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*Published in:*  
Veterinary Parasitology

*DOI:*  
[10.1016/j.vetpar.2010.03.001](https://doi.org/10.1016/j.vetpar.2010.03.001)

*Publication date:*  
2010

*Document version*  
Publisher's PDF, also known as Version of record

*Citation for published version (APA):*  
Becher, A. M., Mahling, M., Nielsen, M. K., & Pfister, K. (2010). Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): an investigation into strongyle egg shedding consistency. *Veterinary Parasitology*, 171(1-2), 116-122. <https://doi.org/10.1016/j.vetpar.2010.03.001>



## Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): An investigation into strongyle egg shedding consistency

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### ARTICLE INFO

#### Article history:

Received 22 October 2009

Received in revised form 24 February 2010

Accepted 1 March 2010

#### Keywords:

Strongyles

Horse

Egg shedding

Consistency

Epidemiology

Selective anthelmintic treatment

### ABSTRACT

For 9 consecutive months (March–November 2008), faecal samples were collected monthly from 129 horses residing within 40 km of Salzburg, Austria. Samples were analysed quantitatively using a modified McMaster egg counting technique. Whenever a faecal egg count (FEC) result exceeded 250 eggs per gram (EPG), the horse was treated with pyrantel, ivermectin or moxidectin.

In 52 of 129 horses (40.3%), no strongyle eggs were ever detected over the course of 9 months. In 39 horses (30.2%), strongyle eggs were detected in at least 1 sample, but the egg count never exceeded 250 EPG. The remaining 38 (29.5%) horses were treated at least once in response to a FEC that exceeded 250 EPG. As a result of this selective anthelmintic scheme, the total number of anthelmintic treatments was reduced to 54% of the number of treatments administered to the same horses in the previous year. Both the maximum and mean FEC dropped significantly after initiation of the study. A statistically significant, negative correlation was demonstrated between the maximum and mean FEC of a horse and its age. Pasture hygiene appeared to reduce FECs, but the effect was not statistically significant. The magnitude of the initial FEC was significantly correlated with the maximum FECs in the subsequent 8 months ( $p < 0.01$ ). The same relationship was observed for the maximum FEC of the first 2 samples. Furthermore, horses which required several anthelmintic treatments had a higher initial FEC and a greater maximum FEC in the first 2 samples than horses which received only one or no treatment. These results suggest that selective anthelmintic treatment accomplished a reduced pasture contamination with strongyle eggs, while simultaneously decreasing the number of anthelmintic treatments. Sustained implementation of a selective treatment strategy has the potential to reduce selection pressure for anthelmintic resistance. These results reported herein will assist equine practitioners in designing and monitoring sustainable anthelmintic treatment programs.

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### 1. Introduction

Since macrocyclic lactone resistance was first reported in small strongyles, no anthelmintic drug can be used in horses without valid concerns about its efficacy (Kaplan, 2004; Trawford et al., 2005). The high prevalence of

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anthelmintic resistance in cyathostomes worldwide mandates that future control efforts should be designed to slow the development of parasite resistance to anthelmintic drugs (Kaplan, 2004; Matthews, 2008).

It is widely accepted that due consideration of the role of parasite *refugia* is key to preserving the efficacy of anthelmintic drugs in worm control programs (van Wyk, 2001; Pomroy, 2006). One way to maximize *refugia* is by applying selective, targeted treatment as part of a sustainable equine nematode control program (Matthews, 2008; Nielsen, 2008).

Selective anthelmintic treatment for equine parasite control was first suggested nearly 20 years ago (Duncan and Love, 1991; Gomez and Georgi, 1991). Selective treatment schemes are based on quantitative analysis of faecal samples from all horses on a given premise. Horses with strongyle FECs that exceed a predefined threshold are treated with anthelmintic drugs, and the remainder of the population is left untreated (Gomez and Georgi, 1991).

One of the basic principles of selective anthelmintic treatment is a consistency of the relative magnitude of strongyle FECs of individual horses over time (Duncan and Love, 1991). Identification of high egg shedders within the herd is an essential goal, and the consistency of egg shedding patterns can be exploited to reduce the number of faecal samples (Gomez and Georgi, 1991; Döpfer et al., 2004; Nielsen et al., 2006; Eysker et al., 2008).

