Acta vet. scand. 1991, *32*, 253–260.

Resistance to Benzimidazole Anthelmintics in Small Strongyles (*Cyathostominae*) of Horses in Denmark

By *H. Bjørn, Chr. Sommer, H. Schougård, Sv. Aa. Henriksen* and *P. Nansen*

Department of Veterinary Microbiology, Laboratory of Parasitology, Royal Veterinary and Agricultural University, Institute of Population Biology, Copenhagen University, Nørlund Hestehospital, and National Veterinary Laboratory, Denmark.

Bjørn, H., Chr. Sommer, H. Schougård, Sv. Aa. Henriksen and P. Nansen: Resistance to benzimidazole anthelmintics in small strongyles (*Cyathostominae*) of horses in Denmark. Acta vet. scand. 1991, 32, 253–260. – This study was undertaken to establish whether anthelmintic resistance was present in nematode parasites of horses in Denmark. Sixteen horse farms were selected for faecal egg count reduction (FECR) tests to measure the efficacy of the anthelmintic used. Resistance to benzimidazole anthelmintics was found on 13 of the 16 farms, with FECR values ranging from 80.0 % to – 101.3 %. On the remaining 3 farms FECR was 100.0 %, 99.3 % and 97.2 %. Results of a questionnaire study on anthelmintic usage, parasite control measures and management practices showed that horses in this study were treated on average 7.1 times/year. Horse owners changed between preparations of drugs but almost only within the same class of anthelmintics. Nine owners gave an anthelmintic treatment to purchased horses before they were introduced on the farm. On 14 farms, the same paddock was grazed every year and the average stocking rate was estimated to be 2.4 horses/ha. Strategies to avoid development of anthelmintic resistance are discussed and recommendations of parasite control on horse farms are presented.

anthelmintic resistance; benzimidazoles; *Cyathostominae* spp.; parasite control.

Introduction

Resistance to anthelmintics in strongyle nematodes is recognized as a problem of major concern in the control of gastrointestinal parasite infections of domestic animals (for review see *Waller & Prichard* 1986). Anthelmintic resistance in strongyles of horses was first described in The United Kingdom by *Gibson* (1960), who found resistance to phenothiazine in small strongyles (*Cyathostomiae*). Later *Drudge & Elam* (1961) discovered similar resistance on 3 studs in Kentucky, USA. In the early sixties, thiabendazole, the first benzimidazole compound, was marketed and already in 1964 resistance in cyathostomes to this drug was reported from Kentucky, USA (*Drudge & Lyons* 1965). Subsequently, resistance to benzimidazole anthelmintics has been reported from England (*Round et al.* 1974), Canada (*Slocombe & Cote* 1977), Australia (*Barger & Lisle* 1979, *Kelly et al.* 1981), USA (*Herd et al.* 1981), Germany (*Bauer et al.* 1983, 1986, *Ullrich et al.* 1988) and Belgium (*Dorny et al.* 1988, *Geerts et al.* 1988). In the Scandinavian countries benzimidazole resistance has been recorded in Norway (*Helle* 1987) and Sweden (*Nilsson et al.* 1989).

Anthelmintic resistance has been defined as "a significant increase in the ability of individuals within a strain to tolerate doses of a compound (toxicant) which would prove lethal to the majority of individuals in a normal population of the same species" (*Prichard et al.* 1980). Recently a working party on anthelmintic resistance in Australia has provided a more precise definition whereby resistance is declared when 1) the observed percentage reduction in faecal egg counts after treatment is less than 95 % and 2) the lower 95 % confidence limit of the percentage reduction is less than 90 % (*Anon.* 1989). This definition was used in the present investigation.

Based on the apparent mode of action, 4 classes of anthelmintics are available for treatment of gastrointestinal parasite infections in horses. These classes include the benzimidazoles and pro-benzimidazoles (Group I), the neuromuscular agents pyrantel and levamisole (Group II), the ivermectins (Group III) and the organophosphorus compounds (Group V).

The purpose of this study was to establish whether resistance to anthelmintics was present in strongyle nematodes of horses in Denmark and further to obtain information on anthelmintic usage and parasite control measures on Danish horse farms.

