SMALL STRONGYLES OF HORSES WITH CROSS RESISTANCE TO BENZIMIDAZOLE ANTHELMINTICS AND SUSCEPTIBILITY TO UNRELATED COMPOUNDS

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Introduction

There are a few reports on the development of tolerance to anthelmintics by strongyles in the horse. Several authors have reported on resistance to phenothiazine (2, 5, 13), tolerance to thiabendazole (TBZ) (3) and cross tolerance with the benzimidazole compounds TBZ and mebendazole (MBZ) (4, 16). Recently, strongyles with resistance to another benzimidazole compound cambendazole (CBZ)[1] were found on a well managed standardbred farm in Ontario. For about two years prior to this recognition of resistance to CBZ, the owner had administered to all horses bimonthly MBZ (Telmin®)[2] except that in November of each year trichlorfon (Anthon®)[3] was used. Prior to that two year period TBZ (Equizole®)[4] was used bimonthly instead of MBZ for about 12 years. This is a report on cross resistance by strongyles in several horses on one farm to benzimidazole compounds and their susceptibility to a variety of unrelated anthelmintics.

Materials and Methods

Fourteen standardbred horses six months to 18 years and naturally infected with strongyles were utilized in this study. Five procedures were attempted. Procedure 1 was completed during the first half of December 1975. Procedure 2 was started in early January 1976 and the other procedures followed at successive seven day intervals. The total time involved for the five procedures was 63 days and the day of commencement of Procedure 1 was labelled Day 1. Each procedure involved two or more groups of horses and in any one group the horses were treated with the same anthelmintic or left untreated. At the time of the administration of the anthelmintic fecal samples were taken from all horses, whether they were treated or not, and again after a further seven days to determine the number of strongyle eggs per gram and for the identification of infective strongyle larvae as described previously (17). Details of the several procedures are given below and summarized in Table I.

Procedure 1. There were two groups. Commencing with the heaviest animal, 12 horses were ranked and then paired by body weight. Each member of a pair was allocated randomly to one of two groups to give six horses per group. On Day 1, each of the animals in one group received orally 20 mg of CBZ/kg body weight. The CBZ was in the form of a paste in a dial-a-dose syringe. The other group was untreated. On Day 7, the procedure was reversed and the horses in the untreated group were treated similarly with CBZ.

Procedure 2. There were four groups. On Day 35, two of the six horses from each of the two groups in Procedure 1 were allocated randomly to one of three groups to give four horses per group. One of the three groups received TBZ (Thibenzole Sheep & Goat Wormer®),[5] the other MBZ (Telmin®) and the third was left untreated. At that time another horse recently returned from the race track was found positive for strongyles, was considered as the fourth group and was treated with CBZ. CBZ was administered as detailed previously. TBZ and MBZ were administered by stomach tube. TBZ was given at the rate of 44 mg of thiabendazole/kg body weight, and MBZ as recommended by the manufacturer. On the day of treatment, the owner unintentionally treated one of the horses in the group classified as untreated with a mixture of TBZ (Equizole®) and trichlorfon (Anthon®) in the feed which was readily consumed.

Procedure 3. There were six groups. On Day 42, each of the four horses in the TBZ and MBZ groups in Procedure 2 was allocated

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[1]Merck Sharp & Dohme, Rahway, New Jersey.

[2]Pitman-Moore Ltd., Don Mills, Ontario.

[3]Chemagro Limited, Mississauga, Ontario.

[4]Merck, Sharp & Dohme Canada Ltd., Mississauga, Ontario.

[5]Merck Sharp & Dohme Canada Ltd., Mississauga, Ontario.

TABLE I

ANTHELMINTICS AND FIVE PROCEDURES USED WITH 14 HORSES. EACH PROCEDURE INVOLVED TWO OR MORE GROUPS OF HORSES. EACH HORSE IS IDENTIFIED BY A CAPITAL LETTER