However, an optimal regimen has yet to be devised for determining the number and frequency of faecal analyses recommended for accurate identification of high egg shedders, especially at the beginning of a selective anthelmintic treatment program. Several approaches have been published. One straightforward suggestion was monthly faecal sampling for 6 months, or sampling at intervals of 4–8 weeks (Matthee and McGeoch, 2004). Several other studies used intervals between 3 weeks and 8 weeks (Duncan and Love, 1991; Gomez and Georgi, 1991; Krecek et al., 1994; Little et al., 2003; Döpfer et al., 2004). In Denmark, faecal samples are usually examined twice annually, between March and May and again during August–September (Nielsen et al., 2006).

The major aim of the present study was to collect data on the quantitative excretion of strongyle eggs by individual horses on several farms during the grazing season, while applying a selective anthelmintic treatment program. An additional goal was to reassess the strongyle egg shedding consistency under conditions of greater sampling frequency. Ultimately, the results might identify a minimum number of faecal samples required, as well as an optimal interval between sampling for evidence-based implementation of a selective anthelmintic treatment scheme.

## 2. Material and methods

### 2.1. Farms and animals

Faecal samples were collected from 129 horses residing on 19 farms within 40 km of Salzburg (Federal State of Bavaria/Germany and Federal State of Salzburg/Austria). Herd sizes ranged from 2 horses per farm to 27 horses per farm. Horse ages ranged between 10 months and 30

**Table 1**

Sex and breed of the 129 horses in the study.

Sex	
Mares	56
Geldings	70
Stallions	3
Breeds	
Warmblood	68
US breeds	23
Pony breeds	12
Haflinger	8
Thoroughbreds	5
Austrian Noriker	5
Arabians	5
Spanish breeds	2
Norwegian Fjordhorse	1

years, with a mean of 11.5 years. Additional information on sex and breed of the horses is presented in Table 1. Information about herd management was solicited, including the number of anthelmintic treatments administered during 2007, and standard pasture hygiene practices. Horses were assigned to 2 groups depending on whether they were grazing on pastures which were cleaned of faeces at least once a week or less. The number of horses in each group was determined by the established management procedures on the farms.

### 2.2. Faecal samples

Fresh faecal samples were collected from individual horses immediately after defaecation. Samples were collected at 4-week intervals between March and November 2008 (i.e., 9 samples per horse). In total, 1161 faecal samples were collected and analysed.

### 2.3. Faecal analysis

Faecal samples collected from March 2008 to October 2008 were analysed quantitatively, using a modified McMaster technique with a sensitivity of 30 eggs per gram (EPG). The last samples collected in November 2008 were analyzed by a modified McMaster procedure with a sensitivity of 20 EPG (Wetzel, 1951).

### 2.4. Treatment

Whenever a FEC result was >250 EPG, the respective horse was always treated 7 days after the FEC analysis. A standard treatment algorithm was followed for all horses. The first time the FEC cut-off value was exceeded, the horse was treated orally with pyrantel embonate (Verminal P<sup>®</sup>, Albrecht GmbH; 19 mg/kg). If the same horse again exceeded the cut-off value, it was treated orally with ivermectin (Diapec P Gel<sup>®</sup>, Albrecht GmbH; 0.2 mg/kg). When a third FEC > 250 EPG was recorded for an individual horse, moxidectin (Equest Orales Gel<sup>®</sup>, Fort Dodge Veterinär GmbH; 0.4 mg/kg p.o.) was administered. Prior to all treatments, contemporaneous body weights were determined by using a girth tape. According to the number of egg counts exceeding 250 EPG, each horse was assigned into 1

**Table 2**

Number of horses exceeding different faecal egg count values in the study.

FEC	Number of occasions	Number of horses (%)	Mean age in years (standard deviation)
>250 EPG	At least once	38 (29.5)	9.5 ± 8.5
<250 EPG	All	91 (60.5)	12.5 ± 5
<250 EPG	All <sup>a</sup>	39 (30.2)	11.5 ± 5
>0 EPG	All	0 (0)	–
0 EPG	All	52 (40.3)	13 ± 5

<sup>a</sup> At least one positive egg count during the study.

of 4 groups (A: 0 × FEC > 250 EPG; B: 1 × FEC > 250 EPG; C: 2 × FEC > 250 EPG; D: 3 × FEC > 250 EPG).

Independent of other anthelmintic treatments, all owners administered an autumn treatment with moxidectin plus praziquantel (Equest Pramox 19.5 + 121.7 mg/g Gel zum Eingeben®, Fort Dodge Veterinär GmbH; Moxidectin: 0.4 mg/kg p.o. and Praziquantel 2.5 mg/kg p.o.) in order to control *Gasterophilus* spp. and tapeworm infections. Horses with a FEC > 250 EPG in October received their autumn treatment 7 days later, but all other horses were administered moxidectin plus praziquantel after the last faecal sample had been collected in November.