Materials and methods

Farms selected for the study

This study consisted of two separate series of investigations on the efficacy of anthelmintics in studs in Denmark. Six (series A) and 10 (series B) studs were selected from 2 equine veterinary practices located in Jutland and Sealand, respectively. The criteria for a stud to be included in the study was that the usage of anthelmintics had been recorded for at least the previous 2 years and a minimum of 10 horses were available for an anthelmintic efficacy test. Further, the test animals had to be left untreated for at least 6 weeks if treated with a group I, II, V drug or 8 weeks if treated with ivermectin (Group III). Two sets of faecal samples were collected per rectum, the first set at the day of treatment and the second set from the same horses 7 days later. All samples were stored at approximately 4°C in a thermostat or cooling box before transport to the laboratory. The following anthelmintics were used at the recommended dose rates: febantel 6.0 mg/kg, fenbendazole 7.5 mg/kg, mebendazole 8.8 mg/kg and pyrantel pamoate 19.0 mg/kg. A questionnaire on the number of horses on the farm, anthelmintic usage (frequency of treatments, drugs used) and grazing management (set stocking, rotational grazing, mixed or alternate grazing) of the horses was completed at the first visit.

Parasitological techniques

Faecal samples of each horse were examined by a modified McMaster technique as described by *Henriksen* (1981) and the number of strongyle eggs per gram (epg) of faeces were estimated. In series A the minimum detection level of the McMaster method was 50 eggs per gram and in series B this level was 5 eggs per gram. Larval cultures of each faecal sample were established according to the technique of *Henriksen & Korsholm* (1983) for production of infective larvae for species differentiation to establish the proportion of larvae belonging to the large strongyles (*Strongylidae*) or small strongyles (*Cyathostomiae*). From each culture at least 100 larvae were differentiated according to morphological criteria (*Lichtenfels* 1975). If less than 100 larvae were present, all larvae were examined.

Calculations and statistical analysis

In series A, only farms where at least 5 horses excreted more than 200 epg and in series B similarly, only farms where at least 5 horses excreted more than 20 epg were included in the calculations. Change in faecal egg output of each horse after treatment was calculated as the egg count ratio (ECR) according to the formula: ECR = $\ln((EPG_2+1)/(EPG_1+1))$, where EPG_2 and EPG_1 denote epg after and before treatment and "ln" is the natural logarithm function. The FECR of the flock was calculated by backtransformation of the mean of ECRs (= $1/n\ (\sum ECR)$) by using the expression: FECR = $(1 - \exp(1/n\ (\sum ECR))) \times 100\ \%$, where "exp" is the exponential function. The lower (LC) and the upper confidence limit (UC) of FECR were calculated as outlined by The Working Party on Anthelmintic Resistance (*Anon* 1989):

$$LC = [1 - \exp(1/n\ (\sum ECR) + ci)] \times 100\ \%$$

$$UC = [1 - \exp(1/n\ (\sum ECR) - ci)] \times 100\ \%,$$

where $ci = t_{05.df} \times SD(ECR) \times n^{1/2}$, $t_{05.df}$ is the t-value at 5 % level at df = n–1 degrees of freedom, SD(ECR) is the standard deviation of ECR and n is the number of observations in the group.

Results

The results of the FECR-test are presented in Table 1. Benzimidazole anthelmintics

Table 1. Results of a faecal egg count reduction (FECR) test at 16 farms of horses in Denmark.

Farm no.	No. of horses treated	Drug tested	EPG_1	EPG_2	FECR (%)	Confidence limits (LC-UC %)
A1	5	PYR	238	0	100.0	98.4–100.0
A2	5	MBZ	273	279	–5.2	–141.3–53.6
A3	10	MBZ	662	1359	–101.3	–236.3–24.4
A4	9	FBZ	237	3	99.3	98.5–99.8
A5	9	MBZ	791	799	–1.0	–62.9–36.7
A6	9	FBZ	475	257	43.5	55.7–80.9
B7	10	FBZ	276	206	23.3	26.9–65.4
B8	15	FBZ	126	8	97.2	89.1–99.3
B9	11	MBZ	480	118	71.7	64.7–89.8
B10	16	MBZ	66	27	42.8	55.2–79.2
B11	5	MBZ	430	62	80.0	–42.5–99.4
B12	5	FB	362	305	14.9	–148.3–69.8
B13	15	MBZ	319	49	78.6	86.0–95.4
B14	7	FBZ	396	155	57.2	–14.8–98.0
B15	16	MBZ	112	57	40.4	47.7–79.8
B16	15	MBZ	794	641	18.6	–16.5–47.1

