Journal of Equine Veterinary Science 33 (2013) 161-164

Journal of Equine Veterinary Science

journal homepage: www.j-evs.com

Original Research

Daily Variability of Strongyle Fecal Egg Counts in Horses

Helena Carstensen DVM [a], Lene Larsen DVM [a], Christian Ritz MSc, PhD [b], Martin K. Nielsen DVM, PhD, Dipl EVPC [a]

[a] Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Taastrup, Denmark
[b] Department of Basic Sciences and Environment, Faculty of Life Sciences, University of Copenhagen, Frederiksberg C, Denmark

ARTICLE INFO

Article history:
Received 23 February 2012
Received in revised form
23 May 2012
Accepted 5 June 2012
Available online 22 August 2012

Keywords:
Horse
Strongyles
Fecal egg count
McMaster
Variability

ABSTRACT

Strongyle parasites are ubiquitous in grazing horses and constitute a potential threat to equine health. Feces were collected from six horses four times daily over a period of 5 days. Fecal egg counts (FECs) were performed to identify any diurnal rhythms in strongyle egg shedding and to quantify variability at the different levels: individual horses, repeated counts, repeated subsamples, different time points, and different days. No significant differences in FECs were found between the different time points ($P = .11$). The variables—horse, day, subsample, and egg count—accounted for a variance of 104.83, 0.10, 7.24, and 5.61, respectively. The apparent lack of additional variability between the four different time points suggests that time of the day chosen for collecting fecal samples does not constitute a source of error in field studies. The majority of variability exists between different subsamples and repeated egg counts on the same subsamples, whereas the variability of FECs between following days can be considered negligible. The findings of this study have implication for designing and performing field surveillance of strongyle FEC levels and applying the FEC reduction test for evaluating anthelmintic efficacy.

1. Introduction

Cyathostomin parasites are ubiquitous in grazing horses worldwide. Although a majority of horses harbor cyathostomins without signs of clinical disease, a life-threatening disease complex called larval cyathostominosis is caused by mass emergence of encysted larvae from the large intestinal walls [1]. Because of this, anthelmintic treatments have been regularly applied in equine establishments over the past decades to reduce the risk of parasitic disease caused by cyathostomins and other parasite categories infecting the horse. Unfortunately, this practice has led to increasing levels of anthelmintic resistance in equine parasites [2], and it is now recommended to reduce the treatment intensity considerably to delay further development of resistance [3]. Several European Union countries have implemented prescription-only legislations to ensure veterinary involvement in the decision process and to encourage parasite diagnostics and surveillance using fecal egg counts (FECs).

An anthelmintic treatment regimen based on the selective therapy principle was first proposed 20 years ago [4,5] and is now being recommended widely for equine strongyle control [3,6]. In this system, FECs are performed from all horses on the farm, and only those exceeding a predetermined threshold egg count are treated, whereas the remaining horses are left untreated. This practice has been supported by studies documenting that adult horses maintain consistent levels of strongyle egg shedding over time, even in the absence of anthelmintic treatment [6-8]. A recent retrospective study found that horses with egg counts in the range of 0-500 eggs per gram (EPG) had

Corresponding author at: Martin K. Nielsen, DVM, PhD, Dipl EVPC, Department of Veterinary Science, M.H. Gluck Equine Research Center, University of Kentucky, Lexington, KY 40546.
E-mail address: martin.nielsen@uky.edu (M.K. Nielsen).

0737-0806/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jevs.2012.06.001

significantly smaller worm burdens compared with horses with egg counts more than this level [9]. Despite this, high levels of variability have been documented in repeated FECs [10,11], and concerns have been raised regarding the reliability of egg counts as criteria for deciding anthelmintic treatment [12].

Questions have been raised whether strongyle egg shedding levels may be consistently different at different times of the day and whether fecal samples collected from the same horses on different days are associated with greater variability [11]. In a study by Warnick [13], more eggs were found on day 4 than on day 1, suggesting a variability in egg shedding between days. Other studies were unable to show any difference in egg counts performed morning and evening from the same horses [14,15]. Similarly, no difference was observed in FECs performed in goats between different time points during the day [16].

