

# Risk Factors for High Endoparasitic Burden and the Efficiency of a Single Anthelmintic Treatment of Danish Horses

By M.M. Larsen<sup>1</sup>, S. Lendal<sup>1</sup>, M. Chriél<sup>1</sup>, S.N. Olsen<sup>2</sup> and H. Bjørn<sup>3†</sup>

<sup>1</sup>Department of Animal Science and Animal Health, Epidemiology, and <sup>2</sup>Department of Clinical Studies, Large Animal Medicine, The Royal Veterinary and Agricultural University, Frederiksberg, and <sup>3</sup>Department of Veterinary Microbiology, Danish Veterinary Institute, Copenhagen, Denmark.

**Larsen MM, Lendal S, Chriél M, Olsen SN, Bjørn H: Risk factors for high endoparasitic burden and the efficiency of a single anthelmintic treatment of Danish horses. Acta vet. scand. 2002, 43, 99-106.** – A questionnaire survey regarding endoparasite control practices in Danish horse herds was carried out in 1995. The participating veterinarians and herd owners were sampled using convenience and purposive sampling. In the analysis of risk factors for development of a high endoparasitic burden (>200 eggs per gram faeces) 903 horses were sampled and the analysis of the efficiency of a single anthelmintic treatment was based on 605 horses. The following factors had a significant effect on the endoparasitic burden: herd type, age of the horses, use of pasture rotation, anthelmintic treatment of horses visiting the herd, use of an adviser in the planning of endoparasite control and advice regarding pasture rotation. An interaction between pasture rotation and advice regarding pasture rotation was found, but due to high collinearity this was not reported. The factors influencing significantly on the reduction of the faecal egg count after a single anthelmintic treatment were the type of herd, the age of the horse, the drug used, and the anthelmintic-resistance-status of the herd. A negative effect of permanent pastures was observed. If pasture hygiene was performed on the advice of the veterinarian, the effect of a single anthelmintic treatment was less compared to a single anthelmintic treatment without any advice. An interaction between the treatment group and the resistance-status of the herd was found. Additional factors, normally accounted for, when endoparasites and anthelmintic resistance is discussed, were investigated, but not found significant in this study.

*endoparasites; epidemiology; anthelmintic resistance.*

## Introduction

In Danish horses resistance to benzimidazole products in the *Cyathostominae* spp. has been reported twice (Bjørn *et al.* 1991, Craven *et al.* 1998) and resistance to pyrantel is suspected (Craven *et al.* 1998). Anthelmintic resistance (AR) is regarded as an increasing problem worldwide, but the factors that contribute to the development of AR are often speculative. Epidemiological techniques have enhanced the

ability to study the complex interactions between factors that may contribute to the occurrence of AR. High frequency of treatment (Wescott 1986), repeated use of anthelmintics with the same mode of actions (Herd 1992), the generation intervals of the parasites (Dangolla 1994) are factors which have been associated with the risk of development of AR in production animals. A low efficiency of a drug might

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be an indication that AR has developed. Some parasites survive treatment, what facilitates selection of AR parasites (Prichard 1994). This makes it necessary to investigate the association between AR and management strategies, which potentially could contribute to its development. Factors previously identified in other production animals are also expected to be important for the development of AR in the horses, but as horses often are handled as a companion animal other factors might contribute to the development of AR.

The objective of this study was 1) to identify risk factors associated with high endoparasite burden and 2) to evaluate the efficiency of a single anthelmintic treatment of Danish horses.

### Materials and methods

In 1994 veterinarians from "The Danish Horse Practising Veterinarian Society" were contacted, and 22 veterinarians entered the survey and selected large (>15 horses) horse herds among their clients. In 1995 a questionnaire was sent to the selected herd owners. Sixty-eight herd owners answered the questionnaire, and 56 of the selected herds with a total of 903 horses entered a Faecal Egg Count Reduction (FECR) test (Craven et al. 1998).

In 1997 the herd owners were interviewed with the aim of validating the questionnaire (Lendal et al. 1998).

### Data analysis

Initially bivariate analyses were performed, and variables having p-values below 0.15 were included in the multivariate analysis (Table 1). A 2-level model was analysed in order to evaluate potential clustering of horses within herds. Due to absence of significant clustering, the data were consequently analysed as a single layer model. Variables were included in the model if  $p \leq 0.05$ .