A1–A6 : series A
B7–B16: series B

PYR: pyrantel pamoate (19.0 mg/kg)
MBZ: mebendazole (8.8 mg/kg)
FBZ: fenbendazole (7.5 mg/kg)
FB: febantel (6.0 mg/kg)

EPG_1: geometric mean eggs per gram faeces before treatment
EPG_2: geometric mean eggs per gram faeces after treatment

FECR: faecal egg count reduction
LC: lower 95 % confidence limit of FECR
UC: upper 95 % confidence limit of FECR

were tested on 15 of 16 farms. On 13 farms the faecal egg count reduction (FECR) met the criteria of anthelmintic resistance being present in a flock. However, on one farm, where pyrantel pamoate had been used regularly, FECR was estimated to be 100.0 % and in two flocks where fenbendazole was tested FECR was found to be 99.3 % and 97.2 % effective, respectively. Only eggs of the strongyloid type were detected and the results of larval cultivation from faeces collected both before and after treatment revealed only the small strongyles (*Cyathostomiae*).
The results of the questionnaire study are summarized in Table 2. Eleven farms had thoroughbred horses and 5 farms had pleasure horses. The number of horses varied from 14 up to 85 with an average of 23.5 horse per farm. The average number of anthelmintic treatments were estimated to be 7.1/farm/year (i.e. a treatment every 7 1/2 weeks on average). Group I anthelmintics were used for 5.3 treatments/farm/year, group II for 0.5 treatments/farm/year, group III for 0.4 treatments/farm/year and group V for 0.7 treatments/farm/year. Some horse owners alternated between anthelmintic within or between years, but the change was most frequently between drugs solely in Group I. Eight horse owners indicated, that before horses were brought into the stud, an

Table 2. Characteristics of 16 horse farms, their anthelmintic treatment practices and grazing management.

Farm no.	Type of farm	Total no. of horses	Number of treatments per year	Times anthelmintics used from group[1]				Treatment of introduced horses	Number of horses/ha	Same paddock for grazing every year	Grazing with horses from other studs
				I	II	III	V				
A1	Tb	13	11	3	6	2		no	1.6	yes	no
A2	Tb	9	13	12			1	yes	1.4	yes	yes
A3	Tb	40	14	13			1	yes	1.6	yes	yes
A4	Tb	85	15	14			1	yes	1.7	yes	no
A5	Tb	24	8	8				no	2.0	ues	no
A6	Tb	11	10	7		2	1	yes	1.7	yes	no
B7	P	16	6	4	1		1	yes	2.4	yes	no
B8	P	28	4	2	1	1		yes	3.4	yes	no
B9	Tb	12	4	3			1	no	7.2	yes	yes
B10	Tb	35	4	2			2	yes	0.8	no	no
B11	Tb	14	6	5			1	yes	–	yes	no
B12	Tb	16	4	3		1		no	3.0	yes	no
B13	P	12	5	4			1	no	3.7	yes	no
B14	P	20	4	3		1		yes	1.3	no	no
B15	P	23	2	1				no	2.4	yes	yes
B16	Tb	18	4	4				no	1.5	yes	yes

[1] group I benzimidazoles and pro-benzimidazoles
group II neuromuscular agents
group III avermectins
group V organophosphates
Tb: thoroughbred
P : pleasure horses

anthelmintic treatment was given, in 4 studs horses were not treated at introduction and 4 owners did not introduce horses. The stocking rate varied from 0.8–7.2 horse/ha with an average of 2.4 horse/ha. On 14 of the farms the same pastures were used for grazing every year and on 1 stud, the owner relocated horses between pastures every year. On 5 properties the horses grazed together with horses from other farms and on 11 farms this did not occur.

