



## Research paper

# Reliability of three common fecal egg counting techniques for detecting strongyloid and ascarid infections in horses



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## ABSTRACT

The detection and quantification of nematode eggs using fecal egg count techniques have an irreplaceable role in equine parasitic control. The reliability, particularly precision and accuracy, of individual techniques have been described only for strongyloid infections. The aim of this study was to compare three fecal egg count techniques used for the detection of the two most common equine nematode infections: strongyloid and ascarid. The Simple McMaster, Concentration McMaster and Mini-FLOTAC techniques were tested on spiked fecal samples with various levels of egg concentration (50, 100, 200, 500, 1000 and 3000 eggs per gram) and naturally infected mixed strongyloid-ascarid samples with 30 replicates. The Simple McMaster, Concentration McMaster and Mini-FLOTAC techniques had precision coefficients of variation of 44.33, 35.64 and 18.25% for the strongyloid infection and 62.95, 35.71 and 18.95% for the ascarid infection, and percent accuracies (mean count/number of eggs spiked) of 97.53, 88.39 and 74.18% for the strongyloid infection and 65.53, 83.18 and 90.28% for the ascarid infection, respectively. Accuracy depended greatly on the type of nematode, but precision did not. The Mini-FLOTAC technique was more precise than the Simple and Concentration McMaster techniques regardless of nematode type. Simple McMaster was the most accurate technique for detecting strongyloid eggs, and Mini-FLOTAC was the most accurate technique for detecting ascarid eggs. Our results indicated that none of the current techniques were universally and sufficiently reliable for the simultaneous quantification of both of these common equine nematodes.

## 1. Introduction

Strongyloids (family Strongylidae), especially cyathostomins (subfamily Cyathostominae), and ascarids (*Parascaris* spp.) are currently the main equine parasites with clinical consequences and increasing levels of anthelmintic resistance around the world (Matthews, 2014). Cyathostomins are pervasive equine nematodes routinely found in nearly all equine operations and are currently considered to be a primary equine pathogen (Love et al., 1999). *Parascaris* spp., the second most important equine parasites, are dominant parasites of foals and weanlings (Nielsen, 2016). Ascarid infection is very rare in adult horses; young horses generally develop immunity over time (Clayton and Duncan, 1979). Poor detection or inappropriate control measures, however, can substantially affect the health of horses and can even have fatal consequences (Tatz et al., 2012). The eggs of both of these types of nematode are detectable by various fecal flotation techniques, but the

results can vary greatly. Using the most reliable techniques and standardizing detection procedures are thus necessary.

The proper detection of strongyloid and/or ascarid eggs, and especially accurate estimates of contamination potential, using fecal egg count (FEC) techniques have an irreplaceable role in functional programs of parasitic control. FEC techniques are necessary for the application of selective therapies for individual adult horses and for the identification of anthelmintic resistance using the FEC reduction test (FECRT) (Nielsen et al., 2016). Many FEC techniques, particularly for detecting ruminant nematodes, and their modifications have been developed over time to achieve the most reliable results. Some procedures require laboratory equipment such as centrifuges, e.g., the Stoll egg-counting (Stoll, 1930), Cornell-Wisconsin (Egwand and Slocombe, 1982), FLOTAC (Cringoli et al., 2010) and Concentration McMaster (Roepstorff and Nansen, 1998) techniques, and other procedures are performed only by passive flotation, e.g., the Simple McMaster

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**Table 1**  
Protocols of the fecal flotation techniques.

Technique	Weight of feces (g)	Flotation solution (SG)	Centrifugation (RCF)	Reading volume (ml)	Flotation time (min)	Multiplication factor
SMM	4	NaCl-glucose 1.28	–	0.3	5	50
CMM	4	NaCl-glucose 1.28	244 × g 5 min	0.3	5	20
MF	5	NaCl-glucose 1.28	–	2.0	10	5

SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; SG, specific gravity; RCF, relative centrifugal force.