Horses receiving any anthelmintic treatment for reasons other than a strongyle FEC > 250 EPG (e.g., high *Parascaris* egg count) were excluded from the study. Similarly, horses which contributed fewer than 9 faecal samples were excluded from the study.

### 2.5. Statistics

Data were analysed using the software package PASW (Predictive Analytics Software) Statistics 17.0. 2. from SPSS/USA. Results of all statistical tests were considered statistically significant if the calculated *p*-values were less than 0.05. Nonparametric tests were selected for most statistical analyses, due to the non-normal distribution of FEC data and the existence of outliers. For analysis of the correlation between two metric variables, Spearman's correlation coefficient was used. In order to find differences among groups A–D, the analysis was done with the Kruskal–Wallis-test. The effect of pasture hygiene on groups A–D was analyzed with a linear regression model (Poisson distribution and log link; covariates age and pasture hygiene).

An analysis of egg shedding consistency was performed with the exact Wilcoxon Rank test for horses that did not receive anthelmintic treatment. At each sampling interval, all horses shedding fewer than 250 EPG up to that point were included in a month-to-month comparison of faecal egg count levels.

## 3. Results

### 3.1. Faecal egg counts

Of the 1161 faecal samples analysed, 71 exceeded 250 EPG, 262 samples yielded FECs between 20 EPG and 250 EPG, and the remaining samples (828) had FEC below the level of sensitivity of the McMaster's technique. On two farms, the results of all analysed samples were below the detection level. Table 2 presents the distribution of pos-

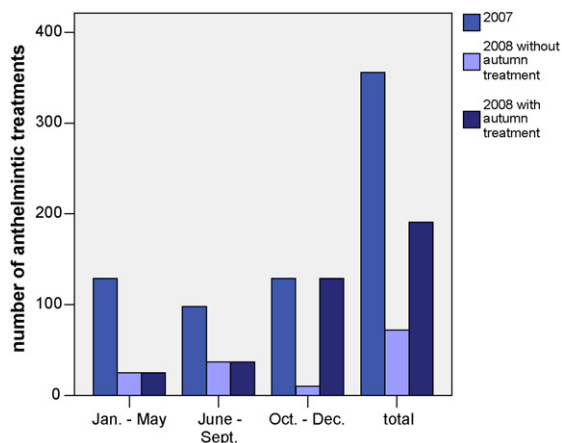
itive FECs among the examined horses. Of the 38 horses with faecal egg counts >250 EPG, the cut-off was exceeded once by 13 animals (group B), and two or three times by 17 (group C) and 8 horses (group D), respectively, during the study period.

### 3.2. Treatments

Faecal egg count results >250 EPG triggered 62 anthelmintic treatments during spring and summer (March–September) of 2008. Not including the autumn treatment for *Gasterophilus* and tapeworms, 71 total anthelmintic doses were administered during the 9-month observation period, which averaged less than one treatment per enrolled horse. When the standard autumn treatment was included, the 129 enrolled horses received a total of 191 treatments. The participating horses had been treated with various anthelmintic drugs between two and four times annually during 2007, but the implementation of a selective anthelmintic treatment program reduced the total number of treatments by 46% on an annual basis (Fig. 1). There was no indication of reduced efficacy by any of the anthelmintic drugs (data not shown).

### 3.3. Maximum and mean faecal egg counts

In March, the faecal egg count results of 5 of 129 horses exceeded 1000 EPG, with a maximum of 4950 EPG. Following initiation of the selective treatment program, no horse exceeded 1000 EPG in any of the 7 subsequent samples. During November, 1 horse exhibited a strongyle egg count of 1740 EPG.



**Fig. 1.** Comparison of the number of anthelmintic treatments in 2007 and 2008 according to the season.

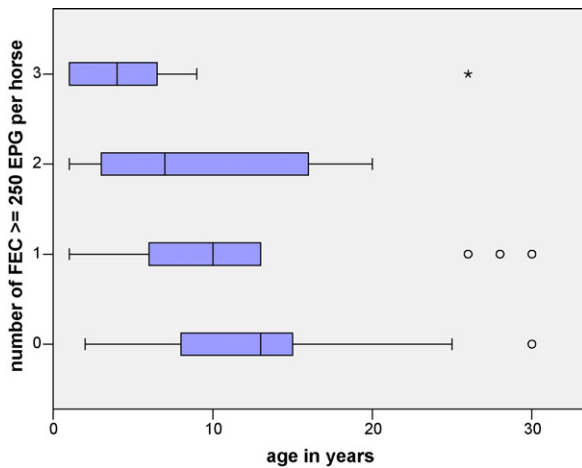


Fig. 2. The number of faecal samples exhibiting >250 EPG per horse, in relation to the age of the same horse.