### Risk factors for high endoparasite burden

The bivariate and the multivariate logistic regression analyses were based on the 903 horses with faecal egg counts measured as "Eggs Per Gram faeces" (EPG). The conceptual model was as follows:

$$Y_i = \beta_0 + X\beta + e_{0i}$$

$e_{0i} \sim N(0, \Omega e_{0i})$ ,  $\Omega e_{0i} = \sigma^2 e_{0i}$  and denotes the residual variation.

$Y_{ijk}$  is binominal distributed  $(\pi_i, 1)$ .

The estimation procedure fixed the dispersion parameter to 1. The dichotomized response variable was defined as EPG pre-treatment count, where one group required anthelmintic treatment (463 horses with  $EPG \geq 200$ ) and the other group did not require treatment (592 horses with  $EPG < 200$ ). The cut off for treatment was chosen because this level allows sufficient infection to induce immunity, but development of massive infection is prevented (Uhlinger, 1991). EPG used in this study consisted only of *Cyathostominae* spp. The ordinary logistic regression model parameter vector  $\beta$  was estimated by maximum likelihood estimation. The confidence interval of the estimates was based on Wald's statistics.

### Efficiency of a single anthelmintic treatment

The bivariate and the multivariate regression analyses were based on 605 horses. The conceptual model was:

$$Y_i = \beta_0 + X\beta + e_{0i}$$

$e_{0i} \sim N(0, \Omega e_{0i})$ ,  $\Omega e_{0i} = \sigma^2 e_{0i}$  and denotes the residual variation.

$Y_i \sim N(X\beta, \Omega)$ .

The response variable was defined as the difference between the natural logarithm of the EPG pre-treatment count and the natural logarithm of EPG 14 days post-treatment count. The ordinary generalised linear model parameter vector  $\beta$  was estimated by maximum likelihood estimation. Only horses that were examined with

Table 1. Variables extracted from the data and evaluated in the study.

Description and levels	
Herd type:	Stud farm Pension Riding school Other type (e.g. racing stables)
Age:	Foals Youngsters Adults
Treatment group:	Control Benzimidazoles Pyrantel Ivermectin
Diagnosed AR towards benzimidazoles in the herd in one or more horses:	Yes No or not known
Pastures are permanent:	Yes No or not known
Pasture rotation used:	Yes No or not known
Horses are grazing together with other species:	Yes No
Horses grazing with horses from other herds:	Yes No or not known
Dung spread on the pastures:	Yes No or not known.
Weight estimation method used:	Eye measure Weigh band Other methods
Weight estimation used in the dosing of anthelmintics:	Mean weight of horses, Weight of heaviest horse Individual weight Others
Anthelmintic treatment at turn out:	Yes No
Anthelmintic treatment at housing:	Yes No
Anthelmintic treatment at pasture rotation:	Yes No
Anthelmintic treatment of new horses bought:	Yes No
Anthelmintic treatment of visiting horses:	Yes No
Problems with diarrhoea are known:	Yes No or not known
Adviser is used in the planning of the endoparasite control:	Yes No
The veterinarian gives recommendations regarding pasture hygienic conditions:	Yes No
The veterinarian gives recommendations regarding time of treatments:	Yes No
The veterinarians recommendations are influenced by the season:	Yes No
The veterinarian gives recommendations regarding number of treatments:	Yes No
The veterinarian gives recommendations regarding choice of anthelmintic product:	Yes No

Table 2. Descriptive statistics for significant risk factors for high endoparasitic burden (EPG&gt;200) and odds-ratios (95% confidence limits) from multivariate analysis (n=903 horses).

Risk factors (levels)	No. of observ.	Bivariate analysis		Multivariate analysis	
		Prev. (%)	p-value	Odds-ratio	p-value
Herd type			0.001		0.0001
Stud farm	539	44		0.57 (0.44-0.75)	
Riding school	241	48		1.65 (1.20-2.27)	
Pension	188	31		0.47 (0.35-0.66)	
Other type	87	61			
Age			0.001		0.0001
Foals	92	35		0.99 (0.76-1.28)	
Youngsters	211	73		4.37 (3.59-5.31)	
Adults	736	38			
The veterinarian gives recommendations regarding pasture hygienic conditions			0.001		0.0003
Yes	473	37		1.80 (1.53-2.12)	
No	582	49			
Anthelmintic treatment of visiting horses			0.001		0.0008
No	283	35		1.91 (1.57-2.33)	
Yes	772	47			
Pasture rotation used			0.025		0.0028
Yes	754	45		1.76 (1.45-2.13)	
No	200	37			
Adviser is used in the planning of the endoparasite control			0.019		0.0062
Yes	538	47		1.54 (1.31-1.80)	
No	517	40			

the FECR test (820 horses) entered the study. A total of 215 horses were withdrawn due to zero egg count pre- and 14 days post-treatment.