Day	Procedures					
	Procedure 1					
	Group 1	Group 2				
1	Horses– A, B, C, D, E, F	Horses– G, H, I, J, K, L				
7	Cambendazole	Untreated				
	Untreated	Cambendazole				
	Procedure 2					
	Group 1	Group 2	Group 3	Group 4		
35	Horses– A, B, G, H	Horses– C, D, I, J	Horses– E, F, K, L	Horse– M		
	Thiabendazole	Mebendazole	Untreated	Cambendazole		
	(Thibenzole Sheep & Goat Wormer®)	(Telmin®)	Owner Treated Horse F Thiabendazole (Equizole®) + Trichlorfon (Anthon®)			
	Procedure 3					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
	Horses– A, I	Horses– B, D	Horses– C, G	Horses– H, J	Horses– E, F, K, L	Horses– M, N
42	Piperazine	Piperazine	Pyrantel	Thiabendazole	Untreated	Dichlorvos
	Carbon disulphide	Carbon disulphide	pamoate	Piperazine		
	Phenothiazine	Phenothiazine				
	(Parvex Plus®)	(Equinan®)	(Strongid-T®)	(Equizole A®)		(Equigard®)
	Procedure 4					
	Group 1	Group 2				
	Horses– E, K	Horses– F, L				
49	Trichlorfon	Untreated				
	Piperazine					
	Phenothiazine					
	(Dyrex T.F.®)					
	Procedure 5					
	Group 1	Group 2				
56	Horse– L	Horse– F				
	Piperazine	Cambendazole				

randomly to one of four groups to give two horses per group. The horses in two of the four groups received piperazine-carbon disulphide complexes with phenothiazine in one of two forms (Parvex Plus®[6] or Equinan®[7]). The third group received pyrantel pamoate (Strongid-T®)[8] and the fourth a mixture of TBZ and piperazine (Equizole A®)[9] The fifth group was composed of the four horses in the untreated group in Procedure 2 and were left untreated in Procedure 3. At that time another horse had returned from stud and together with the horse treated with CBZ in Procedure 2 were assigned to the sixth group and treated with dichlorvos (Equigard®).[10] All the anthelmintics were administered at the rate recommended by the manufacturer. Pyrantel pamoate, TBZ-piperazine and carbon disulphide-piperazine complexes with phenothiazine were administered by stomach tube. Dichlorvos was given by mixing it into a small portion of the ration and was readily consumed.

Procedure 4. There were two groups. On Day 49, two of the four untreated horses in Procedure 3 were allocated randomly to treatment with a mixture of trichlorfon, phenothiazine and piperazine (Dyrex T.F.®)[11] which was administered by stomach tube and at the rate recommended by the manufacturer. The other two horses were untreated.

Procedure 5. There were two groups. On Day 56, each of the two untreated horses in Procedure 4 were allocated randomly to treatment with CBZ paste or piperazine adipate. CBZ was administered as described previously and piperazine was administered at the rate of 200 mg/kg and by stomach tube.

Results

Horses which received CBZ (Procedures 1, 2 and 5) had strongyle eggs in the feces at seven days after treatment although in some horses the counts were reduced (Table II). When these horses were treated with TBZ, MBZ or a mixture of TBZ and trichlorfon (Procedure 2), there was no significant reduction in the number of eggs counted seven days after treatment. However, when these horses were treated with the piperazine-carbon disulphide complexes with phenothiazine, pyrantel pamoate, dichlorvos, TBZ-piperazine (Procedure 3), trichlorfon-phenothiazine-piperazine (Procedure 4) or piperazine (Procedure 5) no strongyle eggs were found in the feces on quantitative analysis seven days after treatment (Table II).

The strongyle eggs from all horses were from small strongyles as determined by analysis of fecal cultures except for one horse where there were in addition some *Strongylus vulgaris*. Following treatment with CBZ, *S. vulgaris* were not identified in fecal cultures but were found subsequently several weeks later. When this horse was treated with piperazine the small strongyles were removed but there were *S. vulgaris* in the fecal culture.

Discussion

Small strongyles resistant to CBZ were found in several horses which had never received the anthelmintic previously. These strongyles were also found to be resistant to other benzimidazole compounds, TBZ and MBZ, which had been used previously. Resistance in strongyles to CBZ has not been reported, but CBZ-tolerant strains of the nematode *Haemonchus contortus* in sheep are known (8, 9). Cross over of tolerance by strongyles to these three benzimidazole compounds, although not reported previously, is not surprising since strongyles have been found tolerant to both TBZ and MBZ (4, 16). Cross resistance with benzimidazole compounds have been found in *H. contortus* (1, 7, 18) and in *Trichostrongylus colubriformis* (6) in sheep.

The fumarase reductase system, which is a metabolic pathway in the respiratory chain of many nematodes, is the likely site of action of benzimidazole compounds. In susceptible strains of *H. contortus*, TBZ (14) and CBZ (12) inhibit the enzyme, but show no effect in resistant strains (15). Resistance to TBZ in *H. contortus* is inherited and probably due to a single gene (10). Le Jambre *et al* (10) suggested that there are similarities in the development of resistance in *H. contortus* with resistance in insects to insecticides. They made no analogy with the helminths but they recognized the conclusions of certain entomologists that several insects develop resistance to a number of insecticides used alternately at the same rate as to a single insecticide. This may be possible in helminths because the anthelmintic l-tetramisole which is not a benzimidazole also inhibits the fumarase reductase system in *H. contortus* (15) as well as in *Ascaris suum*, *Ascaridia galli*, *Toxocara cati* and *Dictyocaulus*

[6]TUCO Products Company, Orangeville, Ontario.