When the study was initiated (March 2008), the mean FEC of all horses was 162 EPG (standard deviation 603 EPG). During the subsequent period of selective anthelmintic treatment, the mean FEC remained between 20 EPG (standard deviation 66 EPG) and 65 EPG (standard deviation 163 EPG).

### 3.4. Age

The mean faecal egg count of each horse was negatively correlated to age ( $R = -0.328$ ;  $p < 0.01$ ). Similarly, the maximum FEC of each horse was negatively correlated with age ( $R = -0.323$ ;  $p < 0.01$ ). The number of FECs exceeding 250 EPG per horse decreased with age as presented in Fig. 2. Asymptotically significant differences were revealed in relation to age among the 4 groups A–D with different numbers of FECs > 250 EPG per horse ( $p < 0.01$ ).

### 3.5. Pasture hygiene

Twenty-nine horses resided on premises where faeces were removed from pasture at least once weekly. The monthly mean faecal egg count of this group was consistently lower than the monthly mean FEC of 100 animals which grazed pastures that were cleaned less frequently or not at all (Fig. 3).

In addition, a difference between the 2 groups of pasture hygiene was observed in terms of number of FECs > 250 EPG per animal. But no effect of pasture hygiene on the number of FEC > 250 EPG per animal was found to be statistically significant ( $p = 0.063$ ; 95% confidence interval between  $-1.437$  and  $0.038$  of the effect of pasture hygiene in comparison to age).

### 3.6. First faecal egg count

The magnitude of the FEC of the initial faecal sample was positively correlated to the maximal FEC of the subsequent 8 samples from the same horse ( $R = 0.636$ ;  $p < 0.01$ ).

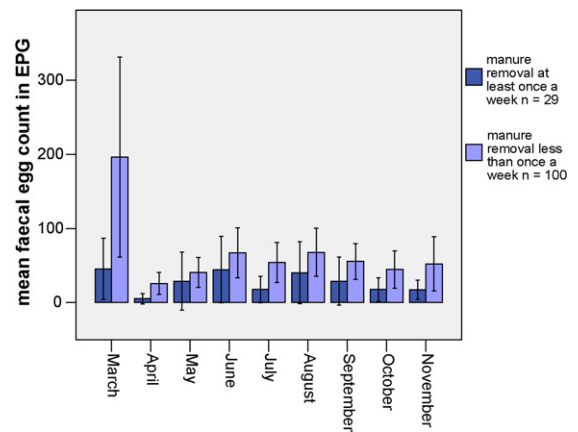


Fig. 3. Comparison of the monthly mean faecal egg counts (with the 95% confidence interval) of 2 groups of horses with different pasture hygiene.

Among groups A–D, there were differences in terms of the magnitude of the initial egg count of the same horse in March as can be seen in Fig. 4. These differences were statistically significant ( $p < 0.01$ ).

### 3.7. First and second faecal egg count

A positive correlation was demonstrated between the maximum FEC of the first 2 samples in March and April and the maximum FECs of the subsequent 7 samples of the same horse ( $R = 0.694$ ;  $p < 0.01$ ). Estimated probabilities for the maximum FEC of the later 7 samples of 1 horse, based on the outcome of the first 2 samples are presented in Table 3.

There were differences in terms of the maximum FEC of the first 2 samples for each individual horse among the 4 groups of horses A–D with different numbers of FECs > 250 EPG and therefore receiving anthelmintic treatment. The differences were statistically significant ( $p < 0.01$ ).