(Roepstorff and Nansen, 1998), Mini-FLOTAC (Cringoli et al., 2017) and FECPAK (Godber et al., 2015) techniques. New technologies such as automated computational egg-counting techniques allow FECs without the need of manual egg-counting but require a computer (Mes et al., 2007; Ghazali et al., 2013) or a smart phone (Slusarewicz et al., 2016) with necessary software and specialized equipment. Processing fecal sample by each standardized protocol thus requires specialized laboratory equipment, time, monetary investment and an experienced technician. The McMaster technique (Gordon and Whitlock, 1939), requiring a McMaster counting chamber, is the most widely used FEC method. Modification of the original protocol (especially centrifugation, weight of sample and dilution ratio) affects the multiplication factor and detection limit (Roepstorff and Nansen, 1998). FLOTAC was introduced to improve these factors (Cringoli et al., 2010). Mini-FLOTAC (Cringoli et al., 2017) is a simplified and more user-friendly version of its predecessor based on passive flotation.

Recent studies have illustrated that the reliability of FEC techniques for quantifying strongylid eggs varies considerably (Slusarewicz et al., 2016; de Castro et al., 2017; Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018; Paras et al., 2018). A reliable FEC technique is characterized by two essential attributes: precision and accuracy. Precision provides information about the variability of replicates from a single sample within one method using the coefficient of variation (CV), and accuracy is the closeness of an FEC to the true value. The Mini-FLOTAC technique is known for both its higher precision and accuracy (Noel et al., 2017; Scare et al., 2017; Paras et al., 2018), but the reliability of the McMaster technique greatly improves with infection intensity. Detection limits affect technique sensitivity, so processing samples with infection intensities below these limits can result in the misinterpretation of FECs, which excludes the application a particular technique for some uses (e.g., FECRTs) (Vidyashankar et al., 2012).

The aim of this study was to provide information on the precision and accuracy of three FEC techniques commonly used in equine parasitology. The Simple McMaster, Concentration McMaster and Mini-FLOTAC techniques were performed on spiked equine fecal samples and on samples from horses with patent, naturally-acquired strongylid and ascarid infections to determine the practical suitability for estimating contamination potential of horses. Many studies have focused on a similar topic, but they have only tested strongylids. This study is the first to include ascarids as another important taxon of equine parasites.

## 2. Materials and methods

### 2.1. Fecal samples and study design

The reliability of three common FEC techniques (Simple McMaster, SMM; Concentration McMaster, CMM; Mini-FLOTAC, MF) for detecting strongylid and ascarid infections was evaluated using both spiked and naturally infected fecal samples. FECs for each technique, nematode type and egg concentration were replicated 30 times – each FEC was performed individually according to protocol with one count done. Fecal samples spiked with strongylid eggs (540), samples spiked with ascarid eggs (540) and naturally infected samples (90) with mixed strongylid-ascarid eggs were processed. All samples (negative, strongylid positive, ascarid positive and mixed strongylid-ascarid positive)

were obtained from a private horse operation, collected immediately after defecation, placed into airtight zip-lock bags and transported in a cooler to the laboratory. A fecal sample from an adult horse recently treated with moxidectin was used as a negative sample for spiking eggs. The absence of eggs (0 eggs per gram (EPG)) in the negative sample was verified by the Mini-FLOTAC technique with 10 replicates. Two positive samples with high intensities of strongylid and ascarid infections, confirmed by SMM, were used separately for egg isolation. A fecal sample from a nine-month-old foal with a mixed strongylid-ascarid infection was used as a naturally infected sample. The negative sample was refrigerated, and the positive samples were promptly processed for egg isolation. A centrifugation-flotation technique described by Coles et al. (2006) was used with slight modification. Isolated eggs were diluted in water and vortexed, and the number of eggs in a specific volume of water was determined. The negative sample was spiked with eggs using a micro-pipette. The negative sample was separately spiked with six concentrations of strongylid or ascarid eggs (50, 100, 200, 500, 1000, 3000 EPG), with 30 replicates for each concentration. A skilled laboratory technician isolated the eggs, spiked the samples and conducted all FECs for the three methods.

### 2.2. Parasitological techniques

SMM (Roepstorff and Nansen, 1998), CMM (Roepstorff and Nansen, 1998) and MF with use of Fill-FLOTAC (Cringoli et al., 2017) were compared. Each technique was processed using the protocols in Table 1. All fecal samples were examined using an Olympus BX51 microscope at a total magnification of 100×. The total processing time (from weighing the feces to the last egg counted including potential 5-minute centrifugation) for each technique was measured, with 10 replicates (one sample at the time), in the naturally infected samples with a mixed infection. Mean egg count was calculated for each technique.