Discussion

Our investigations revealed resistance to benzimidazoles in small strongyles (*Cyathostominae*) of horses in Denmark. Though the results do not give a true estimate of the prevalence of anthelmintic resistance in internal parasites of horses in Denmark, our observations indicate that this problem is widespread. This is in agreement with other studies done elsewhere in the world (*Barger & Lisle* 1979, *Kelly et al.* 1981, *Ulrich et al.* 1988, *Nilsson et al.* 1989) who found low values of FECR at the majority of farms examined. Further, the finding that anthelmintic resistance was indicated against benzimidazoles only is similar to observations in these studies. This may be explained by the fact that benzimidazole anthelmintics during the last 2 decades have obtained a substantial share of the anthelmintic market for horses as well as for other farm animals (see *Borgsteede et al.* 1983). All benzimidazoles have the same mode of action (*Lacey* 1988) which implies that once resistance has developed to one of the benzimidazoles (Group I) this confers resistance to other members of the same group, which is known as side resistance (*Prichard et al.* 1980). In this study it was observed that the majority of the owners alternated between anthelmintics in Group I. If this observation generally holds true, then it probably has contributed to the high numbers of reported instances of benzimidazole resistance in small strongyles of horses.

It is noteworthy that all reported cases of anthelmintic resistance in horses are confirmed to the small strongyles. This may be attributed to the biology of this group of nematodes and possibly to the pharmacokinetic properties of benzimidazoles in the horse. Further investigations are needed to elucidate this area.

Anthelmintic usage and management of livestock are factors of importance for the development of resistance (*Martin* 1986). The general features of the studs in this study include a high frequency of anthelmintic treatments, use of the same class of drugs over successive years, grazing of the same paddocks every year, high stocking rates and no anthelmintic treatment of horses introduced to farms. These are all factors known to contribute to selection for anthelmintic resistance (*Kelly at al.* 1981, *Martin* 1986).

Anthelmintic resistance is virtually only spread by hosts infected with resistant parasites. In this respect, mixed grazing between horses from different farms pose a risk for spreading the problem. Also by introducing new breeding stock to farms the risk of introducing resistant worms must be considered. It is therefore recommended to treat all horses with high doses of an anthelmintic against which no resistance has been recorded, before they are brought into the farm in order to eliminate any parasite worms.

When anthelmintic resistance has been detected the usage of the class of drug in question should be discontinued in order to prevent further selection of the parasite population. In future control an anthelmintic from a different group with a different mode of action should be used to reduce the risk of further selection for resistance. In addition the number of treatments must be reduced to as few as possible. It is advisable to rotate between classes of anthelmintics every second year to avoid continuous selection with the same class of drugs. It has been shown that mixtures of anthelmintics with different modes of action i.e. benzimidazoles plus piperazine or benzimidazoles plus levamisole can be used against benzimidazole resistant nematodes (*Barger et al.* 1985). However, such a strategy may be of limited value, since there has been a report on emergence of piperazine resistance in small strongyles of horses after the use of such mixtures over successive years (*Drudge et al.* 1988).

To avoid development of anthelmintic resistance other measures of parasite control, less dependent on anthelmintics should be considered. Relocation of horses to safe pastures, mixed or alternate grazing with cattle or sheep may also be highly efficient means of control. The removal of dung manually or mechanically as suggested by *Herd* (1981) may be another way to effectively reduce pasture contamination with infective larvae. At this stage anthelmintic resistance has also been described in intestinal nematode parasites of sheep and pigs in Denmark (*Bjørn et al.* in press, *Roepstorff et al.* 1987), though general information on the prevalence of this problem remains to be elucidated. Our observations may suggest that anthelmintic resistance is wide spread in flocks of horses in Denmark and it is likely that the problem will be of increasing importance in the future. To detect early development of anthelmintic resistance and to monitor the efficacy of anthelmintic treatments it is recommended to perform FECR tests at regular intervals. Such tests may also provide information on the parasite status in the stud upon which sound parasite control could be based.

Acknowledgements

This study was supported by Kreaturforsikringsselskabet Danmarks Fond, by grant no 5.23.99.02 from the Danish Agricultural and Veterinary Research Council and by Carlsbergfondet. Veterinary surgeon Poul Hjorth is acknowledged for collaboration in series B of the study. We are most thankful to laboratory technician Margrethe Paermann for her skilful identification of infective larvae. Dr. P. J. Waller, CSIRO, Division of Animal Health, McMaster Laboratory, Sydney, is thanked for reading and commenting the manuscript.

