modification. In the modified critical test with 30 horses, Lyons and colleagues (1) found a 93% mean efficacy for pyrantel pamoate at 13.2 mg pyrantel base/kg BW, not much different from that in the present trial. But there are issues in what is an appropriate critical test to assess the efficacy of any anthelmintic for *A. perfoliata*.

Lyons and colleagues (1) found a 48-h post-treatment period in a preliminary trial "too long to evaluate drug activity against *A. perfoliata*." They presented data for only 2 of those horses and collected feces from only 1 of the 30 horses; 1 worm was found in the feces in the period of 24 to 48 h. In the present study with 13 equids, a 48-h post-treatment period, and examination of all post-treatment feces from all treated equids for tapeworms, a little more than one-third of all tapeworms counted from all equids were in the feces within 24 h. Had the feces not been examined, efficacy of the anthelmintic would have been underestimated. Further, 2 equids had no tapeworms at necropsy: the worms had been expelled in the feces within 24 h. Had the feces not been examined, these animals would have been eliminated from the trial, making for inefficient use of test animals.

Lyons and colleagues (1,2) determined efficacy by comparing the number of tapeworms found in the ileum and cecum and considered as "remaining" and unaffected by the drug with the number of tapeworms found more distally in the large intestine and considered "removed" and affected by the drug. The authors recognized that 24 h

may be insufficient time for worms affected by the drug to be found distal to the cecum. Transit times for ingesta vary and influence movement of tapeworms affected by a medication. However, the authors (1) recovered unattached tapeworms from the cecum of 1 horse and considered this "an indication of at least some drug activity," but because of location and gross morphology those tapeworms were considered "remaining." In the present trial, 3 untreated naturally infected equids were necropsied to visualize with a stereoscope the appearance of normal worms and to note where in the intestine they occurred and whether they were attached to the mucosa or free in the lumen. All tapeworms found appeared normal and were in the cecum; 21.3% were unattached. Williams and associates (8) examined 50 untreated naturally infected horses at necropsy and on gross examination found that more than 81% of *A. perfoliata* were attached to the cecum, 0.2% were attached to the ventral colon, and 16% were detached (but where they were found was not specified). All these worms were presumed to be normal, and those detached did so between the death of the horse and necropsy, which was up to 4 h later. Ihler and coworkers (9) examined 40 untreated naturally infected horses at necropsy within 1 h of death and found almost all worms to be attached. In the present study, the interval from death to necropsy was less than 30 min, and ingesta were removed gently, so as not to dislodge attached worms. Two unattached worms in the cecum, 1 in each of 2 treated horses, were abnormal; if, because of location, such worms were identified as normal, efficacy would be underestimated. One worm attached to the ventral colon of a treated horse was normal in this study. Normally, few *A. perfoliata* are in the ventral colon; if, because of location, such worms were classified as abnormal, efficacy would be slightly overestimated. Subsequently, Lyons and collaborators (10) considered attached worms in the ventral colon as normal.

In the present study, only tapeworms with scolices were included in determining the efficacy of pyrantel pamoate. But major

fragments, or worms without a scolex and with enough segments to be considered a whole worm, accounted for 10% of the total number of intact tapeworms, and 24% of the major fragments were in feces within 24 h after treatment. Exclusion of this significant number of worms from the calculation of efficacy certainly does not favour a drug under investigation and underestimates its efficacy. In a critical test, therefore, major fragments should be considered as tapeworms. Lyons and colleagues (1), in calculating efficacy for 1 horse, included "complete tapeworms except they lacked a scolex" as "removed" by the drug.

Lyons and colleagues' (1,2) "quick-test" method increases the chance of finding tapeworms with scolices still attached, but a too-short post-treatment period may not allow for detection of morphologic change, even microscopic, in worms fatally affected by a drug. In the present study, with a 48-h post-treatment period, examination of all feces after treatment and of all tapeworms in the animal at necropsy for abnormality with a stereoscope, underestimation and overestimation of the efficacy of the anthelmintic were reduced.