### 2.3. Statistical analysis

All statistical analyses used Statistica ver. 13 (StatSoft Inc., 2001, [www.statsoft.com](http://www.statsoft.com)). Basic descriptive statistics (mean, median, standard deviation and coefficient of variation) were calculated. Kruskal-Wallis test with corresponding *P* values was calculated to identify significant differences between the three FEC techniques. If the overall method effect was statistically significant ( $P < 0.05$ ), paired comparisons were made based on rank sums of the Mann-Whitney test. Raw data (i.e. observed egg count) were used as an input data for the statistical analyses. The results of the statistical analyses were visualized using boxplots (indicating medians, percentiles and outliers) in R (Team, R., 2015, [www.R-project.org](http://www.R-project.org)) using the ggplot2 package (Wickham, 2016).

Precision, represented by CV, was calculated for both the spiked and naturally infected samples. The CV for each set of 30 FEC replicates performed by each technique and concentration was calculated as:

$$CV = \left( \frac{\text{standard deviation}}{\text{mean}} \right) \times 100$$

Accuracy was expressed as the mean deviation from the number of spiked eggs and as a percentage for each technique using the formula:

**Table 2**  
Comparison of the three fecal egg count techniques at six egg concentrations in samples spiked with strongylid and ascarid eggs and in the naturally infected sample based on significant differences.

Sample type/concentration/nematode	P	SMM	CMM	MF
SP/50/S	0.3025	–	–	–
SP/100/S	0.0921	–	–	–
SP/200/S	0.0735	–	–	–
SP/500/S**	0.0000	a	b	a,b
SP/1000/S**	0.0000	a	a	a
SP/3000/S	0.1738	–	–	–
NI-/S**	0.0000	a,b	a	b
SP/50/A*	0.0177	a,b	a	b
SP/100/A*	0.0118	a	–	a
SP/200/A*	0.0257	a,b	a	b
SP/500/A**	0.0000	a	b	a,b
SP/1000/A**	0.0000	a	a	a
SP/3000/A**	0.0000	a	a	a
NI-/A**	0.0000	a	b	a,b

SP, spiked samples; NI, natural infection; S, strongylids; A, ascarids; SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; Kruskal-Wallis test with paired comparisons made by Mann-Whitney test \*, P ≤ 0.05; \*\*, P ≤ 0.01.

Different letters within a row indicate significant differences between techniques.

$$Accuracy (\%) = \left( \frac{mean}{spiked\ concentration} \right) \times 100$$

### 3. Results

A total of 1170 fecal samples were examined. Mean processing times (min:s) for the entire protocol, from weighing feces to counting the last egg, were 9:06, 15:54 and 19:31 for SMM, CMM and MF, respectively. Significant differences (P ≤ 0.05 and ≤ 0.01) between techniques and P values are presented in Table 2. Reliability was graphically visualized using heatmaps (Microsoft Excel, version 16.16.1) in Figs. 1 and 2 and boxplots in Figs. 3 and 4.

#### 3.1. Precision

The CVs and means of the three techniques for strongylids and ascarids, for the spiked and the naturally infected samples are presented in Fig. 1. Precision generally improved with higher egg concentrations for all three techniques and both types of nematode. MF was the most

precise technique, SMM was the least precise and CMM was intermediate for both strongylids and ascarids. Precision was comparable for the naturally infected and spiked samples.

#### 3.2. Accuracy

The accuracy for the three techniques for strongylids and ascarids for the spiked and naturally infected samples are presented in Fig. 2 – absolute frequency for direct comparison with data of other authors and relative frequency (%) for comparison of various egg concentrations. Accuracy depended strongly on the type of nematode. SMM was the most accurate technique for strongylid eggs (97.53%), CMM was moderately accurate (88.39%) and MF was the least accurate (74.18%). The results, however, were opposite for ascarid eggs. MF was the most accurate technique for ascarids (90.28%), CMM was moderately accurate (83.18%) and SMM was the least accurate (65.53%).

### 4. Discussion

Our study confirmed that the reliability of FEC techniques depended on various factors, such as nematode type, technique methodology and time invested. The most precise technique was associated with the longest consumption of time. MF required more than twice as much time as SMM, consistent with other results (Noel et al., 2017). Increased time consumption could be associated with a lower multiplication factor, which requires counting more eggs. Evaluating samples with a high number of eggs or mixed infections consumes more time. Flotation time could be another factor. MF required a flotation time of 10 min due to the larger volume, but SMM and CMM required only 5 min each.