The aim of the present study was to investigate variability of repeated equine strongyle FECs performed at four different time points during the day over a period of 5 days. Further, the aims were to identify any diurnal rhythms in strongyle egg shedding, if possible, and to quantify variability at the different levels: individual horses, repeated counts, repeated subsamples, different time points, and different days.

2. Methods

The study was conducted at the Department of Large Animal Sciences, Faculty of Life Sciences, University of Copenhagen, Denmark from May 12-16, 2010. It was designed as a longitudinal prospective analytical cohort study. The target population is strongyle eggs in equine feces, and the study population is strongyle eggs in feces from six horses collected at 6-hour intervals for 5 consecutive days. The study unit is strongyle EPG of feces.

2.1. Horses

Horses were kept at the Large Animal University Hospital, University of Copenhagen and were kept in individual stalls during afternoons and at night, but they were turned out to graze a common pasture for 3 hours every morning. Feces from six mares aged between 7 and 30 years were used in the study. Five of the mares were Standardbred trotters, and one was a pony breed. All horses belonged to the university and were primarily used for teaching purposes. All horses were in good health, and accurate records were kept of all medical treatments, including anthelmintic treatments. None of the horses had received anthelmintic treatment in the previous 6 months. The horses were fed a uniform diet consisting of high-fiber concentrates, vitamin and mineral supplement, and hay.

Feces were collected at 6 AM, 12 PM, 6 PM, and 12 AM. The feces were collected rectally in the same order every time to make the intervals between each sample as close to 6 hours as possible. If no feces were found rectally at the time of collection, a new attempt was made within 1 hour. A sample of approximately 50-100 g was collected at each occasion. The samples were packaged airtight in plastic rectal sleeves and kept refrigerated until further processing within 24 hours.

2.2. Fecal Egg Counts

A McMaster technique with a detection limit of 20 EPG was used to determine the FECs in the samples [17]. This technique uses centrifugation to upconcentrate samples before flotation. All egg counts were performed by the first and second authors. The McMaster chamber (Kruuse, Langeskov, Denmark) was read after 3-10 minutes. All intact strongyle eggs within the lines of the McMaster chamber grid were counted.

Before subsampling, each fecal sample was manually mixed while in the bag. For each fecal sample, three 4-g subsamples were then weighed and processed. From each 4-g subsample, three egg counts were performed. This generated nine egg counts performed from each fecal sample.

Larval cultures were performed to determine the possible presence of large strongyle species in each of the horses. Five grams of feces was cultured from each horse, and 200 larvae were identified, if present, using morphological criteria [18].

2.3. Statistical Analyses

The analysis of data was performed using the statistics program R version 2.10.1 (The R Foundation for Statistical Computing, Vienna, Austria). It was assumed that the four variables—horse, day, subsample, and egg count—gave rise to random variability. The variable time point was assumed to have a fixed effect on FEC.

A mixed model was used to determine the effect of the covariates on FEC. In the mixed model, it is possible to include both random and fixed variables. For the model to describe data satisfactorily, FEC was square root transformed. The description of data was studied by making a residual plot of the predicted FEC values against the differences between predicted and observed FEC values. A normal probability plot was used to examine the assumption that the errors are normally distributed.

The model was used to estimate the amount of variance described by each of the random covariates. An *F*-test was used to determine the potential impact of sampling four times a day on the FEC. Results were interpreted at the 5% significance level.

