

## Comparative studies on faecal egg counting techniques used for the detection of gastrointestinal parasites of equines: A systematic review

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### ARTICLE INFO

#### Keywords:

*Anoplocephala*  
Ascarid  
Comparison  
Equine  
Faecal egg counting technique  
Strongyle

### ABSTRACT

Faecal egg counting techniques (FECT) form the cornerstone for the detection of gastrointestinal parasites in equines. For this purpose, several flotation, centrifugation, image- and artificial intelligence-based techniques are used, with varying levels of performance. This review aimed to critically appraise the literature on the assessment and comparison of various coprological techniques and/or modifications of these techniques used for equines and to identify the knowledge gaps and future research directions. We searched three databases for published scientific studies on the assessment and comparison of FECT in equines and included 27 studies in the final synthesis. Overall, the performance parameters of McMaster (81.5%), Mini-FLOTAC® (33.3%) and simple flotation (25.5%) techniques were assessed in most of the studies, with 77.8% of them comparing the performance of at least two or three methods. The detection of strongyle, *Parascaris* spp. and cestode eggs was assessed for various FECT in 70.4%, 18.5% and 18.5% studies, respectively. A sugar-based flotation solution with a specific gravity of  $\geq 1.2$  was found to be the optimal flotation solution for parasitic eggs in the majority of FECT. No uniform or standardised protocol was followed for the comparison of various FECT, and the tested sample size (i.e. equine population and faecal samples) also varied substantially across all studies. To the best of our knowledge, this is the first systematic review to evaluate studies on the comparison of FECT in equines and it highlights important knowledge gaps in the evaluation and comparison of such techniques.

### 1. Introduction

Helminths are common and important gastrointestinal parasites of equines as they pose a significant threat to equine health and wellbeing, particularly in foals, yearlings and geriatric horses. Strongylid (cyathostom and strongylin) nematodes are the main internal parasites of horses, constituting more than 75% of the total parasite fauna (Tolliver et al., 1987; Bucknell et al., 1996; Lyons and Tolliver, 2004; Lichtenfels et al., 2008). Other major internal parasites found in horses include *Anoplocephala* spp., *Parascaris* spp., *Habronema* spp., *Draschia megastoma*,

*Oxyuris equi* and *Strongyloides westeri* (Tolliver et al., 1987; Lyons and Tolliver, 2004; Saeed et al., 2019). Major clinical signs of parasitism in horses include unthriftiness, reduced stamina, retarded growth, abdominal distension ('pot-belly'), diarrhoea, abdominal pain and death, especially in young and immunocompromised horses (Snyder et al., 1978; Love et al., 1999; Corning, 2009; Peregrine et al., 2014; Saeed et al., 2019).

Since the introduction of benzimidazoles in the 1960s, the control of equine internal parasites has heavily relied upon interval-based (i.e. treating yearlings and older horses regularly at 8-week intervals mostly)

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<https://doi.org/10.1016/j.crpvbd.2021.100046>

Received 2 July 2021; Received in revised form 2 August 2021; Accepted 3 August 2021

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and/or rotational (alternating) use of various anthelmintics (Drudge and Lyons, 1966; Kaplan and Nielsen, 2010). However, the indiscriminate use of anthelmintics has resulted in the emergence of resistant nematode populations and/or shortened egg reappearance periods (an early indication of the development of resistance) for almost all currently available anthelmintics (Kaplan and Nielsen, 2010; Reinemeyer, 2012; von Samson-Himmelstjerna, 2012; Peregrine et al., 2014; Beasley et al., 2017; Martin et al., 2021). Despite the fact that the detection and enumeration of parasite eggs in faecal samples has been the mainstay of clinical and research parasitology for decades, it was only in the 1980s, when the issue of resistant worms was identified, and integrated selective treatment (targeted selected treatment) strategies based upon predetermined thresholds such as faecal egg count (FEC) data obtained from the counting of parasitic eggs within the faecal samples were proposed (Kaplan and Nielsen, 2010; Andersen et al., 2013; Nielsen et al., 2014).

Copromicroscopy, founded by C.J. Davaine in 1857 (Rinaldi, 2014), with the first faecal smear method described by Grassi, Parona and Parona in 1878 (Ballweber et al., 2014), now forms the cornerstone of parasite diagnostics and research. The simple faecal smear lacks sensitivity, and improved methods, such as simple dilution (Stoll, 1923) and a direct centrifugal flotation (DCF) technique (Lane, 1923) for humans and animals (Stoll, 1930) have subsequently been developed. Several modifications made to the Stoll method improved limits of detection and resulted in the development of some of the currently used methods such as Wisconsin (Cox and Todd, 1962) and Cornell-Wisconsin techniques (Egwang and Slocombe, 1982). Along with these centrifugation-based flotation techniques, simple or gravitation-based methods such as the McMaster (Gordon and Whitlock, 1939), the modified McMaster (Whitlock, 1948), FLOTAC® (based on the principle of the Wisconsin technique) (Cringoli, 2006; Cringoli et al., 2010), FECPAK<sup>G1</sup>® (McCoy et al., 2005; Presland et al., 2005), Mini-FLOTAC® (Barda et al., 2013; Cringoli et al., 2017) and the FECPAK<sup>G1</sup>® and FECPAK<sup>G2</sup>® (Tyson et al., 2020), were developed, and are being used in the field. More recently, artificial intelligence-based automated counting techniques have been developed for FEC (Scare et al., 2016; Slusarewicz et al., 2016; Scare et al., 2017; Cain et al., 2020; Elghryani et al., 2020; Nagamori et al., 2020; Cringoli et al., 2021). The available FEC techniques (FECT) vary in performance parameters, including estimates of sensitivity, specificity, precision and accuracy (Ballweber et al., 2014; Nielsen, 2021). Additionally, technical (e.g. loss of eggs during sample processing, type of flotation solution, eggs type and flotation capability and analyst training), and biological (e.g. egg count variation within and between samples and density-dependent fecundity of female worms) sources of variation also play a critical role in determining the performance of FECT (Nielsen, 2021). Given the substantial variation in the performance of various FECT, no single technique is fit for all purposes and the choice of a technique depends upon the intended objective and expected FEC within faecal samples (Ballweber et al., 2014). For example, for studies aimed to evaluate the egg reappearance periods for anthelmintics, the FECT should have a higher diagnostic sensitivity to be able to detect the onset of egg appearance in faeces. On the other hand, a less sensitive technique could suffice the purpose for targeted selective treatments where the objective is generally to identify animals with FEC above a certain threshold value.