Previous studies have indicated that MF was more precise than the McMaster techniques for naturally infected and/or spiked strongylid samples (de Castro et al., 2017; Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018; Paras et al., 2018) and some authors have found Wisconsin as the most precise technique for horses (Paras et al., 2018). The McMaster technique has been variously modified with different multiplication factor. Some studies have used the McMaster technique with a multiplication factor of 25 × (de Castro et al., 2017), and others have used 50 × (Noel et al., 2017; Bosco et al., 2018) or 66.67 × (Scare et al., 2017). Comparing the results of studies is virtually impossible due to high procedural variability of McMaster techniques, human factor, various fecal sample properties, data analysis method, laboratory equipment and different infection intensities of the samples. On that account, authors show dissimilar mean accuracy results – high (Bosco et al., 2018), moderate (Paras et al., 2018) and low (Noel et al.,

Spiked: Strongylids	50 EPG	100 EPG	200 EPG	500 EPG	1000 EPG	3000 EPG	Mean value
SMM	87.23	60.91	48.19	32.33	20.94	16.39	44.33
CMM	70.41	52.50	40.03	18.40	19.12	13.36	35.64
MF	33.32	27.99	16.78	14.70	11.78	4.93	18.25
Spiked: Ascarids	50 EPG	100 EPG	200 EPG	500 EPG	1000 EPG	3000 EPG	Mean value
SMM	167.24	88.40	44.70	33.99	25.34	18.04	62.95
CMM	68.93	51.84	32.08	23.95	22.45	15.03	35.71
MF	35.63	30.85	14.35	11.85	7.71	13.33	18.95
Naturally infected	Strongylids	Ascarids					
SMM	39.53	31.20					
CMM	25.19	17.92					
MF	8.64	14.51					

**Fig. 1.** Precision represented by the coefficient of variation (%) for the three fecal egg count techniques and six egg concentrations in samples spiked with strongylid and ascarid eggs and naturally infected mixed strongylid-ascarid sample. SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; EPG, eggs per gram.

Spiked: Strongylids	50 EPG	100 EPG	200 EPG	500 EPG	1000 EPG	3000 EPG	Accuracy (%)
SMM	0 (37–73)	0 (71–113)	-50 (153–220)	-50 (393–501)	50 (979–1145)	-100 (2666–3014)	97.53
CMM	0 (38–66)	-10 (72–107)	-40 (132–178)	-50 (414–475)	-230 (742–856)	-300 (2589–2861)	88.39
MF	-10 (35–45)	-30 (62–76)	-65 (127–144)	-160 (324–361)	-323 (672–734)	-300 (2638–2737)	74.18

Spiked: Ascarids	50 EPG	100 EPG	200 EPG	500 EPG	1000 EPG	3000 EPG	Accuracy (%)
SMM	-50 (11–49)	-50 (44–86)	-50 (122–171)	-200 (279–361)	-375 (564–682)	-950 (1917–2193)	65.53
CMM	-10 (30–51)	-20 (69–102)	-20 (154–196)	-120 (334–400)	-160 (761–900)	-360 (2498–2794)	83.18
MF	-15 (33–44)	-5 (81–102)	-23 (165–183)	-30 (448–490)	-83 (886–939)	-25 (2873–3174)	90.28

Fig. 2. Accuracy represented by mean deviation from the number of spiked eggs with confidence interval and expressed as percentage in the three fecal egg count techniques and six egg concentrations in samples spiked with strongylid and ascarid eggs. SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; EPG, eggs per gram.

2017; Scare et al., 2017). In contrast, MF has been used in all studies discussed (Barda et al., 2013; Godber et al., 2015; de Castro et al., 2017; Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018; Paras et al., 2018) with a uniform procedure and a multiplication factor of 5 ×, but again, the variable infection intensities of the samples strongly affected the final CVs. We tested both natural infection and spiked samples, with similar results for both models. This study not only confirmed previous results but also provides new data about the precision of FECs with ascarid infections. The precision of the FEC techniques tested was identical for both strongylid and ascarid eggs in the naturally infected and spiked samples.

Both accuracy and precision are integral to the reliability of FEC techniques. MF has been described by some authors as more accurate than the McMaster technique for strongylid infections (Noel et al., 2017; Scare et al., 2017) while other authors reported opposite results (de Castro et al., 2017; Bosco et al., 2018). SMM, however, was the

most accurate technique in our study for detecting strongylid eggs, followed by CMM and MF. In contrast, MF excelled in detecting ascarid eggs, followed by CMM and SMM. Some FEC techniques could thus be more suitable for quantifying strongylid burdens and others for quantifying ascarid burdens in horses.