3. Results

During the study, 1,080 egg counts were performed. FECs were in the range of 0-2,420. Each horse's FEC values are presented in Table 1. Figure 1 presents mean daily egg

Table 1
Range, mean, and median of strongyle fecal egg count values given in eggs per gram of feces for each of the six horses used in the study

Horse	Minimum	Maximum	Mean	Median
a	280	1,600	868	860
b	220	1,060	603	580
c	20	500	253	240
d	480	2,420	1,359	1,360
e	20	540	200	200
f	0	300	102	100

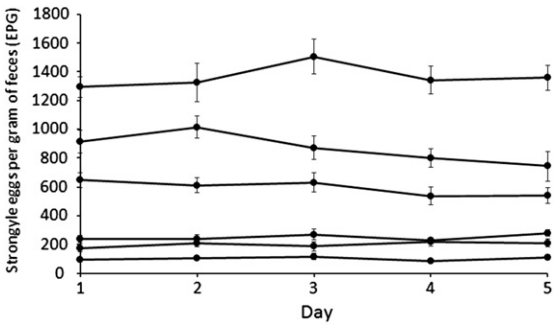

Fig. 1. Mean strongyle egg counts generated for the 36 egg counts performed for each of the six horses each day. Error bars designate 95% confidence intervals. There were no statistical differences between the 5 days in the study.

counts and 95% confidence intervals for each horse during the study. Cyathostomins were found in the larval cultures of all six horses, with *Triodontophorus* spp identified in horses e (0.5%) and f (2%), respectively. No other large strongyle species were encountered.

Egg counts were not significantly different at the four different time points during the study ($P = .11$). The variances explained by the four random covariates are presented in Table 2.

4. Discussion

We demonstrated that over a 5-day period, the day-to-day variability in egg counts was negligible, with the majority of variability existing between different subsamples and repeated counts on the same subsamples. In addition, we found no evidence of a diurnal rhythm in strongyle egg shedding. To our knowledge, this is the first study to compare FECs in horses at four different times of the day, and no significant differences were found.

The results are in agreement with findings of Denwood et al. [15], who reported a low variance between FECs counted in feces collected twice daily. The difference between the two studies is that Denwood et al. [15] examined the daily variability as a random effect on FECs, which makes them capable of determining size of the daily variability, whereas our study was designed to determine whether there is a fixed daily variability. However, both studies have come to the conclusion that the time of day for collection of fecal samples has no effect on FEC.

It should be noted that the level of variability associated with FECs is partly dependent on the egg-counting technique used. More sensitive techniques designed to detect low egg count levels can be expected to have less variability than techniques with higher detection limits. To illustrate this point, the FLOTAC technique has a limit for detection of one EPG and is reported to have significantly lower variability levels than other egg-counting techniques [19].

Table 2
Total variance of repeated strongyle egg counts explained by the random variables in the analysis: horse, day, 4-g subsample, and egg count

Covariate	Horse	Day	4-g Sample	Egg Count
Variance	104.83 (89.01%)	0.10 (0.09%)	7.24 (6.15%)	5.61 (4.76%)

The percentage of the total variance of each covariate is presented in parentheses.

In the present study, the maximum difference in FEC at different times of the day was 43 EPG (data not shown), but this difference was not significant. A larger number of horses in the study could possibly result in the difference becoming statistically significant. However, this difference is small and would not have clinical relevance. Similarly, Bennett [14] found a nonsignificant difference in large strongyle egg shedding of 24 EPG between samples collected morning and evening. Our study revealed a larger variability between subsamples than between individual egg counts. Thus, one approach to reduce the variability of egg counts could be to perform two or more repeated egg counts from the same fecal sample and then calculating the average.

One study evaluated trichostrongyle egg counts in goat feces performed every second hour for 24 hours every 3 weeks through 14 months [16]. The findings suggested that the time of year did not have an impact on whether there is a daily variability in the shedding of strongyle eggs. Whether this is the case for equine strongyle egg shedding has yet to be determined. In the present study, the strongyle eggs were primarily from cyathostomins, but the composition of cyathostomin species is not known. This composition would be relevant to determine in future studies, as the variation in egg shedding could be dependent on the species. Other herds might be infected with a different composition of strongyle species, and it is therefore important to determine whether the lack of a significant daily variability in shedding of strongyle eggs applies to all strongyle species in horses. However, multiple studies have found the same strongyle species to be predominant in horses worldwide [20-27].

