Veterinary Parasitology 228 (2016) 103–107

Contents lists available at ScienceDirect

Veterinary Parasitology

journal homepage: www.elsevier.com/locate/vetpar

Research paper

Repeatability of strongyle egg counts in naturally infected horses

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ARTICLE INFO

Article history:
Received 30 March 2016
Received in revised form 22 August 2016
Accepted 27 August 2016

Keywords:
FEC
Egg counts
Horses
Repeatability
Negative-binomial
Egg-shedding consistency
Selective anthelmintic treatment

ABSTRACT

The selective treatment of horses is used to decrease the number of anthelmintic treatments by only treating a proportion of animals in the population. One way to select animals for treatment is to identify low and high egg-shedders using faecal egg counts (FEC); then to treat only the high egg-shedders. The value of this method is enhanced if differences among individuals in the level of egg-shedding remain consistent over time. One way to assess the stability of the rankings of animals over time is to measure the repeatability which is defined as the variance between horses divided by the total variance. The repeatability varies between 0 (no consistency in the values) to 1 (perfect consistency). To determine the repeatability of egg-shedding in naturally infected horses over time, 2637 FEC and raw egg counts (REC; i.e. originally counted eggs without multiplication factor) from 303 horses were analysed. The distribution of FEC was more overdispersed than a Poisson distribution. Therefore, a negative-binomial model was used. The within-horse-repeatability of RECs was 0.52. In a second analysis, we excluded horses that were treated with anthelmintic drugs during the study by eliminating all REC within the egg-reappearance-period. Here, the within-horse-repeatability was very similar at 0.53. The results show that egg-shedding of individual horses stays fairly consistent over time. They also show that animals which shed relatively high numbers of nematode eggs can be identified and targeted for treatment.

1. Introduction

The main goals of parasitological management are to prevent clinical disease and to reduce the intensity of infection below a disease-threatening threshold. In the past, regular anthelmintic treatment was used to control strongyle infections in horses and ruminants. However, this was accompanied by the spreading of anthelmintic resistance (Sangster, 1999; Wolstenholme et al., 2004). Targeted selective anthelmintic treatment schemes are one option to delay the development of resistance by only treating animals with high faecal egg counts (FEC) (Coles, 2002; Hoste et al., 2002; van Wyk, 2001; van Wyk et al., 2006; Vercruysse et al., 2009). FEC are already broadly used in targeted treatment and selective breeding in cattle and sheep (Bishop, 2012; Kenyon et al., 2009; Saddiqi et al., 2012; Stear et al., 1995). Variation in egg excretion in horses is said to be also influenced by genetic variation (Kornas et al., 2015). Since 1999, the use of FEC has become important in the Selective Anthelmintic Therapy (SAT) of horses, where only horses that exceed a certain FEC threshold are treated (Becher et al., 2010; Matthews, 2008; Nielsen, 2012; Nielsen et al., 2014a).

For practical implementation of SAT in horses the intervals between FEC should be as long as possible and as short as necessary. The aim of such strategies is therefore that during long-term implementation of SAT, high and low egg shedders within a group of horses should be identified to allow the extension of the intervals between sampling and treatment for low egg shedders and to keep high egg shedders under close observation. For this, a reliable identification of low and high egg-shedders is essential. Therefore, the value of SAT depends in part upon the stability of FEC over time. In immune-competent, healthy, adult horses that have been previously exposed to cyathostomin infection, egg-shedding consistency develops over time (Becher et al., 2010; Nielsen et al., 2006; Wood et al., 2013). This consistency can be estimated by measuring the repeatability of egg-shedding.

The objective of this study was to determine the repeatability of egg-shedding in horses over time, in order to determine if FEC can

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http://dx.doi.org/10.1016/j.vetpar.2016.08.021
0304-4017/

Table 1
Number of horses by gender and age.

	Number of horses	Percent [%]	
Gender	Female	138	45.54
	Male	165	54.46
Age Groups (years)	Young (1–6)	68	22.44
	Adult (7–15)	149	49.17
	Old (16–33)	86	28.38

be used as a criterion for identifying horses that consistently shed relatively high numbers of eggs.

2. Materials and methods

2.1. Horses

The study contains data from 303 naturally infected horses at 35 riding stables in South-East Bavaria, Germany. All stables were customers of the veterinary practice that conducted the sampling for routine diagnostic purposes. No special selection for breeds was done. Horses came from a variety of breeds including warmbloods, US breeds, Norico-Pinzgauer, pony, Arab, and Haflinger. The composition of the data set according to gender and age is shown in Table 1.