The precision and accuracy of the three techniques, however, may also have been affected by homogenization (Went et al., 2018), flotation solution and sample dilution (Cringoli et al., 2004), use of centrifuges (Zajac et al., 2002) or a technician. The technician was not blinded to the true egg concentration in spiked samples in our study which could affect the results.

### 5. Conclusion

Our findings indicated that MF was more precise than SMM and CMM for performing FECs in equine samples, regardless of the type of

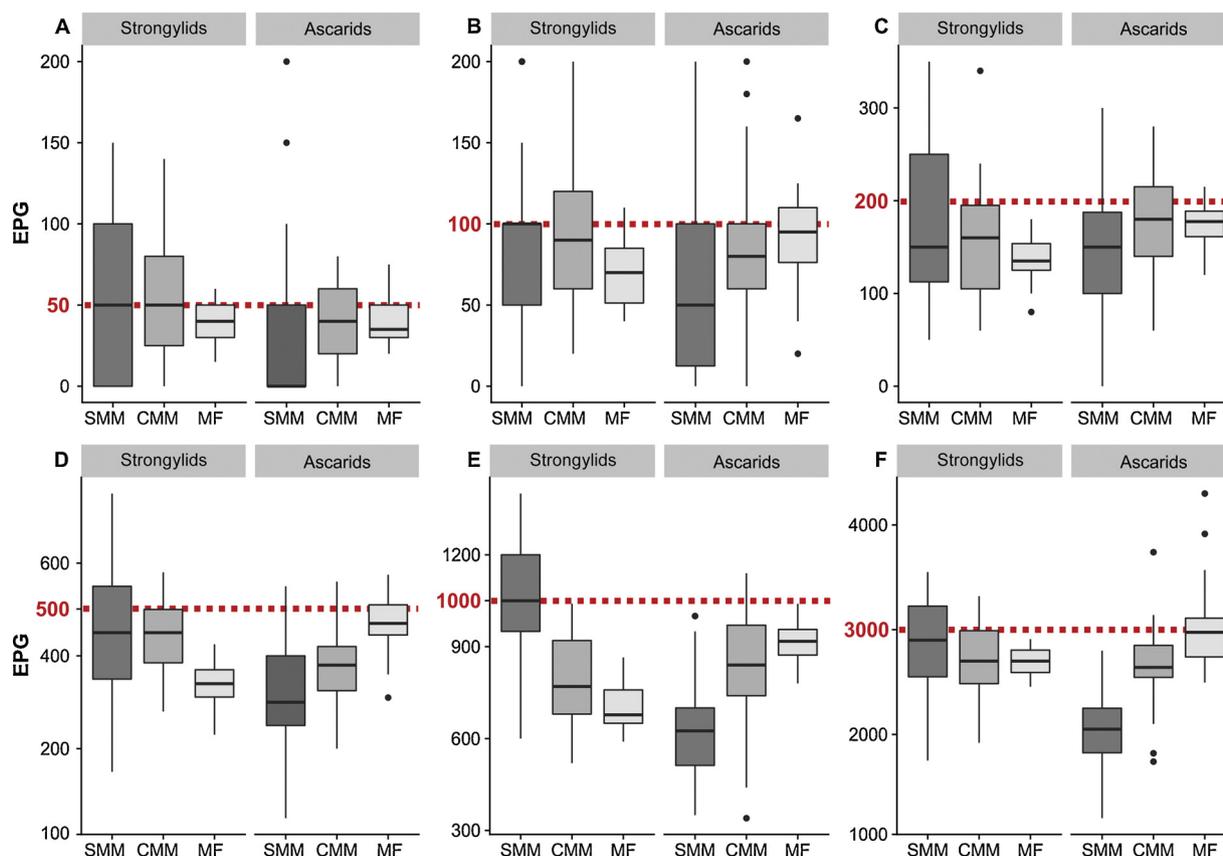
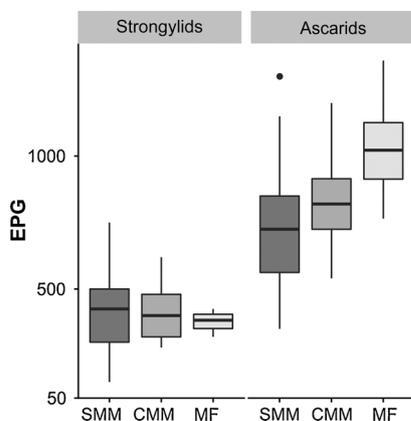


Fig. 3. Boxplots of the reliability of the three fecal egg count techniques at six egg concentrations (A–F) in samples spiked with strongylid and ascarid eggs. The boxplots represent medians (central lines), 25–75 percentiles (boxes), non-outliers (whiskers) and outliers (dots). SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; EPG, eggs per gram.