## Results

### *Risk factors for high endoparasitic burden*

The factors influencing the endoparasitic burden are presented in Table 2. Strong collinearity was found between the factors "use of pasture rotation" and "veterinarian gives advice regarding pasture hygienic measures" ( $p < 0.001$ ).

### *Efficiency of a single anthelmintic treatment*

The multilevel model analysis revealed no sig-

nificant clustering between veterinarians or herds (data not shown). Factors having a significant influence on the efficiency of an anthelmintic treatment are presented in Table 3 and 4. Collinearity between the variable "the veterinarian gives advice regarding the choice of anthelmintics" and the variable "veterinarian gives advice regarding pasture hygienic measures" was seen ( $p < 0.007$ ).

## Discussion

### *Risk factors for high endoparasitic burden*

The risk for high endoparasitic burden was significantly increased for horses stabled in riding schools compared to horses from "other types".

Table 3. Descriptive statistics for risk factors associated with efficiency of anthelmintic treatment (FECR test). Estimates of the average Egg Per Gram faeces (EPG) reduction (difference between the logarithm of EPG pre-treatment and EPG post-treatment) with standard errors (SE) and P-values from bivariate and multivariate analysis are listed.

Risk factors (levels)	N	Bivariate analysis		Multivariate analysis	
		Mean	p-value	Estimate (SE)	p-value
Herd type			0.0001		0.0001
Stud farm	329	0.4614		-0.0321 (0.0682)	
Riding school	130	0.5911		0.1780 (0.0836)	
Pension	88	0.1781		-0.2844 (0.0810)	-
Other types	58	0.5556		0 (0)	
Age			0.0001		0.0044
Foals	55	0.3415		-0.1326 (0.0673)	
Youngsters	174	0.5223		0.0918 (0.0432)	
Adults	376	0.4438		0 (0)	
Pastures are permanent			0.1699		0.0012
Yes	438	0.4476		-0.1527 (0.0471)	
No	167	0.4819		0 (0)	
The vet gives recommendations regarding pasture hygienic conditions			0.0650		0.0001
Yes	240	0.3995		-0.1666 (0.0433)	
No	365	0.4950		0 (0)	
Treatment group			0.0001		
Control	233	0.0070		1	
Benzimidazole	221	0.5719		1	
Pyrantel	81	0.9470		1	
Ivermectin	70	1.0261		1	
Anthelmintic resistance towards benzimidazoles in the herd			0.1542		
Yes	482	0.4388		1	
No	123	0.5286		1	

<sup>1</sup> See Table 4.

The risk is decreased for horses in pensions and stud farms. Regarding the pensions it might be explained by the fact that the proportion of young horses was small in the pensions or because the horses often are private owned and therefore, the most efficient product is used even though it is the more expensive. In riding schools the economic output is important and therefore a tendency to use cheaper drugs and perhaps fewer treatments could be seen.

The findings regarding the age of the horse in this study are consistent with the findings in a study by *Love & Duncan* (1992). They indicated that the age of the host had an effect on the cyathostome infection level; the risk of having a high endoparasitic burden was higher in youngsters compared to adult horses. The fact that foals have lower endoparasitic burdens could, according to *Herd & Gabel* (1990), be due to the fact that foals have not yet acquired

Table 4. Estimations and standard errors (SE) of the interaction ( $p=0.0037$ ) between anthelmintic resistance (AR) towards benzimidazoles in the herd and treatment group ( $n=605$  horses).

	AR towards benzimidazoles in the herd	
	Yes	No
Treatment group		
Benzimidazole	0,5257 (0,0484)	0,8552 (0,0764)
Pyrantel	0,9976 (0,0695)	0,8700 (0,1049)
Ivermectin	1,0482 (0,0663)	0,8594 (0,2084)
Control	0 (0)	0,1360 (0,0746)

any immunity, which means that a greater accumulation of encysted cyathostominae in the caecal and colonic mucosa is allowed. In this stage they do not yet contribute with massive faecal egg output.

The stocking density is often mentioned when risk factors for high endoparasitic burden are discussed (Herd 1987, Bjørn et al. 1991). In this study it was not possible to calculate and evaluate the exact stocking density. However, a previous study indicated that the average stocking density was lowest in pensions (Lendal et al. 1998), and therefore the horses would not be forced to graze close to the roughs (Herd 1987) and consequently have a lower risk of endoparasitic infections than horses stabled in riding schools.