Among the four random variables studied, the horse is the variable that gives rise to the largest variance. This finding is not surprising, as individual horses are known to maintain different levels of strongyle egg shedding over time [6-8]. This appears to be partly explained by host immunity, as younger horses tend to have higher FECs than adult horses although their luminal worm burden is roughly the same [28-30]. However, it should be pointed out that younger horses were not included in the present study, and it is possible that findings could be different with this age-group owing to the higher egg count levels. Thus, results from this study cannot necessarily be extrapolated to younger horses.

One study reported a larger day-to-day variability than found in this study [13]. This difference might be partly explained by the fact that the study did not account for the variability associated with laboratory techniques by performing repeated egg counts and analyzing several subsamples per time point. Despite the variability between days, Warnick [13] concluded that a single FEC is sufficient to determine the level of strongyle egg shedding in a horse. Although this statement may appear somewhat unjustified, later studies have verified that levels of strongyle egg shedding in adult horses are consistent over time [6-8]. It is important to note that a seasonal variation in the egg shedding should be expected [31], but that our study suggests that such effects can be considered negligible over shorter periods. This is important when performing the FEC

reduction tests for detecting anthelmintic resistance. Here, pre- and posttreatment FECs are performed 10-14 days apart, and it is important to exclude the possible impact of external sources of variability. With this in mind, it could be relevant to evaluate egg count variability over a 14-day period. Furthermore, future studies could be used to determine whether the low day-to-day variability can be found at different times of the year and under different climatic conditions.

5. Conclusions

In summary, this study provides useful observations for veterinarians and horse owners using FECs for selective therapy purposes and/or FEC reduction test on equine establishments. We conclude that there is no significant within-day variability in the shedding of strongyle eggs in horses, and the time of day for collection of fecal samples does not represent a source of error in research studies. In addition, the day-to-day variability of FECs can be considered negligible over a period of 5 days, so there is no additional benefit from performing repeated egg counts over consecutive days to estimate levels of egg shedding.

Acknowledgments

The authors thank laboratory technicians Tina Roust and Maria Rhod, as well as Drs. Mette Ernebjerg Almind and Lene Hedegaard Sommer, for their valuable help in the laboratory.