To date, several studies have assessed the performance parameters of FECT for equines (Bello and Allen, 2009; Fukumoto et al., 2011; de Castro et al., 2017; Napravnikova et al., 2019). Although these studies have demonstrated varying levels of performance of different techniques, there has not been a systematic appraisal of such comparative studies in equine parasitology. Therefore, the objectives of this systematic review were to critically appraise the current knowledge on the comparison of various coprological techniques and/or modifications to assess the parasite burden in equines and to identify the knowledge gaps and future research directions in equine parasitology.

## 2. Materials and methods

### 2.1. Database searches

This systematic review was completed as per the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Supplementary Table S1). We searched three databases: Web of Science, Google Scholar and PubMed. The primary literature search strategy was designed for Web of Science and then customised for other databases. The literature search was completed for studies published on the comparison of various coprological techniques used for the detection of eggs of equine internal parasites until February 28th, 2021. We used various combinations of key search terms relevant to the topic to retrieve all peer-reviewed research articles, conference proceedings and postgraduate theses published in the English language. The main search terms included “gastrointestinal parasites/nematodes/helminths of equine(s)/horse(s)/donkey(s)”, “comparison/validation/assessment”, “f(a)ecal egg count(s) in equine(s)/horse(s)/donkey(s)”, “flotation/sedimentation”, “McMaster”, “Wisconsin”, “FLOTAC®”, “FECPAK®”, “Mini-FLOTAC®”, “automated counting and Parasight System®”. Additionally, we examined the reference lists of retrieved articles and reviews to identify any other articles that could be relevant to the scope of this review.

### 2.2. Assessment of studies and data extraction

The retrieved references were imported into EndNote X9.2 and duplicates were removed using a built-in function, and remaining references were assessed for the selection of relevant studies. The assessment criteria were based upon three main components, including (i) language: English, (ii) article type: original research articles, conference proceedings and postgraduate theses, and (iii) study topic: studies on the comparison/validation/assessment of coprological techniques for equine FEC. In the first screening step, titles and abstracts were screened for the removal of irrelevant studies. Subsequently, full-text articles/studies were retrieved for selected references through the library of the University of Melbourne and inter-library loans. The full-text studies were subjected to the second screening step using the set assessment criteria.

The relevant data were compiled from each of the selected studies into a predesigned Microsoft Excel® spreadsheet. Data were extracted for study type, publication title, year of publication, country, coprological methods used, the number of equine samples tested, information about flotation/sedimentation solutions used, any other host species included in the study, parasites detected, parameters assessed, detailed methodology, key findings and conclusions. The definitions of important epidemiological and performance-related terms are provided in Table 1, and those of analytical and diagnostic performance parameters are summarised in Tables 2 and 3.

## 3. Results and discussion

### 3.1. Database searching, assessment, and screening

Our systematic search of the three databases for studies published on the topic produced a total of 14,005 results (Google Scholar, number of studies  $n = 7460$ ; PubMed,  $n = 3982$  and Web of Science,  $n = 2563$ ). Following the removal of duplicates ( $n = 7573$ ), the title/abstract screening step of 6432 references resulted in 75 studies. Subsequently, the full-text screening step yielded a total of 27 studies using our inclusion criteria (Fig. 1). These 27 studies were published between 1974 and 2021 and included 18 original research articles, five short communications, three conference proceedings/abstracts and one postgraduate thesis (Fig. 1).

**Table 1**  
Definitions of commonly used terms for the validation and comparison of diagnostic assays

Term	Definition <sup>a</sup>
Repeatability	The level of agreement between results of replicates of a sample both within and between runs of the same test method in a given laboratory
Reproducibility	The ability of a test method to provide consistent results, as determined by estimates of precision, when applied to aliquots of the same samples tested in different laboratories, preferably located in distinct or different regions or countries using the identical assay (protocol, reagents and controls)
Analytical specificity	The ability of the assay to distinguish the target analyte (e.g. antibody, organism or genomic sequence) from non-target analytes, including matrix components
Analytical sensitivity	The estimated amount of analyte in a specified matrix that would produce a positive result at least a specified percent of the time
Diagnostic sensitivity	The proportion of samples from known infected reference animals that test positive in an assay
Diagnostic specificity	The proportion of samples from known uninfected reference animals that test negative in an assay
Accuracy	The closeness of a test value to the expected (true) value (mean or median) for a reference standard reagent of known concentration or titre
Precision	The degree of dispersion (variance, standard deviation or coefficient of variation) within a series of measurements of the same sample tested under specified conditions

<sup>a</sup> Source: Jacobson and Wright (2019).

### 3.2. General characteristics of studies

Of the 27 studies on the comparison or assessment of techniques for assessing FEC in equines, the majority originated from North America ( $n = 13$ ), followed by Europe ( $n = 11$ ), Australasia ( $n = 2$ ), South America ( $n = 1$ ) and Asia ( $n = 1$ ) (one study was conducted in two regions including Europe and Australasia) (Fig. 2), and most were conducted during the last decade (2011–2021) (Fig. 3). Twenty-three studies focused on the comparison of different techniques and/or variations of the same technique, whereas the assessment of new techniques and different variables were investigated in two and five studies, respectively (Supplementary Table S2).