However, post-treatment periods shorter than 48 h should be examined. In the present trial, the mean number of tapeworms in untreated horses (231.3) was considerably higher than the mean number in treated horses (80.6), with major fragments included in the count. Was that fortuitous or were there more worms in treated equids that had fragmented and were not recovered? These means are considerably higher than those in recent surveys of untreated horses in Australia, Norway, England, and Ireland (8,9,11,12). In Spain (13), a mean count for untreated horses was not identified, but the maximum number in a horse was far lower than in the present study. However, the mean and range for the treated horses in the present study are not unlike those for untreated horses in the United States and Sweden (14,15). If one assumes that most tapeworms, including major fragments, were recovered from treated equids in the present trial, only 0.5% were found in feces up to 12 h after treatment. Thus, 12 h may also be too short for recognition of tapeworms affected by an anthelmintic. In the 12- to 24-h period, 29.7% of the combined total of tapeworms and major fragments were in feces; the proportions were 43.5% in the 24- to 36-h period and 15.8% in the 36- to 48-h period. A protocol with 24- or 36-h post-treatment periods and examination of all feces and of all worms in the animal at necropsy may be appropriate to reduce the number of major fragments, to allow sufficient time for worms affected by medication to be detected microscopically, and to increase the chance of finding intact worms.

Detectable morphologic change in tapeworms following treatment may occur at different times with different anthelmintics, and when that change occurs needs to be determined to identify the appropriate post-treatment period. Imirie and Jacobs (16), using pyrantel embonate in horses, found that one-half of the tapeworms were recovered in feces within 24 h after treatment. In the present study and a previous one (3) with pyrantel pamoate, most worms were expelled between 24 and 48 h after treatment. Gauderon and colleagues (17) found only 83.6% efficacy for pyrantel pamoate at 13.6 mg/kg BW against *A. perfoliata* in foals. That report describes a controlled test lasting 18 to 20 d and documents that the efficacy of pyrantel pamoate was highly variable.

Coprologic techniques for diagnosing tapeworm infection in horses have low sensitivity, especially when horses harbour fewer than 100 tapeworms (9,15,18,19). Hearn and Hearn (4), using the Cornell-Wisconsin centrifugation flotation technique (7), advised that post-treatment fecal samples with eggs released from disintegrating worms would provide a better indication of infection in a herd than pretreatment samples. They treated 24 horses with pyrantel pamoate at 19.8 mg pyrantel base/kg BW and examined fecal samples at the time of treatment and 17, 24, 39, and 48 h thereafter. At least one-half of the horses showed negative results throughout that study, but the largest number of positive results was at 24 h. The authors recommended this time for estimating the prevalence of tapeworms in a herd. In the present study, fecal samples were examined every 6 h after treatment, and few equids had negative results during the 48 h; 2 or fewer had negative results between 12 and 36 h. Further, the mean egg count peaked at 18 to 24 h; results were positive for all 13 equids at 18 h and for 12 at 24 h. From these observations, fecal sampling would be most useful at 18 to 24 h after treatment.

Proudman and Edwards' (18) centrifugal flotation technique appeared to be the most useful technique for recovering tapeworm eggs from horse feces, with a sensitivity of 61% and a specificity of 98%. In the present study, equids were acquired when they were found with *A. perfoliata* eggs on fecal examination with use of the Cornell-Wisconsin centrifugal flotation technique. Before entering the trial, the equids were checked at least once more with that technique to confirm their status. That technique's sensitivity in a critical assessment with trichostrongylid eggs in bovine feces was 100% at 3 to 70 eggs/g feces and 93% at 1.5 eggs/g (7). In the present study, the specificity of that technique was 100%, and at the time of treatment of the equids its sensitivity was 62%. At 18 h post-treatment the sensitivity was 100%, and at 24, 30, and 36 h after treatment the sensitivity was 92%. Tapeworm eggs are seen easily with this technique, which is much simpler, uses less feces, and is less time-consuming than that advocated by Proudman and Edwards.

In the present study, as in others (15,18,19), there appeared to be no correlation between number of eggs counted before treatment and number of tapeworms in the equids, and 2 of the equids in the present study had substantially more than 100 worms. Meana and associates (19) considered the normal tapeworm's sporadic discharge of gravid tapeworm segments, and their uneven distribution in the fecal mass could explain the lack of correlation between tapeworm burden and egg detection. There appeared also to be no relationship between number of eggs counted at any period after treatment or number of times a fecal sample was positive during that period and number of tapeworms in an animal. These discrepancies are probably related to how quickly tapeworms disintegrate and where that disintegration occurs after the use of the anthelmintic. The more proximal in the large intestine fragmentation occurs, the more likely eggs released would be more evenly distributed in the ingesta and detectable on fecal analysis.

Acknowledgments

This study was supported with financial assistance from Pfizer Canada, Kirkland, Quebec.

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