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Short communication

Effects of homogenizing methods on accuracy and precision of equine strongylid egg counts

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ABSTRACT

Recommendations for control of equine strongylid parasites are based on regular determination of fecal egg counts to identify high strongylid shedders and to evaluate treatment efficacy. The McMaster technique has long been used as the standard egg counting technique in equine veterinary practice in most parts of the world, but recent work has found the Mini-FLOTAC technique to perform with significantly better accuracy and precision. The Mini-FLOTAC system comes with a homogenizing device, termed the Fill-FLOTAC, and it has been hypothesized that this device might have a significant impact on accuracy and precision. The aim of the present study was to investigate the impact of the Fill-FLOTAC homogenizer in comparison with the classical McMaster approach, where samples are suspended in flotation medium by stirring with tongue depressor in a plastic cup. The study compared the McMaster and Mini-FLOTAC techniques, but also included cross-over versions where the Fill-FLOTAC was used with the McMaster chamber, and the tongue depressor and plastic cup homogenizing method was used with the Mini-FLOTAC counting disc. Fecal samples were collected from horses naturally infected with mixed strongylid species. Five samples were included from each of the following egg count levels: 0–500, 501–1000, and > 1000 eggs per gram (EPG). Each sample was then analyzed with all four set-ups with three subsamples collected from the same suspension, and three repeated counts determined on each subsample. Both the Fill-FLOTAC homogenizer ($p = 0.0098$) and the McMaster counting chamber ($p = 0.0298$) were significantly associated with higher strongylid egg counts, whereas the Mini-FLOTAC chamber was associated with a lower coefficient of variation ($p < 0.0001$). Precision, however, was not associated with homogenization method ($p = 0.9341$). Taken together, this study suggests that while the homogenizing method has a positive effect on egg count accuracy, the counting chamber appears to primarily affect precision.

1. Introduction

Fecal egg counts (FEC) are important tools in equine parasite control as they can be used to evaluate anthelmintic treatment efficacy and to identify high strongylid egg shedders in need of treatment (Nielsen et al., 2014). A principle of selective therapy has been proposed, where strongylid fecal egg counts are determined from all horses in a given population and only those exceeding a pre-determined threshold receive treatment (Nielsen et al., 2014). Today, the American Association of Equine Practitioners (AAEP) is recommending routine determination of fecal egg counts in horses as an integral part of a surveillance-based parasite control program (Nielsen et al., 2016).

While useful and important, strongylid FECs are also subject to confusion. A multitude of techniques and modifications of these exist and it is often difficult to discern what advantages these may offer relative to each other. The McMaster technique is by many considered the standard go-to technique in equine veterinary practice (Uhlinger, 1993), given its relative ease of use. The principle of this method was first described about 80 years ago (Gordon and Whitlock, 1939), and it typically involves passive flotation within a McMaster counting chamber. As such, the McMaster is one of the recommended techniques in the AAEP parasite control guidelines (Nielsen et al., 2016). More recently, the Mini-FLOTAC technique has been developed as an alternative to McMaster and has found wide-spread use in both veterinary and human parasitology (Cringoli et al., 2017). Like McMaster, the Mini-FLOTAC is based on passive flotation within a counting chamber, but a substantially larger volume of fecal suspension is examined under the microscope, leading to a much lower limit of detection (Cringoli et al., 2017). Typically, the Mini-FLOTAC technique has a detection limit of 5 or 10 eggs per gram (EPG) (Cringoli et al., 2017), whereas simple McMaster techniques used in veterinary practice most often employ detection limits of 25 or 50 EPG (Uhlinger, 1993).

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Accuracy and precision are important parameters for quantitative tests such as fecal egg counting methods. Precision, also referred to as repeatability, is defined by how close repeated measures are to each other, whereas accuracy, also known as the inverse of bias, is a measure for how close a given method measures to the true value (Levecke et al., 2012). The McMaster technique is generally known to vary as much as +/- 50% between repeated equine strongylid counts from the same samples (Uhlinger, 1993). This variability, in turn, can confound the interpretation of the fecal egg count reduction test (FECRT) when evaluating anthelmintic treatment efficacy (Vidyashankar et al., 2012). Recent work has illustrated that the precision of the FECRT is affected by the choice of egg counting technique, especially at lower pre-treatment egg count levels (Levecke et al., 2012). Thus, it is important to seek to improve precision of current egg counting techniques, and to develop new and improved protocols.

A recent comparison of Mini-FLOTAC to McMaster for determining equine strongylid egg counts found the Mini-FLOTAC to have significantly higher precision and accuracy (Noel et al., 2017), and this is in agreement with similar studies done evaluating determination of ovine trichostrongylid egg counts (Godber et al., 2015) and counts of canine hookworm, whipworm and ascarid eggs (Lima et al., 2015). While some of this improved performance is likely due to the larger Mini-FLOTAC counting chamber (2 mL), the system also comes with a separate homogenizing and filtering device, termed the Fill-FLOTAC (Cringoli et al., 2017). It is not known how this device affects accuracy and precision in comparison with more traditional manual approaches for homogenizing and suspending fecal samples in flotation media (Noel et al., 2017).