### 3.3. Techniques/methods used/tested

Most of the studies used the simple McMaster method or modifications of this technique ( $n = 22$ ), followed by Mini-FLOTAC® ( $n = 9$ ),

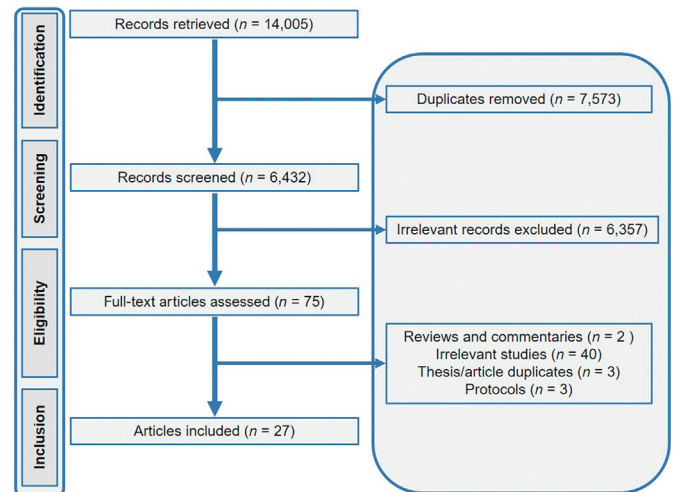
**Table 2**  
Principle, advantages and limitations of commonly used faecal egg counting techniques

Method	Principle	Advantages	Limitations
Direct smear	The small amount of fresh faeces mixed with saline (or iodine) on a microscope slide	Cheap, fast processing time	Qualitative, very low accuracy, precision and sensitivity
Cornell-Wisconsin	Based on centrifugal flotation of eggs in a salt solution in a tube, collection onto a coverslip and counting under a microscope	Cheap, high limit of detection	Time-consuming, low accuracy and very low precision
McMaster	Faeces mixed in a flotation solution are loaded onto chambers of a slide and the floated eggs are counted	Cheap, medium processing time	Low sensitivity
FLOTAC®	Based on centrifugal-flotation of eggs in a specialised apparatus and subsequent translation of the top layer	Cheap, high sensitivity, very high accuracy and precision	Time-consuming, special equipment (centrifugation rotors) required
Mini-FLOTAC®	A modified version of FLOTAC without centrifugation step and reduced reading volume	High sensitivity, accuracy and precision, medium processing time	Detection of some parasites (e.g. trematodes) requires centrifugation
FECPAK®	Eggs are floated in a flotation solution, accumulated into a single viewing area and imaged	Does not require technical skills as eggs identified and counted remotely, digitalised images	Low accuracy, precision and medium sensitivity, time-consuming
Parasight System®	A faecal sample mixed in water is filtered to remove debris, eggs are labelled with a fluorescent dye, imaged and counted using an automated algorithm	High precision, does not require technical skills, fast, automated counting, digitalised images	Expensive, some results need to be confirmed visually, does not detect overlying eggs, cannot differentiate with high debris background

Note: Sources: Cringoli et al. (2017); Sukas et al. (2019).

**Table 3**  
Key features and performance parameters of commonly used faecal egg counting techniques

Method	Faeces (g)	Dilution (ml)	Reading volume (ml)	Limit of detection
McMaster	4	56	0.3	50
Modified McMaster	4	56	1	15
Cornell-Wisconsin	5	55	2	1
FLOTAC	5	45	10	1
Mini-FLOTAC	5	45	2	5
FECPAK <sup>G1</sup>	~20	230	1	25
FECPAK <sup>G2</sup>	~20	210	0.88	45
Parasight	6	54	4	2.5



**Fig. 1.** Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram showing the number of articles at each stage and the exclusion criteria applied in this study.

flotation ( $n = 7$ ), modified Wisconsin ( $n = 5$ ), sedimentation ( $n = 4$ ), smartphone-based/automated ( $n = 4$ ), combined sedimentation-flotation ( $n = 3$ ), FECPAK® ( $n = 2$ ), and Parasight System® ( $n = 1$ ) techniques (Supplementary Table S2). Performances of two, three and four techniques were assessed and/or compared in 11, 10 and 2 studies, respectively, whereas 6 studies investigated the effect of various variables (including specific gravity, mesh size, counting time, flotation time, flotation solution, sample weight and homogenisation, number of