References

Anon: Report of the working Party for the Animal Health Committee of the Standing Committee on Agriculture: Anthelmintic resistance. Ed. PJ Waller, SCA Technical Report Series – No. 28, CSIRO Australia, East Melbourne.

Barger IA, Lisle IA: Benzimidazole resistance in small strongyle of horses. Aust. vet. J. 1979, *55,* 594–595.

Barger IA, James RE, Davies HI: Evaluation of paste formulations of fenbendazole plus piparazine against benzimidazole-resistant cyathostomes in horses. Aust. vet. J. 1985, *62,* 139–140.

Bauer C, Merkt JC, G Janke-Grimm G, Bürger H-J: Prevalence and control of benzimidazole-resistant small strongyles on german thoroughbred studs. Vet. Parasitol. 1986, *21,* 189–203.

Bauer C, Gandras R, Stoye M, Bürger H-J: Eine Feldstudie zur Anthelmintika-Resistenz von Strongyliden bei Pferden. (A field study on anthelmintic resistance in strongylids of horses). Berl. Münch. Tierärztl. Wschr. 1983, *96,* 312–316.

Bjørn H, Monrad J, Nansen P: Levamisole resistance in Ostertagia circumcincta from a sheep flock in Denmark. Acta vet. scand. 1991, *32,* 145–154.

Borgsteede FHM, Henriksen SvAa, Over HJ: Facts and reflections IV. Resistance of Parasites to Anthelmintics. Central Veterinary Institute, Lelystad, The Netherlands, 1983.

Dorny P, Vercruysse J, Berghen P: Resistance of equine small strongyles to benzimidazoles in Belgium. J. Vet. Med. B. 1988, *35,* 72–75.

Drudge JH, Elam G: Preliminary observations on the resistance of horse strongyles to phenothiazine. J. Parasit. 1961, *47,* 38–39.

Drudge JH, Lyons ET: Newer deveolpments in helminth control and Strongylus vulgaris research. Proc. 115th Meeting Am. Ass. Eq. Prac. 1965, 378–383.

Drudge JH, Lyons ET, Tolliver SC, Lowry SR, Fallon EH: Piparazine resistance in population-B equine strongyles: A study of selection in Thoroughbreds in Kentucky from 1966 through 1983. Amer. J. vet. Res. 1988, *49,* 986–994.

Geerts S, Guffens G, Brandt J, Kumar V, Eysker M: Benzimidazole resistance of small strongyles in horses in Belgium. Vlaams Diergeneesk. Tijdschrift 1988, *57,* 10–26.

Gibson TE: Some experiences with small daily doses of phenothiazine as a means of control of strongylid worms in the horse. Vet. Rec. 1960, *72,* 37–41.

Helle O: Tiabendazol og fenbendazol til bekjempelse av parasitter hos unghingster. Resistens mot fenbendazol hos små strongylider. (Treatment against parasites with thiabendazole and fenbendazole in young stallions. Development of resistance against fenbendazole in small strongyles). Norsk Veterinærtidsskrift 1986, *98,* 623–631.

Henriksen SvAa: A modified and simple McMaster technique. In: Epidemiology and Control of Nematodiasis in Cattle. (Eds. P. Nansen, R. J. Jørgensen & E. J. L. Soulsby), pp. 45–50, Martin Nijhof Publishers, London 1981.

Henriksen SvAa, Korsholm H: A method for culture and recovery of gastrointestinal strongyle larvae. Nord. Vet. Med. 1983, *35,* 429–430.

Herd RP, Miller TB, Gabel AA: A field evaluation of pro-benzimidazole, benzimidazole and non-benzimidazole anthelmintics in horses. J. Amer. Vet. Med. Assoc. 1981, *179,* 686–691.

Kelly JD, Webster JH, Griffin DL, Whitlock HV, Martin ICA, Gunawan M: Resistance to benzimidazole anthelmintics in equine strongyles. 1. Frequency, geographical distribution and relationship between occurrence, animal husbandry procedures and anthelmintic usage. Aust. vet. J. 1981, *57,* 163–171.