If the herd owner used an adviser in the planning of endoparasite control or if the veterinarian gave advice regarding pasture hygienic measures there was an increased risk of high endoparasitism. This is probably caused by selection procedure, since 53% of the herds were included due to suspicion of endoparasitic problems in the herd.

In other studies when factors influencing the endoparasitic burden are discussed, factors like pasture rotation (Bjørn et al. 1991), alternate grazing (Eysker et al. 1986), permanent pastures (Herd 1987) are included as preventive measures. These factors were also investigated

in this study. It was seen that the risk for high endoparasitic burden increased if pasture rotation was used. This could have something to do with the negative effect of the veterinarians' recommendations regarding pasture hygienic measures. An explanation could be that horses perhaps return to a pasture where they have already been earlier in the grazing season and therefore a correct pasture rotation procedure has not been used.

Strategic treatments (Herd et al. 1985) could also act as a preventive measure. In this study it was investigated whether there was an effect of treatment at turn out, pasture rotation, at housing, introduction of new horses either bought or visiting horses. Only treatment of visiting horses was significant and the risk for high endoparasitic burden was increased if visiting horses were not treated with anthelmintics.

It is important to bear in mind that although low faecal egg counts are found, cyathostomes can still be present because it is possible to find zero egg counts even if the horse is severely infected (Herd 1992). This means that some horses in this analysis might be false negatives. Fifty horses (5%) had zero egg counts pre-treatment and positive egg count post-treatment and they indicate the minimum number of false negatives. Clinical signs of diarrhoea can be associated with cyathostomes (Love 1992), but in this study no association between diarrhoea and high endoparasitic burden was found.

#### *Efficiency of a single anthelmintic treatment*

Horses stabled in riding schools, stud farms or other types have a higher reduction in the endoparasitic burden after a single anthelmintic treatment than horses stabled in pensions. Youngsters have a larger reduction and foals a smaller compared to adult horses. The fact that there is reduced efficiency of anthelmintics in foals could, according to Herd & Gabel (1990), be due to accumulation of encysted cyathos-

tomes in the caecal and colonic mucosa and in this stage they are not affected by anthelmintics.

The use of permanent pastures (not ploughed or renewed between grazing seasons) lowered the efficiency of a single anthelmintic treatment compared to pastures renewed between grazing seasons. This is consistent with *Herd* (1987) who mentioned that ploughing expose the larvae on the pastures and will lead to death as a result of desiccation.

If the veterinarians gave advice regarding pasture hygienic measures, the reduction would be smaller than if the veterinarian did not give this kind of advice. This might be caused by selection bias because 53% of the herds were actually selected by the veterinarians due to suspicion of endoparasitic problems.

A statistical significant interaction between the pharmacological group and the AR-status of the herd was found. As expected benzimidazole treated horses in herds with a diagnosed AR have smaller efficiency of the anthelmintic treatment than ivermectin or pyrantel treated horses.

The effect of strategic seasonal treatments (turn out, pasture rotation or housing) was evaluated but all were non-significant. Likewise no effect of a single treatment of horses when introduced (newly bought or visiting) was found. The effect of pasture rotation, alternate grazing, grazing with horses from other herds or the spread of horse dung on the pasture was also investigated but not significant. There were no indications of problems with underdosing even though eye-measure was used as weight estimation method. This has been mentioned previously as a risk factor for development of AR (*Besier & Hopkins* 1988). Advice obtained from veterinarians regarding annually number of treatments, choice of anthelmintic, or time of treatment, did not have any significant effect on the reduction.

## Conclusion

The herd type and the age of the host are the most important factors both regarding risk for high endoparasitic burden was increased endoparasitic burden and effect of an anthelmintic treatment. Unfortunately, advice obtained from veterinarians regarding pasture hygienic measures had a negative effect on both the risk for high endoparasitic burden and the effect of treatment. This means that more emphasis has to be put on the recommendations provided from veterinarians on the subject. The herd owners need to be more aware about the life cycle and epidemiology of endoparasites in order to obtain a better understanding of the recommendations given by veterinarians in order to take advantage of these in a more efficient way. It should be made perfectly clear that the use of alternative control measures should be extended and that the use of anthelmintics should be under more strict control in order to avoid AR towards the few still effective products.

## Acknowledgements

This study was supported by Merial A/S (formerly MSD-AgVet), Copenhagen, Denmark. The authors would like to thank the participating veterinarians and herd owners for taking time to answer the questionnaires.