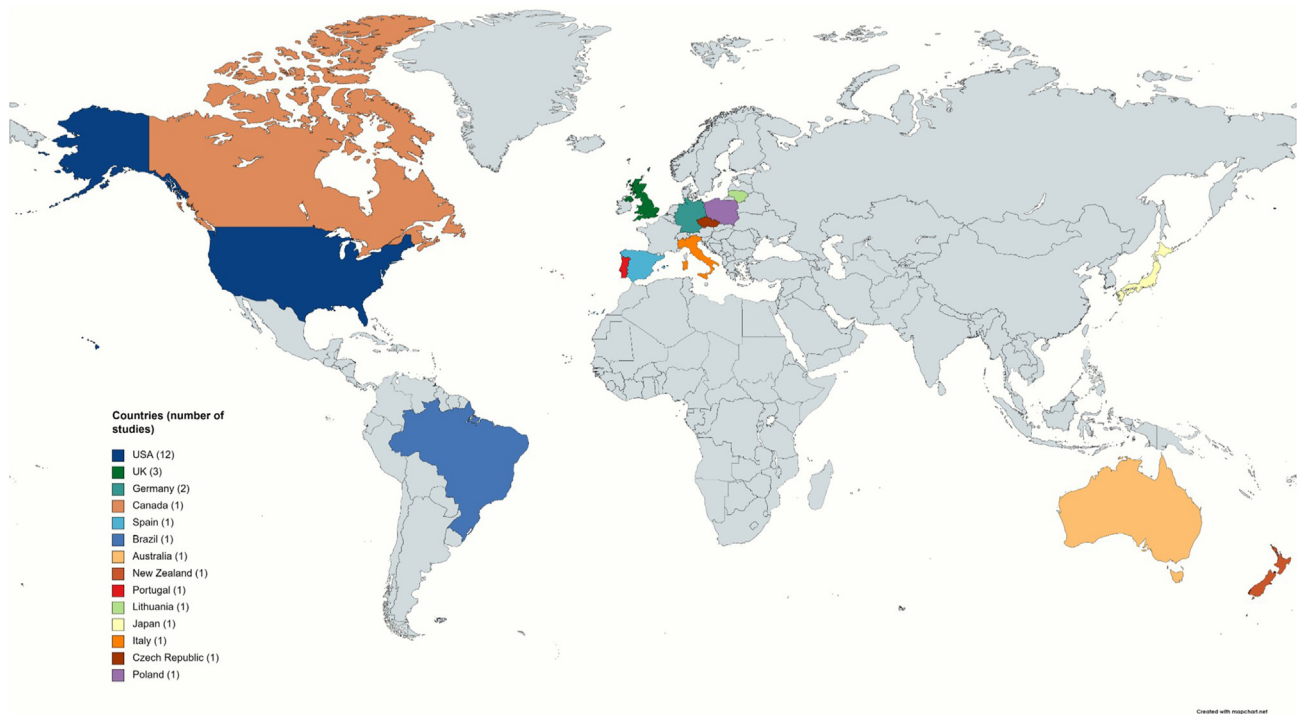


Fig. 2. Geographical distribution of studies (n = 27) included in this systematic review. One study was conducted in two countries (see Supplementary Table S2); hence, 28 studies are listed in the map.

chambers counted and diurnal variations) on the performance of a single technique (Supplementary Table S2).

3.4. Flotation solutions and parasites studied

Solutions used for the preparation of faecal slurries and/or suspension of eggs included sodium chloride and sugar (specific gravity (sg) = 1.25–1.28, n = 8), sugar (sg = 1.2–1.3, n = 8), sodium chloride (sg = 1.2, n = 7), sodium nitrate (sg = 1.11–1.40, n = 7), zinc sulphate (sg = 1.18–1.30, n = 4), and sodium dichromate (sg = 1.35, n = 1) (see Supplementary Table S2). O’Grady and Slocombe (1980) investigated the effect of specific gravity (1.11–1.38) of one flotation solution (sodium nitrate) on FEC and found the optimal flotation (maximum mean eggs per gram (epg) after eight minutes flotation time) of strongyle and ascarid eggs using a flotation solution with a specific gravity of 1.22–1.35. Moreover, the authors also reported that the flotation time (4–12

minutes) had no effect on FEC, using a solution of 1.27 specific gravity. In other studies, sugar solution was found to be more efficient (i.e. higher egg and lower % coefficient of variation (CV%)) compared to salt solutions for performing Mini-FLOTAC® and the McMaster technique for strongyle eggs (Silva et al., 2019), the double centrifugation technique for Anoplocephala spp. eggs (Rehbein et al., 2011) and the McMaster technique for Parascaris spp. eggs (McQueary, 1976).

Strongyles (cyathostomins and strongylins) were the most commonly investigated parasites (n = 19), followed by Parascaris spp. (n = 5), and cestodes (Anoplocephala spp., n = 5). The majority of studies investigated FEC in horses only (n = 19), whereas a few studies included other hosts, such as sheep (n = 7), cattle (n = 4), dogs (n = 4), cats (n = 2), pigs (n = 2), goats (n = 1), and llamas (n = 1) (see Supplementary Table S2).

3.5. Performance parameters

Various diagnostic parameters assessed and/or compared for different techniques included estimates of accuracy (n = 10), precision (n = 10), sensitivity (n = 6), and specificity (n = 1) (Tables 4 and 5; Supplementary Table S2). Moreover, six studies also investigated factors including mesh size, flotation time, counting time, specific gravity, the weight of the faecal sample, as well as variations arising in FEC due to technical and biological factors (Supplementary Table S2).

Out of 15 studies that investigated the accuracy and precision (expressed as percentage precision and coefficient of variation) of FECT, five studies included both measures of diagnostic test performance (Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018; Went et al., 2018; Napravnikova et al., 2019) whereas the remaining studies included one only (Table 4). The accuracy and precision of the McMaster technique and Mini-FLOTAC® were compared in five and eight studies, respectively, and reported variable results, with accuracy being higher for the McMaster method in one study (Went et al., 2018) but lower in three studies (Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018). One study reported greater accuracy of the McMaster technique for strongyles but lower accuracy for Parascaris spp. eggs when compared with Mini-FLOTAC® (Napravnikova et al., 2019). Conversely, Mini-FLOTAC® was

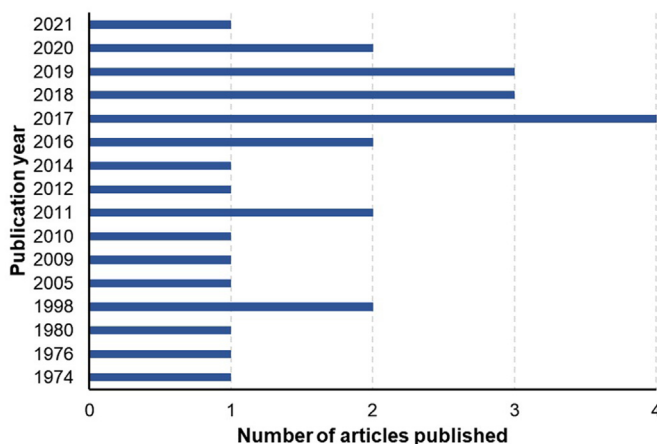


Fig. 3. Year-wise distribution of number of published articles (n = 27) selected in this systematic review.