**Fig. 4.** Boxplots of the precision of the three fecal egg count techniques in the naturally infected mixed strongylid-ascarid sample. The boxplots represent medians (central lines), 25–75 percentiles (boxes), non-outliers (whiskers) and outliers (dots). SMM, Simple McMaster technique; CMM, Concentration McMaster technique; MF, Mini-FLOTAC technique; EPG, eggs per gram.

nematode. Our precision results were supported by previous studies. Accuracy depended on nematode type, and the results differed between the strongylid and ascarid eggs. More studies are needed to clarify the differences in the detection of various types of nematode. Our study also indicated that no FEC technique was universally applicable for quantifying equine nematode infections; a suitable technique must be chosen for each purpose. Performing FECRTs requires a highly precise technique with consistent results, so MF may be the most suitable technique, but high precision requires more time. Routine FECs for adult horses should most likely use SMM, due to the shorter time required and the high accuracy for strongylids.

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## References

Barda, B.D., Rinaldi, L., Ianniello, D., Zepherine, H., Salvo, F., Sadutshang, T., Cringoli, G., Clementi, M., Albonico, M., 2013. Mini-FLOTAC, an innovative direct diagnostic technique for intestinal parasitic infections: experience from the field. *PLoS Negl. Trop. Dis.* 7, 8.

Bosco, A., Maurelli, M.P., Ianniello, D., Morgogliano, M.E., Amadesi, A., Coles, G.C., Cringoli, G., Rinaldi, L., 2018. The recovery of added nematode eggs from horse and sheep faeces by three methods. *BMC Vet. Res.* 14, 7.

Clayton, H.M., Duncan, J.L., 1979. The development of immunity to *Parascaris equorum* infection in the foal. *Res. Vet. Sci.* 26, 383–384.

Coles, G.C., Jackson, F., Pomroy, W.E., Prichard, R.K., von Samson-Himmelstjerna, G., Silvestre, A., Taylor, M.A., Vercruyse, J., 2006. The detection of anthelmintic resistance in nematodes of veterinary importance. *Vet. Parasitol.* 136, 167–185.

Cringoli, G., Rinaldi, L., Veneziano, V., Capelli, G., Scala, A., 2004. The influence of flotation solution, sample dilution and the choice of McMaster slide area (volume) on

the reliability of the McMaster technique in estimating the faecal egg counts of gastrointestinal strongyles and *Dicrocoelium dendriticum* in sheep. *Vet. Parasitol.* 123, 121–131.

Cringoli, G., Rinaldi, L., Maurelli, M.P., Utzinger, J., 2010. FLOTAC: new multivalent techniques for qualitative and quantitative copromicroscopic diagnosis of parasites in animals and humans. *Nat. Protoc.* 5, 503.

Cringoli, G., Maurelli, M.P., Leveck, B., Bosco, A., Vercruyse, J., Utzinger, J., Rinaldi, L., 2017. The Mini-FLOTAC technique for the diagnosis of helminth and protozoan infections in humans and animals. *Nat. Protoc.* 12, 1723–1732.

de Castro, L.L.D., Abrahão, C.L., Buzatti, A., Molento, M.B., Bastianetto, E., Rodrigues, D.S., Lopes, L.B., Silva, M.X., de Freitas, M.G., Conde, M.H., Borges, F.A., 2017. Comparison of McMaster and Mini-FLOTAC fecal egg counting techniques in cattle and horses. *Vet. Parasitol.* 10, 132–135.

Egwand, T.G., Slocombe, J.O., 1982. Evaluation of the Cornell-Wisconsin centrifugal flotation technique for recovering trichostrongylid eggs from bovine feces. *Can. J. Comp. Med.* 46, 133–137.