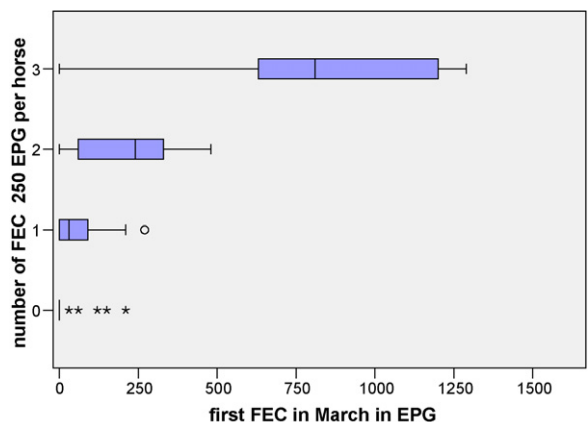


Fig. 4. Level of the first FEC and the number of FECs > 250 EPG for the same horse. 0 FECs > 250 EPG:  $n = 91$ ; 1 FECs > 250 EPG:  $n = 13$ ; 2 FECs > 250 EPG:  $n = 17$ ; 3 FECs > 250 EPG:  $n = 8$ . Not depicted are one outlier at 4950 EPG with 3 times > 250 EPG and two outliers at 3210 EPG and 3000 EPG with 2 times > 250 EPG.

**Table 3**

Estimated probability for the maximal FEC of the following 7 samples depending on the outcome of the first 2 samples of the same horse.

Results of first 2 samples	Maximum of the later 7 samples (EPG)	Probability (%)
0 EPG, 0 EPG	0	62
0 EPG, 0 EPG	<200	88
0 EPG, 0 EPG	<250	92
<250, <250 EPG <sup>a</sup>	<250	82

<sup>a</sup> There was no horse with the first two FECs > 250 EPG, as all horses with the first FECs > 250 EPG received anthelmintic treatment.

### 3.8. Month-to-month comparison

The results of all horses not exceeding 250 EPG until the first analysed month were compared with the results of the same horses in the following month. As presented in Table 4 statistically significant differences were found between May and June as well as between July and August.

## 4. Discussion

The present results clearly demonstrate that a selective treatment program under field conditions can lead to a reduction of the number of anthelmintic treatments without undue risk of excessive egg shedding. Our data provide further evidence that the egg shedding levels are influenced by both the age of the horse and level of pasture hygiene. In addition, the data support the consistency of strongyle egg shedding by individual horses. In the present study, the first and second FECs of a horse strongly predicted the level of egg shedding by the same horse during the subsequent 7 months. However, the results also suggest that a sampling interval of 4 weeks during the grazing season was often too frequent to detect differences between samples.

### 4.1. Treatment

In this study, implementation of selective therapy required a total of only 71 anthelmintic treatments for FEC > 250 EPG. Compared to a strategy of treating all herd members four times annually, this represents a reduction of 86% in the cumulative number of doses. When the special autumn treatments of all horses are included, selective treatment still provided a 63% reduction in treatment numbers. For horses enrolled in the study, the present program led to a 46% decrease compared to the number of treatments administered during 2007. Other authors have reported that selective anthelmintic therapy reduced

**Table 4**

Results of the month-to-month comparison with the exact Wilcoxon Rank tests: horses shedding less than 250 EPG up to the first of the 2 months were included, as all other horses were treated between the 2 samples.

Months	Mean FEC in the first month in EPG	n	p (two sided)
March–April	19.91	113	0.402
April–May	11.35	111	0.088
May–June	15.14	109	0.001
June–July	17.06	102	0.360
July–August	16.67	99	0.000
August–September	19.59	95	0.350
September–October	17.74	93	0.732
October–November	16.31	93	0.446

anthelmintic treatment numbers by 36–77% (Gomez and Georgi, 1991; Krecek et al., 1994; Little et al., 2003; Matthee and McGeoch, 2004). A comparison of the various studies is difficult because different EPG thresholds were used and the selective treatment method was compared to different strategies ranging from four (Krecek et al., 1994), five or more (Matthee and McGeoch, 2004) or six treatments per year (Gomez and Georgi, 1991; Little et al., 2003). Additionally, all cited studies were based on a 12-month period in contrast to the 9 months of the present study. Furthermore, autumn treatments were not administered in these studies.

### 4.2. Reduction of pasture contamination

Decreases in both the maximum and the mean faecal egg count after the first sampling indicate that a selective treatment program is a potent tool for reducing pasture contamination with strongyle eggs.

### 4.3. Age

The slightly negative correlation between mean and maximal faecal egg count and the age of the respective, individual horse means that younger horses shed more strongyle eggs. Additionally, horses with one or more FECs > 250 EPG, and therefore receiving treatment, tended to be younger than horses for which all FECs were < 250 EPG. These findings are consistent with the well-known relationship of age to the level of egg shedding that had been reported previously (Herd, 1993; Uhlinger, 1993; Little et al., 2003; Döpfer et al., 2004; Matthee and McGeoch, 2004).