**Table 4**  
Accuracy and precision of various faecal egg counting techniques used for equine faecal samples

Methods used	Spiked epg/flotation solution	Parasite detected	Accuracy (%)	Precision (%)	Reference
Remodified McMaster (Sheather's sugar)	Not applicable	Ascarids	6,387 <sup>a</sup>	NA	McQueary (1976)
Direct centrifugal flotation (Sheather's sugar)		Strongyles	1,403 <sup>a</sup>		
Remodified McMaster (sodium chloride)		Ascarids	90 <sup>b</sup>		
Direct centrifugal flotation (sodium chloride)		Strongyles	86 <sup>b</sup>		
Sedimentation	1 epg 2 epg	<i>Anoplocephala perfoliata</i>	7,413 <sup>a</sup>	NA	Williamson et al. (1998)
			1,456 <sup>a</sup>		
Flotation 1	10 epg 20 epg 100 epg 200 epg		45 <sup>b</sup>		
			97 <sup>b</sup>		
			0.6		
			0.6		
			0		
			2.4		
Flotation 2	1 epg 2 epg 10 epg 20 epg 100 epg 200 epg		3		
			12		
			0		
			0.18		
			0.2		
			0.7		
FECPAK and McMaster	1 epg 2 epg 10 epg 20 epg 100 epg 200 epg	Strongyles	1.9	FECPAK had lower variance and thus higher precision	Presland et al. (2005)
			3.9		
			0.6		
			1		
			2.2		
			1.2		
Centrifugal flotation McMaster	% of eggs recovered using centrifugal flotation	Cyathostomins	100	NA	Bello and Allen (2009)
			68–81		
McMaster	CV associated with the level of faecal pile, faecal bolus and sample from a faecal bolus, as well as the McMaster procedure	Cyathostomins	NA	CV more dependent on individual animal and higher than faecal bolus and McMaster CV	Denwood et al. (2012)
Combined zinc sulfate sedimentation-flotation	1 epg 5 epg 10 epg 20 epg 40 epg 60 epg 80 epg	Cyathostomins & <i>A. perfoliata</i>	1.20 & 0	NA	Becker et al. (2016)
			2.12 & 1.60		
			3.46 & 1.32		
			2.00 & 0.80		
			4.13 & 0.90		
			2.22 & 0.91		
McMaster	1 epg 30 epg 50 epg 80 epg 100 epg 500 epg 1000 epg		3.51 & 1.00		
			0 & 0		
			22.20 & 22.20		
			44.40 & 26.60		
			61.00 & 30.50		
			37.70 & 39.90		
Smartphone prototype (accuracy: mean % epg; precision: 100–CV) <sup>c</sup>	5 epg 50 epg 500 epg 1000 epg	Strongyles	75.90 & 14.90	71.6	Scare et al. (2017)
			40.70 & NA		
			18.91		
			57.02		
McMaster (accuracy: mean % epg; precision: 100–CV) <sup>c</sup>	5 epg 50 epg 500 epg 1000 epg		25.02	49.12	
			29.15		
			Not applicable		
			11.11		
Mini-FLOTAC (accuracy: mean % epg; precision: 100–CV) <sup>c</sup>	5 epg 50 epg 500 epg 1000 epg		32.22	64.34	
			22.22		
			75.56		
			72.33		
McMaster (accuracy: mean % epg; precision: 100–CV) <sup>c</sup>	5 epg 50 epg 500 epg 1000 epg	Strongyles	87.94	53.7	Noel et al. (2017)
			0		
			16.67		
			43.33		
Mini-FLOTAC (accuracy: mean % epg; precision: 100–CV) <sup>c</sup>	5 epg 50 epg 500 epg 1000 epg		34.16	83.24	
			33.33		
			28.33		
			52.33		
Mini-FLOTAC (accuracy: mean % epg; precision: % CV) <sup>c</sup>	10 epg 50 epg 200 epg 500 epg	Strongyles	56.25	49.6	Bosco et al. (2018)
			90		
			90		
			96		
			82	8.1	
				3.1	

(continued on next page)

Table 4 (continued)

Methods used	Spiked epg/flotation solution	Parasite detected	Accuracy (%)	Precision (%)	Reference
McMaster (chamber) (accuracy: mean % epg; precision: % CV) <sup>c</sup>	10 epg	Strongyles	70	135.6	
	50 epg		78	51.4	
	200 epg		84	23.1	
	500 epg		92	10.9	
McMaster (grid) (accuracy: mean % epg; precision: % CV) <sup>c</sup>	10 epg	Strongyles	80	248.6	
	50 epg		98	90.5	
	200 epg		90	39.9	
	500 epg		98	17.3	
Cornell–Wisconsin (accuracy: mean % epg; precision: % CV) <sup>c</sup>	10 epg	Strongyles	40	33.4	
	50 epg		38	16.6	
	200 epg		52	51.8	
	500 epg		50	5.2	
McMaster (precision as variance) <sup>c</sup>	including FEC < 50 epg excluding FEC < 50 epg	Cyathostomins	NA	0.71	Britt et al. (2017)
Mini-FLOTAC (precision as variance) <sup>c</sup>	including FEC < 50 epg excluding FEC < 50 epg			0.52	
Modified-Wisconsin (precision as variability) <sup>c</sup>	Not applicable	Strongyles	NA	0.045	Paras et al. (2018)
Three-chambered McMaster (precision as variability) <sup>c</sup>				0.311	
Mini-FLOTAC (precision as variability) <sup>c</sup>				0.143	
McMaster (precision as CV) <sup>c</sup>	Not applicable	Strongylid	3rd highest egg count	0.45	Went et al. (2018)
Mini-FLOTAC (precision as CV) <sup>c</sup>			2nd highest egg count	0.23	
McMaster with Fill-Flotac (precision as CV) <sup>c</sup>			highest egg count	0.45	
Mini-FLOTAC with tongue depressor and cup (precision as CV) <sup>c</sup>			4th highest egg count	0.20	
Simple McMaster (accuracy: mean % epg; precision: % CV for spiked and natural infections, respectively) <sup>c</sup>	Not applicable	Ascarids	65.53	62.95 & 31.20	Napravnikova et al. (2019)
		Strongyles	97.53	44.33 & 39.53	
Concentration McMaster (accuracy: mean % epg; precision: % CV for spiked and natural infections, respectively) <sup>c</sup>		Ascarids	83.18	35.71 & 17.92	
		Strongyles	88.39	35.64 & 25.19	
Mini-FLOTAC (accuracy: mean % epg; precision: % CV for spiked and natural infections, respectively) <sup>c</sup>		Ascarids	90.28	18.95 & 14.51	
		Strongyles	74.18	18.25 & 8.64	
Simple flotation (precision as % CV) <sup>c</sup>	Salt	Strongyles	NA	43.15	Silva et al. (2019)
	Sugar		52.43		
Centrifuged flotation (precision as % CV) <sup>c</sup>	Salt			68.97	
	Sugar			86.07	
McMaster (precision as % CV) <sup>c</sup>	Salt			95.75	
	Sugar			53.96	
Mini-FLOTAC (precision as % CV) <sup>c</sup>	Salt			98.16	
	Sugar			50.23	
FECPAK <sup>G1</sup>	Not applicable	Strongyles	100	NA	Tyson et al. (2020)
FECPAK <sup>G2</sup> (accuracy as a percentage of mean FECPAK <sup>G1</sup> egg count) <sup>c</sup>			101		