[7]Rogar/STB, London, Ontario.

[8]Roger/STB, London, Ontario.

[9]Merck Sharp & Dohme Canada Ltd., Mississauga, Ontario.

[10]Shell Canada Limited, Toronto, Ontario.

[11]Fort Dodge Laboratories Inc., Fort Dodge, Iowa.

TABLE II
SMALL STRONGYLE EGGS PER GRAM OF FECES IN 14 HORSES

Horse	Age in Years	Day 1	7	14	35	42	49	56	63
A	11	CBZ*	—	—	TBZ*	PAR†	—	—	—
		100	100	150	250	200	0	0	0
B	11	CBZ*	—	—	TBZ*	EQU†	—	—	—
		1150	200	300	600	300	0	0	0
C	1/2	CBZ*	—	—	MBZ*	STT†	—	—	—
		3500	950	750	1650	1200	0	0	0
D	3/4	CBZ*	—	—	MBZ*	EQU†	—	—	—
		900	550	350	1500	1400	0	0	0
E	18	CBZ*	—	—	—	—	DTF†	—	—
		150	150	400	350	550	350	0	0
F	4	CBZ*	—	—	EAA†	—	—	CBZ*	—
		200	100	150	200	150	200	250	300
G	3	—	CBZ*	—	TBZ*	STT†	—	—	—
		800	750	300	400	200	0	0	0
H	4	—	CBZ*	—	TBZ*	EQA†	—	—	—
		800	800	150	400	300	0	0	0
I	2	—	CBZ*	—	MBZ*	PAR†	—	—	—
		1100	500	200	150	150	0	0	0
J	5	—	CBZ*	—	MBZ*	EQA†	—	—	—
		800	350	150	400	450	0	0	0
K	18	—	CBZ*	—	—	—	DTF†	—	—
		750	500	300	1850	550	650	0	0
L	3/4	—	CBZ*	—	—	—	—	PIP†	—
		4200	1600	50	250	150	700	1300	0
M	3	—	—	—	CBZ*	EQG†	—	—	—
		—	—	—	200	50	0	0	0
N	6	—	—	—	—	EQG†	—	—	—
		—	—	—	—	350	0	0	0

*Benzimidazole anthelmintics
CBZ : Cambendazole
TBZ : Thiabendazole
MBZ : Mebendazole
PAR : Parvex Plus®
EQU : Equinan®
EAA : Equizole and Anthon®

†Non-benzimidazole anthelmintics
STT : Strongid-T®
EQA : Equizole A®
EQG : Equigard®
DTF : Dyrex T.F.®
PIP : Piperazine

viviparus (19). It may well be, of course, that the development of resistance does not involve a single mechanism.

In our study several anthelmintics unrelated to the benzimidazoles were found to be useful against the resistant strongyles. Piperazine, pyrantel pamoate, dichlorvos, piperazine-carbon disulphide complexes with phenothiazine, piperazine with TBZ and trichlorfon with phenothiazine and piperazine were highly effective. Piperazine which is relatively inexpensive and non toxic is effective also against phenothiazine resistant small strongyles (2) and is an extremely useful compound. Lyons *et al* (11) have demonstrated that pyrantel pamoate was effective in reducing the fecal egg count in two horses with small strongyles reported to be resistant to TBZ. No potentiation could be found when trichlorfon (Anthon®) was mixed with TBZ (Equizole®), but the trichlorfon-phenothiazine-piperazine (Dyrex T.F.®) mixture was effective. The level of trichlorfon in Anthon® is similar to that in Dyrex T.F.® so it would appear that it was the piperazine or the synergism of piperazine and phenothiazine were the useful ingredients against the small strongyles. Only the manufacturers of Dyrex T.F.® claim efficacy against strongyles.

Control programs involving different classes of anthelmintics available for horses appears, therefore, to be more effective than a program with a single anthelmintic. However, regardless of the anthelmintics used, routine fecal

analysis must be an integral part of the program. The development of tolerance to anthelmintics will occur, and monitoring by analysis of fecal samples taken strategically is the only way to gain some estimate of the effectiveness of a control program for strongyles.