### 4.4. Pasture hygiene

Pasture transmission is crucial to the epidemiology of equine strongyle infections, so it was important to consider this variable in the present trial, even though the available data were limited. Various authors have recommended pasture hygiene in addition to selective treatment because removal of faeces from grazing areas interrupts the strongylid life cycle (Herd, 1986; Matthee and McGeoch, 2004; Matthews, 2008). This study provided partial evidence to support this hypothesis because the mean FECs of horses grazing pastures which were cleaned weekly generally were lower than horses grazing partially or non-cleaned pastures, although the differences were not significant ( $p > 0.05$ ).

#### 4.5. Shedding consistency

For individual horses, the magnitude of the initial egg count was significantly correlated to the maximal egg count of the subsequent 8 samples. The same relationship was demonstrated for the maximal FEC of the first two egg counts and the maximal egg count of the succeeding 7 samples. These findings support the consistency of strongyle egg shedding patterns of individual horses. In addition, the present findings are consistent with the results of two recent studies: In The Netherlands, 2 samples were collected from 484 horses at an interval of 6 weeks, and examined quantitatively with a detection limit of 50 EPG. This trial revealed that 55.2% of horses had consistently low (<100 EPG) egg counts (Döpfer et al., 2004). A Danish study with 424 horses examined 3 samples from the same horse at intervals up to 7 months, using a technique with a sensitivity of 50 EPG. This study demonstrated an 84% probability of a FEC < 200 EPG after two prior FECs from the same horse were both <200 EPG (Nielsen et al., 2006).

Demonstration of consistency in egg shedding patterns means that an initial faecal sample before the start of a selective anthelmintic therapy program is predictive of the level of egg shedding and number of treatments required during the following months. These results are consistent with those of a study in the US (Gomez and Georgi, 1991). Because the correlation between the maximal FEC of the first 2 samples and the maximal FECs of the succeeding 7 samples from the same horse was even higher, the predictive value of egg shedding patterns can be improved by analysing 2 faecal samples from the same horse. However, during most months of the grazing season, a sampling interval of 4 weeks apparently was too brief to demonstrate markedly different results.

#### 4.6. Practical considerations

A reduction in anthelmintic treatment intensity, as achieved in the present study, is one possibility for decreasing the rate of selection for resistance to macrocyclic lactones (Kaplan, 2002). From a practical standpoint, however, it is still unclear how many FECs are needed to implement a selective anthelmintic treatment program.

The data presented herein suggest that horses with a low first and second faecal egg counts are less likely to shed high numbers of worm eggs in the following months, and therefore require fewer FECs and anthelmintic treatments. Results from Denmark (Nielsen et al., 2006) concluded that two negative or low faecal egg counts, independent of the sampling interval, can identify a majority of low-shedding horses.

But, this begs the question: How many additional FECs are needed to identify those high egg shedders that exhibit low first and second FECs?

An optimal interval between faecal samples cannot be determined from the results of this study. According to the month-to-month comparison in spring and autumn, an interval of 4 weeks seems to be too short. On the other hand a 4-week interval can be useful in the summer. Other variables should be considered as well, including the result

of the first and second faecal egg count, the age of the horse and the frequency of pasture hygiene measures. The present study suggests that young horses with high first FEC on a pasture where manure is not removed should be monitored more frequently by FECs than older horses with a low first FEC on a pasture that is cleaned weekly.

Further studies are needed to determine a precise interval between FECs and a precise number of FECs in the first year of a selective treatment program. A possible approach could be to compare selectively treated groups with untreated control groups. This is especially important for the analysis of the precise role of pasture hygiene.

Additional FEC data are needed during the second and later years of a selective treatment program to determine whether the frequency of faecal monitoring can be reduced in subsequent grazing seasons.

Ultimately, implementation of selective anthelmintic therapy will help to maintain the current efficacy of macrocyclic lactones against cyathostomins, particularly because no new products for horse nematodes appear to be imminent.

#### Acknowledgments

The authors thank Dr. E. Müller for implementing and supporting this study from the very first beginning. We are grateful for the encouragement and collaboration of various horse and stable owners. We acknowledge H. Husoska, E. Kiess, M. Scheuerle and K. Simon for the reliable and tireless work in the laboratory. We thank Dr. Ray Kaplan and Dr. Craig Reinemeyer for critically reviewing the manuscript.

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