Abbreviations: CV, coefficient of variation; epg, eggs per gram; NA, not assessed.

<sup>a</sup> Mean epg.

<sup>b</sup> Percent eggs recovered on four successive coverslips based on McMaster counts.

<sup>c</sup> Measure of each of the corresponding parameter given in the published article.

found to be more precise than the McMaster technique for strongyles in seven studies (Britt et al., 2017; Noel et al., 2017; Scare et al., 2017; Bosco et al., 2018; Paras et al., 2018; Went et al., 2018; Napravnikova et al., 2019) whereas one study reported similar precision for both techniques (Silva et al., 2019) (Table 4).

The sensitivity of the McMaster technique was compared with other techniques such as FECPAK®, Wisconsin, flotation, sedimentation, combined sedimentation-flotation, Mini-FLOTAC® and automated egg counting in six studies: five of these studies (Presland et al., 2005; Fukumoto et al., 2011; Tomczuk et al., 2014; Becker et al., 2016; Bosco et al., 2018) reported lower sensitivity of the McMaster technique, while one study (Cain et al., 2020) reported similar sensitivity levels for

McMaster, Wisconsin and an automated egg counting technique (Table 5).

Among studies on other important factors affecting the performance of FECT (Supplementary Table S2), one study reported the effect of using salt and sugar-based flotation solutions and found that Sheather's sugar solution provided better flotation of equine *Parascaris* spp. eggs (McQueary, 1976). In another study conducted by O'Grady and Slocombe (1980), the specific gravity range 1.22–1.35 was found to optimise the recovery of strongyle and ascarid eggs, with no effect of flotation time using a flotation solution of 1.27 specific gravity. Moreover, the same study reported the recovery of the greatest number of strongyle eggs using a mesh size of 500 µm<sup>2</sup>, whereas the recovery of *Parascaris* spp.

**Table 5**  
Sensitivity of various faecal egg counting techniques used for equine faecal samples

Method used	Parameter definition	Spiked epg/worm burden	Parasite detected	Sensitivity (%)	Reference
Sedimentation	Eggs detected in % samples from infected animals	Not applicable	<i>Anoplocephala perfoliata</i>	22.5	Williamson et al. (1998)
Flotation 1				25.0	
Flotation 2				37.5	
Method A (McMaster)	Detection of eggs at different worm burden levels	<100 >100	<i>A. perfoliata</i>	11.8	Meana et al. (1998)
Method B (Modified McMaster)		<100 >100		0	
Method C (Tube and coverslip)		<100 >100		35.3 57.1	
FECPAK	Percentages of various spiked epg levels detected	50 epg 100 epg 200 epg	Strongyles	35.3 42.9 100	Presland et al. (2005)
McMaster	Percentages of various spiked epg levels detected	50 epg 100 epg 200 epg		100 100 100	
McMaster	Detection of eggs at different worm burden levels	5, 9, 11, 18, 23, 104, 156, 160, 162, 1700		<i>A. perfoliata</i>	
Modified Wisconsin (sodium nitrate)				Eggs detected at worm burdens of 23, 104, 156, 160 and 1700	
Modified Wisconsin (zinc sulfate)				Eggs detected at worm burdens of 23, 104, 156, 160 and 1700	
Modified Wisconsin (sucrose)				Eggs detected at worm burdens of 23, 104, 156, 160, 162 and 1700	
Flotation	No. of total tapeworms No. of tapeworms with gravid proglottids	NA	<i>A. perfoliata</i>	16.7 20.7	Tomczuk et al. (2014)
Sedimentation	No. of total tapeworms No. of tapeworms with gravid proglottids			8.3 10.3	
Modified sedimentation-flotation	No. of total tapeworms No. of tapeworms with gravid proglottids			58.3 72.4	
McMaster	No. of total tapeworms No. of tapeworms with gravid proglottids			2.8 3.4	
Combined zinc sulfate sedimentation-flotation	Percentages of various spiked epg levels detected for cyathostomins and <i>A. perfoliata</i> , respectively	1 epg 5 epg 10 epg 20 epg 40 epg 60 epg 80 epg	Cyathostomins & <i>A. perfoliata</i>	6.7 & 0 46.7 & 26.7 86.7 & 35.3 86.7 & 60.0 100 & 80.0 100 & 86.7 100 & 100	Becker et al. (2016)
McMaster		1 epg 30 epg 50 epg 80 epg 100 epg 500 epg 1000 epg		0 & 0 13.3 & 13.3 60 & 26.7 60 & 60.0 80 & 80.0 100 & 100 100 & 100	
Mini-FLOTAC	Percentages of various spiked epg levels detected	10 epg 50 epg	Strongyles	100 100	Bosco et al. (2018)
McMaster (chamber)		10 epg 50 epg		41.7 100	
McMaster (grid)		10 epg 50 epg		25.0 75.0	
Cornell-Wisconsin		10 epg 50 epg		100 100	
McMaster Wisconsin Automated egg counting technique	Percentages of various spiked epg levels detected	0–200; 201–500; 501–1000; >1000 epg	Strongyles	99.40 99.40 98.00	Cain et al. (2020)