The aim with the current study was to evaluate the impact of the Fill-FLOTAC homogenizer on equine strongylid egg count magnitude and precision and compare it to the traditional homogenization approach, where the fecal sample is suspended into the flotation solution by stirring with a wooden tongue depressor in a cup (Nielsen et al., 2016).

2. Materials & methods

2.1. Egg counting techniques evaluated

Four egg count methodologies were evaluated in this study: 1) the Mini-FLOTAC with the Fill-FLOTAC homogenizer, 2) the Mini-FLOTAC with plastic cup and tongue depressor, 3) the McMaster with plastic cup and tongue depressor, and 4) the McMaster with the Fill-FLOTAC homogenizer. An overview of these methodologies is presented in Table 1.

Table 1
The four egg counting set-ups evaluated in the study. Two represented the normal set-ups (Mini-FLOTAC with Fill-FLOTAC and McMaster with plastic cup) and the other two were cross-overs between the counting chambers and the homogenizing steps.

Egg count technique	Homogenizer	Amount of feces (g)	Volume of flotation medium (mL)	Volume examined (mL)	Detection limit (EPG[a])
Mini-FLOTAC	Fill-FLOTAC	5	45	2.0	5.00
McMaster	Fill-FLOTAC	5	45	0.3	33.33
McMaster	Plastic cup	4	26	0.3	25.00
Mini-FLOTAC	Plastic cup	4	26	2.0	3.75

[a] Eggs per Gram of feces.

2.2. Fecal samples

Fecal samples were collected from a herd of horses naturally infected with mixed-species strongylid parasites. This herd has been maintained without anthelmintic treatment since 1979 (Lyons et al., 1990). Fresh samples were collected, placed in a cooler, and transported to the laboratory, where they were immediately refrigerated (4 °C).

Fecal samples were pre-screened with triplicate counts determined with the Mini-FLOTAC method (Noel et al., 2017) and five samples representing each of the following three strongylid egg count levels were selected for the study; 0–500, 501–1000, and > 1000 EPG.

2.3. Egg count data

For each of the 15 fecal samples, three subsamples were weighed and suspended in glucose-salt flotation medium (specific gravity 1.25). Triplicate counts were determined from each suspension by counting all strongylid eggs present in both counting chambers of the Mini-FLOTAC and two-chambered McMaster slides (area under the grids) and multiplying with the detection limit (*i.e.*, multiplication factor) given in Table 1. This generated a total of 540 egg counts or 135 for each of the four methods described in 2.1.

All 4 and 5 g subsamples were weighed on a laboratory balance (0.1 g accuracy). With the Fill-FLOTAC apparatus, 50 pumps were applied with the plunger to achieve homogenization. The two chambers on the Mini-FLOTAC disc were then filled with suspension, and left for 10 min before counting according to published protocols (Cringoli et al., 2017). The plastic cup homogenizing approach involved introducing the feces into the flotation medium in a disposable solo plastic cup and subsequently stirring 50 times with a wooden tongue depressor to achieve suspension. The suspension was poured through double-layered cheesecloth before the two counting chambers on a McMaster slide were filled and left for 5 min until counting as per the AAEP guidelines (Nielsen et al., 2016).

2.4. Statistical analysis

To determine method precision, coefficients of variation (CV) were calculated for each triplicate count, and mean CVs with 95% confidence intervals were calculated for each method and each of the three egg count categories. Statistical Analysis System (SAS) version 9.3 software (Cary, North Carolina, USA) was used to analyze the impact of homogenization method (Fill-FLOTAC *versus* plastic cup) and counting slide (Mini-FLOTAC *versus* McMaster) on the CV values. A mixed model analysis was conducted using the mixed procedure in SAS with CV as outcome variable, and egg counting slide (McMaster, Mini-FLOTAC), homogenizing technique (plastic cup, Fill-FLOTAC), and strongylid egg count level (0–500, 501–1000, and > 1000 EPG) as explanatory variables. Sample ID was kept as a random effect. If any the evaluated explanatory variables were statistically associated with the outcome, a Tukey's pairwise comparison of least squared means was performed.

The effects of methods for homogenization and counting slide/disc on strongylid egg count magnitude were also evaluated with mixed linear model analysis using the mixed procedure in SAS. In these analyses, egg count magnitude was interpreted as a reflection of accuracy, as neither McMaster nor Mini-FLOTAC have been found to overestimate equine strongylid egg counts (Noel et al., 2017). Strongylid egg count was the outcome variable, while egg counting slide, homogenizing technique, and strongylid egg count level were explanatory variables. Sample ID and replicate number were kept as random effects. If any the evaluated explanatory variables were statistically associated with the outcome, a Tukey's pairwise comparison of least squared means was performed. Normal distribution of variables was assessed with generation of normal plots as well as Shapiro–Wilks and Kolmogorov–Smirnov tests. All results were interpreted at the 0.05 significance