## References

- Besier RB, Hopkins DL*: Anthelmintic dose selection by farmers. *Aust. Vet. J.* 1988, 65, 193-194.
- Bjørn H, Sommer C, Schougaard H, Henriksen Sv Aa, Nansen P*: Resistance to benzimidazole anthelmintics in small strongyles (*Cyathostominae*) of horses in Denmark. *Acta. Vet. Scand.* 1991, 32, 253-260.
- Craven J, Bjørn H, Henriksen Sv Aa, Nansen P, Larsen M, Lendal S*: A survey of anthelmintic resistance on Danish horse farms, using 5 different methods of calculating faecal egg count reduction. *Equine Vet. J.* 1988, 30, 289-293.
- Dangolla A*: Epidemiological analysis of helminthological data from Danish sow herds with emphasis on occurrence of anthelmintic resistance. Ph.

- D. Thesis. The Royal Veterinary and Agricultural University, Copenhagen. 1994.
- Eysker N, Jansen J, Mirck MH: Control of strongylosis in horses by alternate grazing of horses and sheep and some other aspects of the epidemiology of strongylidae infections. *Vet. Parasitol.* 1986, *19*, 103-115.
- Herd RP: Pasture hygiene. In: Robinson, N.E. (edi.): Current therapy in equine medicine 2. W.B. Saunders Company. 1987, 334-336.
- Herd RP: Choosing the optimal equine anthelmintic. *Veterinary Medicine.* 1992, *87*, 213-239.
- Herd RP, Willardson KL, Gabel AA: Epidemiological approach to the control of horse strongyles. *Eq. Vet. J.* 1985, *17*, 202-207.
- Herd RP, Gabel AA: Reduced efficacy of anthelmintics in young horses compared with adult horses. *Eq. Vet. J.* 1990, *22*, 164-169.
- Lendal S, Larsen MM, Bjørn H, Craven J, Chriél M, Olsen SN: A questionnaire survey on nematode control practices on horse farms in Denmark and the existence of risk factors for the development of anthelmintic resistance. *Vet. Parasitol.* 1998, *78*, 49-63.
- Love S: Parasite associated equine diarrhoea. *Comp. Cont. Educ. Pract. Vet.* 1992, *14*, 642-649.
- Love S, Duncan JL: The development of naturally acquired cyathostome infections in ponies. *Vet. Parasitol.* 1992, *44*, 127-142.
- Prichard R: Anthelmintic resistance. *Vet. Parasitol.* 1994, *54*, 259-268.
- Uhlinger CA: Equine small strongyles: Epidemiology, pathology and prevention. *Comp. cont. Educ. Pract. Vet.* 1991, *13*, 863-869.
- Wescott RB: Anthelmintics and drug resistance. *Vet. Clin. North. Am. [Equine Pract.]* 1986, *2*, 367-380.

## Sammendrag

*Risikofaktorer for høj endoparasitær belastning og effekt af en enkelt anthelmintisk behandling af danske heste.*

I 1995 blev der foretaget en undersøgelse af praksis for endoparasitkontrol i danske hestebesætninger. Data fra hestebesætningerne blev indsamlet gennem dyrlæger, der havde givet tilsagn om at deltage i undersøgelsen. Der indgik ialt 906 heste i undersøgelse af risikofaktorer for høj parasitbyrde (>200 æg pr. gram fæces), og 605 heste i undersøgelse vedrørende effekten af behandling med anthelmintika (forskellen mellem før og efter behandling). Signifikante risikofaktorer for høj parasitbyrde var besætningskategori, hestens alder, brug af foldrotation, strategisk anthelmintikabehandling af nye heste, rådgivning om mulige kontrolforanstaltninger og foldrotation. Der var vekselvirkning mellem foldrotation og råd om samme, men på grund af høj grad af collinearitet, er dette ikke anført. Faktorer, der havde signifikant sammenhæng med effekten af anthelmintikabehandling vurderet ved reduktion i antallet af æg pr. gram fæces, var besætningskategori, hestens alder, anvendte præparat og besætningens anthelmintika-resistens status. Besætninger med permanente græsgange havde større effekt af en enkelt behandling end andre besætninger. Effekten af en enkelt behandling var mindre, såfremt besætningsejeren gennemførte græsmarkshygiejniske tiltag som følge af råd fra den praktiserende dyrlæge. Der var vekselvirkning mellem det anvendte præparat og besætningens anthelmintika-resistens status.

*(Received December 6, 2001; accepted January 21, 2002).*

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