Abbreviations: CV, coefficient of variation; epg, eggs per gram; NA, not assessed.

eggs appeared to not be influenced by the use of mesh sizes between 200 and 500  $\mu\text{m}^2$  (O'Grady and Slocombe, 1980). Similarly, Rehbein et al. (2011) investigated the effect of flotation solution type and the weight of the faecal sample on the recovery of tapeworm eggs and found that the use of larger sample sizes in sugar-based flotation solutions provided greater FEC. Slusarewicz et al. (2019) investigated the effect of duration of egg counting (at-leisure, two-minutes and one-minute) on FEC of the

McMaster technique compared to Parasight system® and reported a substantial decrease in the precision (one-third, assessed by the coefficient of variation) and FEC (50–60% decrease) for the former method following one-minute counting instead of at-leisure counting. Another study used a Fill-FLOTAC device to investigate the effect of sample homogenisation on FEC and reported that homogenisation resulted in improved accuracy with no effect on precision (Went et al., 2018). The

diagnostic specificity was compared in a single study which reported higher values for the McMaster method than that of Mini-FLOTAC (Noel et al., 2017) (Supplementary Table S2).

To the best of our knowledge, this is the first systematic review to critically appraise studies that assessed and/or compared the performance of FECT in horses. The findings of this review highlight the potential opportunities for improvements in future studies aiming to validate and/or compare various FECT for horses and other animal species. It is clear from the results presented above that there is no consensus on the methodology and assessed parameters (and their interpretations) in FECT comparison studies. Although the McMaster technique has been used as a reference standard (a test of known and high accuracy) by most researchers when assessing and comparing other techniques, it lacks diagnostic sensitivity for samples with lower egg counts. Additionally, the analytical and diagnostic capabilities of the McMaster technique are affected by a number of factors (e.g. the amount and type of faecal samples, volume and specific gravity of flotation solutions) (Roepstorff and Nansen, 1998). More recently, some studies have used FLOTAC® (Cringoli, 2006) and Mini-FLOTAC® (Cringoli et al., 2017) as reference standards because of reportedly higher sensitivities in comparison to the McMaster technique. However, there is also considerable variation in the performance of these methods and other similar techniques due to a variety of factors that need to be thoroughly investigated for intended species in order to use them as reference standards (the best test available at a time for comparison) (Duggan, 1992; Claassen, 2005).

The choice of flotation solution is one of the key factors that may affect the FEC of a sample, but it often receives less consideration. Several studies have reported that the sucrose-based solutions provide more accurate FEC in horses and other animals than other flotation solutions (McQueary, 1976; Cringoli et al., 2004; Rehbein et al., 2011; Silva et al., 2019). However, no single flotation solution is ideal for all FECT and/or different egg types in faeces. For example, Cringoli et al. (2017) reported that for Mini-FLOTAC®, sodium chloride with a specific gravity of 1.20 and glucose-salt solutions with a specific gravity of 1.24–1.26 were the most efficient flotation solutions for equine strongyles, whereas zinc sulphate with a specific gravity of 1.35 and glucose-salt solutions with the specific gravity of 1.24–1.26 were better for *Parascaris* spp. eggs.

Another important factor to consider for FECT is the specific gravity used for a flotation solution. Previously, O'Grady and Slocombe (1980) demonstrated that the best flotation of parasite eggs was achieved with solutions with a specific gravity between 1.22 and 1.35. Currently, the majority of FEC methods utilise flotation solutions with specific gravity  $\geq 1.2$ . This value is also in agreement with the estimated mean specific gravities calculated for strongylid, *Parascaris* spp. and *Anoplocephala* spp. eggs by Norris et al. (2018) as 1.0453, 1.0903 and 1.0636,

respectively. The only other evidence available is about the specific gravity of *Parascaris equorum* eggs (1.0969) (David and Lindquist, 1982). Exceeding the upper limit of specific gravity (1.35) usually results in the flotation of faecal debris and rapid crystallisation of the flotation solution (O'Grady and Slocombe, 1980). Additionally, Denwood et al. (2012) investigated the sources of variability in FEC results (both within and between animals) and reported that within animal variation (unequal distribution of eggs between faecal piles) was the most important factor responsible for variation in FEC. They also reported that no diurnal variation was found in FEC and these findings were in agreement with those of Carstensen et al. (2013). Recently, Wilkes et al. (2019) reported significant variability in strongyle and ascarid FEC in individual foals between different portions of a faecal pile, different faecal piles, and samples collected across different days. Given the significance of these important factors for FEC, studies aiming to validate and compare various FECT should take into account differences arising from using various flotation solutions, the size and the number of faecal (sub)samples used for testing.

The reported higher performance (accuracy and precision) of Mini-FLOTAC® compared to the McMaster technique is probably because the former technique requires and analyses a larger, uniformly homogenised (using a Fill FLOTAC device) faecal sample; thereby, it has a lower multiplication factor than that of the McMaster method. The only study which reported higher accuracy for McMaster was probably due to the use of Fill-FLOTAC in combination with the McMaster technique, resulting in a better homogenisation of eggs within faecal samples (Went et al., 2018). This study also reported that the Fill-FLOTAC affected accuracy, but not precision, while the counting chamber (Mini-FLOTAC vs McMaster) affected precision, but not accuracy (Went et al., 2018). However, further testing would be required to support these preliminary findings. Similarly, the sensitivity and efficiency of different FECT are directly related to faecal quantity, dilution factor and the volume of suspension analysed (Lester and Matthews, 2014; Daş et al., 2020).