Ghazali, K.H., Alsamraai, R.S.H., Mohamed, Z., 2013. Automated system for diagnosis intestinal parasites by computerized image analysis. *Mod. Appl. Sci.* 7, 98.

Godber, O.F., Phythian, C.J., Bosco, A., Ianniello, D., Coles, G., Rinaldi, L., Cringoli, G., 2015. A comparison of the FECPAK and Mini-FLOTAC faecal egg counting techniques. *Vet. Parasitol.* 207, 342–345.

Gordon, H.M., Whitlock, H.V., 1939. A new technique for counting nematode eggs in sheep faeces. *J. Coun. Sci. Ind. Res.* 12, 50–52.

Love, S., Murphy, D., Mellor, D., 1999. Pathogenicity of cyathostome infection. *Vet. Parasitol.* 85, 113–122.

Matthews, J.B., 2014. Anthelmintic resistance in equine nematodes. *Int. J. Parasitol. Drugs Drug Resist.* 4, 310–315.

Mes, T.H.M., Eysker, M., Ploeger, H.W., 2007. A simple, robust and semi-automated parasite egg isolation protocol. *Nat. Protoc.* 2, 486.

Nielsen, M.K., Mittel, L., Grice, A., Erskine, M., Graves, E., Vaala, W., Tully, R.C., French, D.D., Bowman, R., Kaplan, R.M., 2016. AAEP parasite control guidelines. *Am. Assoc. Equine Pract.*

Nielsen, M.K., 2016. Evidence-based considerations for control of *Parascaris* spp. infections in horses. *Equine Vet. Educ.* 28, 224–231.

Noel, M.L., Scare, J.A., Bellaw, J.L., Nielsen, M.K., 2017. Accuracy and precision of Mini-FLOTAC and McMaster techniques for determining equine strongyle egg counts. *J. Equine Vet. Sci.* 48, 182–187.

Paras, K.L., George, M.M., Vidyashankar, A.N., Kaplan, R.M., 2018. Comparison of fecal egg counting methods in four livestock species. *Vet. Parasitol.* 257, 21–27.

Roepstorff, A., Nansen, P., 1998. *Epidemiology, Diagnosis and Control of Helminth Parasites in Swine*. FAO Animal Health Manual, Rome.

Scare, J.A., Slusarewicz, P., Noel, M.L., Wielgus, K.M., Nielsen, M.K., 2017. Evaluation of accuracy and precision of a smartphone based automated parasite egg counting system in comparison to the McMaster and mini-FLOTAC methods. *Vet. Parasitol.* 247, 85–92.

Slusarewicz, P., Pagano, S., Mills, C., Popa, G., Chow, K.M., Mendenhall, M., Rodgers, D.W., Nielsen, M.K., 2016. Automated parasite faecal egg counting using fluorescence labelling, smartphone image capture and computational image analysis. *Int. J. Parasitol.* 46, 485–493.

StatSoft Inc, 2001. STATISTICA (Data Analysis Software System), Version 13.0. Tulsa, USA, [www.statsoft.com](http://www.statsoft.com).

Stoll, N.R., 1930. On methods of counting nematode ova in sheep dung. *Parasitology* 22, 116–136.

Tatz, A.J., Segev, G., Steinman, A., Berlin, D., Milgram, J., Kelmer, G., 2012. Surgical treatment for acute small intestinal obstruction caused by *Parascaris equorum* infection in 15 horses (2002–2011). *Equine Vet. J.* 44, 111–114.

Team, R., 2015. *Core: R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, version 3.5.2. Vienna, Austria [www.R-project.org](http://www.R-project.org).

Vidyashankar, A.N., Hanlon, B.M., Kaplan, R.M., 2012. Statistical and biological considerations in evaluating drug efficacy in equine strongyle parasites using fecal egg count data. *Vet. Parasitol.* 185, 45–56.

Wickham, H., 2016. ggplot2 – elegant graphics for data analysis. *J. Stat. Softw.*

Went, H.A., Scare, J.A., Steuer, A.E., Nielsen, M.K., 2018. Effects of homogenizing methods on accuracy and precision of equine strongylid egg counts. *Vet. Parasitol.* 261, 91–95.

Zajac, A.M., Johnson, J., King, S.E., 2002. Evaluation of the importance of centrifugation as a component of zinc sulfate fecal flotation examinations. *J. Am. Anim. Hosp. Assoc.* 38, 221–224.