Summary

Thirteen standardbred horses, all from one farm, were found with a natural infection of small strongyles which were resistant to cambendazole when it was first administered to the horses. The small strongyles were found resistant also to two other benzimidazole compounds thiabendazole and mebendazole. It is presumed that the strongyles had developed a resistance to the two latter compounds which had been used previously and routinely for many years and that the resistance was uncovered when cambendazole was first used. The small strongyles were found to be susceptible to the following non-benzimidazole compounds: piperazine, pyrantel pamoate, dichlorvos, piperazine-carbon disulphide complexes with phenothiazine, and mixtures of piperazine-thiabendazole and piperazine-trichlorfon-phenothiazine. Some reasons for the development of resistance in nematodes and some considerations for control programs are discussed.

Résumé

Cette expérience clinique concernait 13 chevaux Standardbred d'une même ferme, atteints d'une infection naturelle par des petits strongles qui s'avérèrent résistants à un traitement initial au cambendazole. Ces parasites se révélèrent aussi résistants à deux autres composés du benzimidazole : le thiabendazole et le mébendazole. Les auteurs prétendent que les strongles auraient développé une résistance aux deux derniers anthelminthiques que le propriétaire utilisait de façon routinière depuis plusieurs années; ils découvrirent cette résistance, lors de la première utilisation du cambendazole. Les petits strongles s'avérèrent par ailleurs vulnérables aux anthelminthiques suivants, dépourvus de benzimidazole : la pipérazine, le pamoate de pyrantel, le dichlorvos, des complexes pipérazine-carbosulfure, enrichis de phénothiazine, et des mélanges de pipérazine-thiabendazole et de pipérazine-trichlorfon-phénothiazine. Les auteurs commentent certains facteurs qui expliquent le développement d'une résistance de la part des nématodes et quelques suggestions relatives à l'élaboration de programmes d'éradication de ces parasites.

Acknowledgments

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ANALYSE DE VOLUME/BOOK REVIEW

Veterinary Physiology. Edited by J. W. Phillis. Published by W. B. Saunders Limited, Toronto. 1976. 882 pages. Price $36.00.

La tendance actuelle, qui se vérifie dans presque tous les programmes d'enseignement vétérinaire, est à la réduction de l'importance des sciences de base, à la condensation de leur contenu. Ce volume s'inscrit dans cette orientation. L'éditeur a imposé un choix visant à ne retenir de toutes les nouvelles acquisitions physiologiques des dernières décennies, que les éléments d'intérêt vétérinaire.

Pour conserver l'unité de style et la continuité d'un sujet à l'autre, l'éditeur a réduit le nombre de collaborateurs. Chaque section a cependant été préparée par un spécialiste afin de fournir à l'étudiant le maximum d'information dans un minimum de texte.

L'ordre de présentation qui a été retenu permet d'acquérir les connaissances nécessaires à la compréhension des chapitres successifs. Le livre commence par une analyse de la physiologie cellulaire de base et par l'étude des propriétés des cellules musculaires et nerveuses. Le fonctionnement du système nerveux central et périphérique est ensuite discuté suivi de l'étude du système endocrinien. La circulation, la respiration et les systèmes gastro-intestinal et rénal sont abordés dans cet ordre. Puis, viennent l'étude du métabolisme et de la bio-énergétique, de la reproduction et de la lactation. Le livre se termine par un résumé de biométrie, indispensable à la compréhension de la littérature scientifique. Tous les chapitres ou sections ont une courte liste de références choisies pour leur intérêt complémentaire.

Ce livre dont la présentation est sobre mais bien faite, est sans aucun doute l'ouvrage de référence par excellence pour l'étudiant vétérinaire et l'étudiant en sciences animales.

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This book has been written bearing in mind the constraints imposed by the recent trend towards shorter curricula in the basic sciences in veterinary schools.

The editor has limited the number of contributors to the book in order to give the reader a consistency and a continuity of style. The text of each contributor presents a clear and concise account of the more recent knowledge in veterinary physiology.

Each chapter or section includes a short list of references. The order in which the material is introduced has been retained to give the information necessary for an understanding of the successive chapters.

The book begins with an analysis of basic cellular physiology and by a study of nerve and muscle cells functions. A detailed discussion of the functioning of the peripheral and central nervous systems is followed by an account of the regulation of the body by the endocrine glands. Circulation, respiration, gastrointestinal and urinary systems are treated that, preceding the sections on metabolism and bioenergetics, reproduction and lactation. The final section is devoted to biostatistics, which is always of significance for an intelligent comprehension of scientific literature.

This textbook is presented with sobriety, and it should constitute an excellent basic text for the veterinary student. However, in a subsequent edition, a little more emphasis should be devoted to the circulatory and digestive systems and to the reproductive functions which are more relevant to the veterinary profession. *A. Dallaire.*