2.2. Faecal analysis

Monthly faecal samples were collected from each horse by the veterinarian for nine consecutive months (March to November) and stored at 10 °C for a maximum of 5 days until analysis. Faecal egg counts (FEC) were performed according to the modified McMaster-Method used in the Diagnostic Center of Comparative Tropical Medicine and Parasitology, LMU Munich. The flotation solution used was a saturated sodium chloride solution with a specific gravity of 1.2. Two McMaster chambers were counted for each sample and each egg counted represented 20 eggs per gram (epg). A total of 2606 FEC were obtained (121 samples were missing). In addition to the FEC in eggs per gram, the results of the analyses were also recorded as the number of eggs counted (raw egg counts; REC), which correspond to the individual FEC in eggs per gram divided by the multiplication factor 20.

2.3. Statistical analysis

For the assessment of the stability of animal ranking the data was analysed with SAS® 9.3 and 9.4 (SAS Institute). The analysis was performed for the faecal egg count values (FEC) and for raw egg counts (REC). For each horse up to 9 consecutive FEC were available to assess the repeatability. In total three datasets were analysed: dataset 1 contained 2606 FEC (in epg) from 303 horses. Dataset 2 contained 2604 REC from 303 horses and was derived by dividing the individual FEC by 20 to regain the original raw counts. This was done to exclude an inflation of the variance due to the multiplication factor. Due to the inflated variance, the number of eggs counted does not conform to the same statistical distribution as the number of eggs per gram. In particular, if the number of eggs counted follows a negative binomial distribution, the number of eggs per gram will not. Dataset 3 (n = 2430 REC) was derived from dataset 2 by dropping all results from samples within the specific egg reappearance period (ERP) of the used anthelmintic compound. This was to exclude the effect of anthelmintic treatment. Since all horses were treated either with pyrantel or ivermectin the ERPs were set at 4 or 8 weeks, respectively, according to the AAEP Parasite Control Guidelines (Nielsen et al., 2013).

2.3.1. Analysis of epg values (dataset 1)

The FREQUENCY and UNIVARIATE procedures were used to derive summary statistics for dataset 1.

2.3.2. Analysis of raw egg count data (datasets 2 and 3)

Raw egg counts (REC) were used to determine the distribution and the repeatability. The repeatability is defined as the variation between animals divided by the total variation (1). The CORR and UNIVARIATE procedures were used to describe datasets 2 and 3 and to calculate Spearman's rank correlation coefficient for both sets.

In the REC datasets, no evidence for zero-inflation could be found using a likelihood ratio test in the GENMOD and the FMM procedures. Analysis with the GLIMMIX procedure indicated variation greater than the Poisson distribution. Therefore the negative binomial was used to model the additional variation. In SAS the parameterization of the negative binomial defines the variance as $[\mu + (k^*\mu^2)]$. The symbol μ represents the mean and k is the overdispersion parameter and was estimated by maximum likelihood. The GLIMMIX procedure was used to partition the variance into within-horse and between-horse components. The ratio of the between-horse variation to the total variation defines the repeatability (Falconer and Mackay, 1996).

3. Results

Horses were sampled for 9 consecutive months. Table 2 shows the mean faecal egg count at each sampling date of all horses. Mean FEC at the sampling dates ranged from 50.1 epg to 112.8 epg. The standard errors of the FEC ranged from 121.9 to 342.1 and the samples ranged from 0 to a maximum of 3240 epg. The frequency of horse samples with egg counts of zero ranged from 63% (October) to 78% (April). The mean number of horses at each sample date averaged over all sample dates with a FEC greater than 200 epg was 21.7. These animals were treated with an anthelmintic after sampling. This was 7.4% (195/2606) of the samples.

The total mean REC of all horses was 3.7 counted eggs. The means of the REC of all horses at each of the nine sampling occasions ranged from 1.9 to 5.6 counted eggs. The distribution of raw egg counts can be seen in Fig. 1 for sampling date 7 (September), as an example.

Table 2
Mean FEC of all horses at sampling dates 1–9 (FEC_1–9), their distribution and the variance of REC. Minimum FEC was 0 epg for each sampling date.

	Month	Horses [n]	Variance of REC	Mean FEC [epg]	Max FEC [epg]	FEC >200 epg [n]
FEC_1	March	300	292.6	107.7	2320	29
FEC_2	April	299	93.1	50.1	1560	14
FEC_3	May	297	37.2	37.9	960	13
FEC_4	June	295	63.1	51.5	1720	16
FEC_5	July	292	303.8	112.8	2740	30
FEC_6	August	284	143.6	68.2	2720	23
FEC_7	September	281	239.7	91.3	3240	23
FEC_8	October	278	171.5	96.2	2220	28
FEC_9	November	280	170.6	72.6	2300	19