#### 4. Future implications

With the growing concerns of anthelmintic resistance and, importantly, the lack of new anthelmintics available to control equine parasites, there is an increased interest amongst veterinary professionals and animal-health officials in the development and validation of veterinary assays for accurate diagnosis, surveillance and monitoring of parasitic infections (Nielsen, 2021). It is therefore important to properly validate a FEC method prior to its comparison with existing standard techniques. For an assay to be considered validated for an intended purpose, there are

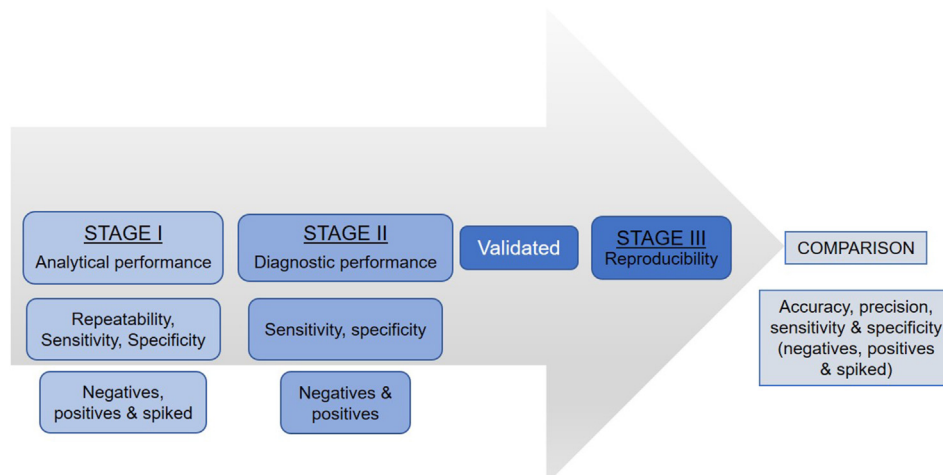


Fig. 4. Flowchart diagram of steps involved in validation and comparison of a diagnostic assay.



certain critical requirements that must be met, as outlined in guidelines for the development and validation of diagnostic assays by the World Organisation for Animal Health (Office International des Epizooties: OIE) (Jacobson and Wright, 2019). The developmental stage of a diagnostic test involves the description of the intended purpose, standardisation and optimisation of the assay. Test validation is critical and is often not completed properly for veterinary diagnostic tests in general and FECT in particular. This stage includes measuring analytical parameters (repeatability, analytical sensitivity and specificity), diagnostic parameters (diagnostic sensitivity and specificity) and reproducibility (Jacobson and Wright, 2019). Once an assay completes both of these stages, it is considered validated and can then be used in the target population for its intended purpose (Jacobson and Wright, 2019). For calculating the required number of samples to be tested through each stage of the assay development and validation, prior consultation with a (bio)statistician should be made as per the recommendations of the OIE. After the test is validated, its comparison with existing FEC methods can be made, and the important parameters which need to be compared include accuracy, precision, repeatability, and diagnostic sensitivity and specificity (Fig. 4). Additionally, the FECT comparison studies should also pay attention to other important sources of variation (both technical and biological as outlined in this article) in FEC and their potential impact on the performance of FECT in designing and conducting such studies. It is anticipated that this proposed assessment method of developing and validating a new diagnostic method and comparing a new test with existing test(s) would help the parasitological community standardise methods for parasite diagnostics as per the OIE guidelines.

## 5. Conclusions

In conclusion, this systematic review has clearly demonstrated that there is a lack of consensus on the methodology used, assessed performance parameters, and interpretation of results for studies on the assessment and comparison of FECT. Most studies used the McMaster technique to compare the performance of FECT and reported higher estimates of accuracy, precision and sensitivities for other techniques. Sugar-based solutions were reported to perform better for egg recovery in most of the techniques. This systematic review highlights the need for thorough validation studies which characterise the analytical and diagnostic parameters of existing and new FECT as outlined by the OIE standards. For example, for future studies aiming to validate or compare a new technique, analytical and diagnostic parameters should be tested experimentally.

## Funding

The financial assistance for this project was provided by the AgriFutures Australia, Thoroughbred Breeders Australia and Boehringer Ingelheim Animal Health Australia Pty. Ltd. Boehringer Ingelheim did not have any role in the design or content of this manuscript.

## CRedit author statement

Abdul Ghafar, Abdul Jabbar: Conceptualization, Data curation & Writing - Original Draft. Abdul Jabbar: Funding and Supervision; Ghazanfar Abbas, Justine King, Caroline Jacobson, Kristopher J. Hughes, Charles El-Hage, Anne Beasley, Jenni Bauquier, Edwina J.A. Wilkes, John Hurley, Lucy Cudmore, Peter Carrigan, Brett Tennent-Brown, Martin K. Nielsen, Charles G. Gauci, Ian Beveridge: Writing - Reviewing & Editing. All authors read and approved the final manuscript.

## Data availability

All data reported in this paper are either included the manuscript or presented as a supplementary material.

## Declaration of competing interests

The authors are members of the Australian Equine Parasitology Advisory Panel supported by AgriFutures Australia and Boehringer Ingelheim Animal Health Australia. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. Given his role as a Co-Editor, Abdul Jabbar had no involvement in the peer-review of this article and has no access to information regarding its peer-review. Full responsibility for the editorial process for this article was delegated to Luis Cardoso and Aneta Kostadinova.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.crpvbd.2021.100046>.

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