Lacey E: The role of the cytoskeletal protein, tubulin, in the mode of action and mechanism of drug resistance to benzimidazoles. Int. J. Parasitol. 1988, *18,* 885–936.

Lichtenfels JR: Nematode Parasites of Domestic Equids. Proc. helminth. Soc. Wash. 1975, *42,* special issue.

Nilsson O, Lindholm A, Christensson D: A field evaluation of anthelmintics in horses in Sweden. Vet. Parasitol. 1989, *32,* 163–171.

Martin PJ: Development and control of resistance to anthelmintics. In: Parasitology – Quo Vadit? Proc. IV. Int. Congr. Parasitol. (Eds. MJ Howell, pp. 493–501). Australian Academy of Science, Canberra 1986.

Prichard RK, Hall CA, Kelly JD, Martin ICA, Donald AD: The problem of anthelmintic resistance in nematodes. Aust. vet. J. 1980, *56,* 239–251.

Roepstorff A, Bjørn H, Nansen P: Resistance of Oesophagostomum spp. in pigs to pyrantel citrate. Vet. Parasitol. 1987, *24,* 229–239.

Round MC, Simpson DJ, Haselden CS, Glendinning ESA, Baserville RE: Horse strongyles tolerance to anthelmintics. Vet. Rec. 1974, *95,* 517–518.

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.

Ullrich D, Bauer C, Bürger H-J: Benzimidazol resistenz bei kleinen Strongyliden (*Cyathostominae*): Verbreitung in Pferdebeständen Nordrhein-Westfalens. (Benzimidazole resistance in small strongyles (*Cyathostominae*): Occurrence in horse farms in Nordrhein-Westfalen). Berl. Münch. Tierärztl. Wschr. 1988, *101,* 406–408.

Waller PJ, Prichard RK: Drug Resistance in Nematodes. In Chemotherapy of Parasitic Diseases. (Eds. W. C. Campbell & R. S. Rew), pp. 339–362, Plenum Publishing Corporation, New York 1986.

Sammendrag

Resistens overfor benzimidazolormemidler hos hestens små strongylider (Cyathostominae) i Danmark.

Formålet med dette arbejde var at undersøge, om ormemiddelresistens var tilstede hos indvoldsorm i danske heste. Der udvalgtes 16 hestebesætninger, hvor alle anthelmintiske behandlinger var registreret ved præparat og behandlingstidspunkt mindst to år tilbage før undersøgelsestidspunktet. I besætningerne gennemførtes en såkaldt "faecal egg count reduction" (FECR) test med henblik på at måle effektiviteten af det ormemiddel ejerne havde anvendt gennem de seneste år og endvidere foretoges en spørgeskemaundersøgelse over anvendelse af ormemidler, parasitbekæmpelse samt afgrænsningsforholdene i besætningen. I 13 besætninger blev der fundet resistens overfor benzimidazoler med FECR værdier varierende mellem 80.0 og −101.3 %, mens FECR i de 3 øvrige besætninger fandtes til henholdsvis 100.0 %, 99.3 % og 97.2 %. Resultaterne af spørgeskemaundersøgelsen viste, at hestene i gennemsnit behandledes 8.2 gange om året. I hovedparten af besætningerne anvendtes samme ormemiddel år efter år. Otte ejere ormebehandlede nyindkøbte heste, 5 ejere udførte ikke sådanne behandlinger og 3 ejere indkøbte aldrig heste. I 14 besætninger anvendtes samme areal til afgrænsning år efter år og belægningsgraden blev beregnet til 2.4 hest/ha i gennemsnit. Ormebekæmpelsesprogrammer, hvor risikoen for resistensudvikling er søgt minimeret, diskuteres ligesom bekæmpelsesmetoder uden anvendelse af ormemidler omtales.

(Received March 7, 1990; accepted June 28, 1990).

Reprints may be requested from: H. Bjørn, Department of Veterinary Microbiology, Laboratory of Parasitology, Royal Veterinary and Agricultural University, 13 Bülowsvej, DK-1870 Frederiksberg C, Denmark.