References

[1] Love S, Murphy D, Mellor D. Pathogenicity of cyathostome infection. Vet Parasitol 1999;85:113-22.
[2] Kaplan RM. Drug resistance in nematodes of veterinary importance: a status report. Trends Parasitol 2004;20:477-81.
[3] Kaplan RM, Nielsen MK. An evidence-based approach to equine parasite control: it ain't the 60s anymore. Equine Vet Educ 2010;22:306-16.
[4] Duncan JL, Love S. Preliminary observations on an alternative strategy for the control of horse strongyles. Equine Vet J 1991;23: 226-8.
[5] Krecek RC, Guthrie AJ, Van Nieuwenhuizen LC, Booth LM. A comparison between the effects of conventional and selective antiparasitic treatments on nematode parasites of horses from two management schemes. J S Afr Vet Assoc 1994;65:97-100.
[6] Becher A, Mahling M, Nielsen MK, Pfister K. Selective anthelmintic therapy of horses in the Federal states of Bavaria (Germany) and Salzburg (Austria): an investigation into strongyle egg shedding consistency. Vet Parasitol 2010;171:116-22.
[7] Dopfer D, Kerssens CM, Meijer YG, Boersema JH, Eysker M. Shedding consistency of strongyle-type eggs in Dutch boarding horses. Vet Parasitol 2004;124:249-58.
[8] Nielsen MK, Haaning N, Olsen SN. Strongyle egg shedding consistency in horses on farms using selective therapy in Denmark. Vet Parasitol 2006;135:333-5.
[9] Nielsen MK, Baptiste KE, Tolliver SC, Collins SS, Lyons ET. Analysis of multiyear studies in horses in Kentucky to ascertain whether counts of eggs and larvae per gram of feces are reliable indicators of numbers of strongyles and ascarids present. Vet Parasitol 2010;174:77-84.
[10] Mes TH. Technical variability and required sample size of helminth egg isolation procedures. Vet Parasitol 2003;115:311-20.
[11] Uhlinger CA. Uses of fecal egg count data in equine practice. Compend Contin Educ Pract Vet 1993;15:742-9.
[12] von Samson-Himmelstjerna G, Ilchmann G, Clausen PH, Schein E, Fritzen B, Handler J, et al. Recommendations for a sustainable control of gastro-intestinal worm infections in horses in Germany [in German]. Pferdeheilk 2011;27:127-40.
[13] Warnick LD. Daily variability of equine fecal strongyle egg counts. Cornell Vet 1992;82:453-63.
[14] Bennett MF. Is there a morning-evening difference in egg production of bloodworms, strongyle parasites of equines? Prog Clin Biol Res Br 1990;341:729-33.
[15] Denwood MJ, Love S, Reid SW, Innocent GT. Investigating the reliability of a single faecal egg count to quantify the true egg shedding rate of a horse. In: Proceedings of the British Equine Veterinary Association, Liverpool, England; 2008.
[16] Rinaldi L, Veneziano V, Morgoglione ME, Pennacchio S, Santaniello M, Schioppi M, et al. Is gastrointestinal strongyle faecal egg count influenced by hour of sample collection and worm burden in goats? Vet Parasitol 2009;163:81-6.
[17] Roepstorff A, Nansen P. Epidemiology, diagnosis and control of helminth parasites of swine. In: FAO Animal Health Manual. Rome, Italy: Food and Agriculture Organization of the United Nations; 1998.
[18] Russell AF. The development of helminthiasis in thoroughbred foals. J Comp Pathol Ther 1948;58:107-27.
[19] Cringoli G, Rinaldi L, Maurelli MP, Utzinger J. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. Nat Protoc 2010;5:503-15.
[20] Bucknell DG, Gasser RB, Beveridge I. The prevalence and epidemiology of gastrointestinal parasites of horses in Victoria, Australia. Int J Parasitol 1995;25:711-24.
[21] Eysker M, 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;19:103-15.
[22] Gawor JJ. The prevalence and abundance of internal parasites in working horses autopsied in Poland. Vet Parasitol 1995;58:99-108.
[23] Krecek RC, Reinecke RK, Horak IG. Internal parasites of horses on mixed grassveld and bushveld in Transvaal, Republic of South Africa. Vet Parasitol 1989;34:135-43.
[24] Lind EO, Eysker M, Nilsson O, Uggla A, Höglund J. Expulsion of small strongyle nematodes (cyathostomin spp) following deworming of horses on a stud farm in Sweden. Vet Parasitol 2003;115:289-99.
[25] Reinemeyer CR, Smith SA, Gabel AA, Herd RP. The prevalence and intensity of internal parasites of horses in the U.S.A. Vet Parasitol 1984;15:75-83.
[26] Silva AV, Costa HM, Santos HA, Carvalho RO. Cyathostominae (Nematoda) parasites of *Equus caballus* in some Brazilian states. Vet Parasitol 1999;86:15-21.
[27] Torbert BJ, Klei TR, Lichtenfels JR, Chapman MR. A survey in Louisiana of intestinal helminths of ponies with little exposure to anthelmintics. J Parasitol 1986;72:926-30.
[28] Chapman MR, French DD, Klei TR. Prevalence of strongyle nematodes in naturally infected ponies of different ages and during different seasons of the year in Louisiana. J Parasitol 2003;89:309-14.
[29] Klei TR, Chapman MR. Immunity in equine cyathostome infections. Vet Parasitol 1999;85:123-36.
[30] Lyons ET, Tolliver SC, Drudge JH. Historical perspective of cyathostomes: prevalence, treatment and control programs. Vet Parasitol 1999;85:97-112.
[31] Poynter D. Seasonal fluctuations in the number of strongyle eggs passed by horses. Vet Rec 1954;66:74-8.