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Research paper

# Evaluation of two *Fasciola hepatica* faecal egg counting protocols in sheep and cattle

Claire Reigate [a,*], Hefin W. Williams [a], Matthew J. Denwood [b], Russell M. Morphew [a], Eurion R. Thomas [c], Peter M. Brophy [a]

[a] Institute of Biological, Environmental & Rural Sciences, Aberystwyth University, Penglais Campus, Aberystwyth, Ceredigion, SY23 3FG, United Kingdom
[b] Department of Veterinary and Animal Sciences, Faculty of Health and Medical Sciences, University of Copenhagen, Frederiksberg, Denmark
[c] Techion UK, Peithyll Centre, Capel Dewi, Aberystwyth, Ceredigion, SY23 3HU, United Kingdom

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ABSTRACT

Fascioliasis causes significant economic losses and is a constant challenge to livestock farmers globally. Fluke faecal egg counts (flukeFECs) are a simple, non-invasive method used to detect the presence of patent liver fluke infection. Many flukeFEC techniques exist but they vary in complexity, precision and accuracy. The objective of this study was to evaluate the egg recovery capabilities of two simple flukeFEC methods at different egg concentrations in two ruminant species, using artificially spiked faecal samples. We added *Fasciola hepatica* eggs to sheep and cattle faeces at 2, 5 10 and 20 epg and utilised the Flukefinder® (FF) and a simple sedimentation method (referred to as the Becker method) to investigate the effects of methods, species and egg density on egg recovery. We calculated the proportion of fluke eggs recovered using each technique, and determined the lowest reliable egg detection threshold of each flukeFEC method. The performance of the flukeFEC methods were also compared using faecal samples collected from naturally infected animals. The egg-spiking study revealed that both FF and the Becker sedimentation method are significantly more likely to recover eggs from cattle faeces than sheep ($P < 0.001$). Overall, FF recovered more eggs than the Becker method ($P < 0.001$), and importantly has a reliable low egg detection threshold of 5 epg in sheep and cattle. The kappa coefficient indicated a substantial agreement between FF and the Becker method in naturally infected faecal samples collected from cattle (0.62, $P < 0.05$) and a moderate agreement in sheep (0.41, $P < 0.05$). This study demonstrated that FF has a low egg detection threshold and therefore has promising potential for the future of on-farm liver fluke diagnostics.

## 1. Introduction

Fascioliasis is a debilitating parasitic flatworm disease of livestock reared on pasture-based systems. Prevalence is global and increasing due to a changing climate, emerging anthelmintic resistance and adaptations in agricultural practices (Beesley et al., 2017; Fairweather, 2005; Fox et al., 2011). In the UK, where the majority of production systems are grassland-based, the cost of fascioliasis to the UK livestock sector was estimated to be almost €300 m annually (Jones et al., 2015; Williams, 2014). On-farm diagnosis and monitoring of ruminant fascioliasis is challenging. In the UK, indication of liver fluke presence on a farm often comes from liver condemnation reports (Hanley et al., 2020), but these are based on visual evaluations and therefore not standardised between abattoirs, thus making them variable (Mazeri et al., 2016) and potentially erroneous. There are several ante-mortem fluke diagnostic tests available, but no one test can be considered as having adequately high sensitivity and specificity in the field setting (Mazeri et al., 2016; Rapsch et al., 2006), and none can be considered as 'pen-side' for use directly on farms. The serological tests are blood, sera and milk ELISAs and thus rely on circulating antibodies (Arifin et al., 2016). These antibody-based tests are able to provide early detection of *F. hepatica* infection in previously naïve animals, and have high sensitivity and reproducibility (Arifin et al., 2016; Beesley et al., 2017), making them valuable support tools for managing infection in imported stock or animals in the first year of exposure. However, antibody-based tests are incapable of clearly distinguishing between active, post-treatment or historic infections (Salimi-Bejestani et al., 2005), and results regarding a possible correlation between specific antibody levels and fluke burden is conflicting (Alvarez Rojas et al., 2014; Kuerpick et al., 2013; Reichel, 2002). Coproantigen tests are additional promising tools in fluke diagnostics, they have been

* Corresponding author.
*E-mail address:* clr19@aber.ac.uk (C. Reigate).

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shown to be 100 % sensitive at detecting artificial ovine fasciolosis, and do confer some correlation between fluke burden and coproantigen levels (Brockwell et al., 2013). However, they too require a laboratory that has the capability of performing ELISAs, cannot be performed directly on-farm and have not been extensively tested under field conditions to confirm the same sensitivity as seen in experimental infections (Calvani et al., 2018; Gordon et al., 2012). In contrast, fluke faecal egg counts (flukeFECs) are simple, non-invasive and the results are immediate. They can be performed on-farm as they do not require any specialist sampling techniques nor expensive sophisticated laboratory equipment, just a light based microscope and a water supply. FlukeFECs are generally regarded as being almost 100 % specific and are widely used in veterinary practice although a major limitation is they will only detect patent infections (Mazeri et al., 2016). There is no single gold-standard diagnostic in fluke infection so often a combination of clinical signs, grazing history, abattoir reports, serological, coproantigen and flukeFECs are used depending on circumstances. FlukeFECs therefore remain a valuable diagnostic tool. A large number of different variations of flukeFECs have previously been described, using either simple sedimentation (Becker et al., 2016; Conceição et al., 2002), sedimentation with fine filtration (Arifin et al., 2016; Dorsman, 1956a,b; Happich and Boray, 1969; Kleiman et al., 2005) or sedimentation followed by flotation (Charlier et al., 2008; Malrait et al., 2015; Rinaldi et al., 2015).

The operational characteristics of diagnostic tests are commonly evaluated in terms of diagnostic sensitivity and specificity. Sensitivity reflects the ability of a test to correctly identify those individuals that are infected and specificity is the ability of a test to correctly identify those uninfected as negative. Confusingly, FEC methods can also be evaluated in terms of 'detection sensitivity', but this is not a direct measure of its ability to correctly identify positive animals, but rather the lowest faecal egg load that can be reliably measured using a given method (Paras et al., 2018). Therefore, detection sensitivity (which we refer to as the egg detection limit for the avoidance of confusion with sensitivity) with respect to parasitic helminths needs to be determined by artificially spiking faecal samples with a known number of eggs and evaluating the test performance prior to field testing for sensitivity.

Flukefinder® (FF) is a commercially available egg detection device based on a modified sedimentation and fine filtration technique (Dixon and Wescott, 1987). It is commonly used in veterinary diagnostic laboratories across North America (Zajac and Conboy, 2012). To date, FF evaluation against other coprological methods has been fragmented: using spiked human stool samples (Zárate-Rendón et al., 2019), experimentally infected rats (Duthaler et al., 2010) or naturally infected cattle (Kleiman et al., 2005). To our knowledge there are no studies comparing FF to alternative flukeFEC methods in artificially spiked sheep and cattle samples. Becker and colleagues (2016) previously assessed the egg detection limit and efficiency of a simple sedimentation technique using sheep faeces spiked with *F. hepatica* eggs ranging from 1 to 30 eggs per gram (epg). The Becker protocol requires minimal lab equipment and can easily be adapted for on-farm use. Given this potential for use by farmers it represents a possible alternative to FF. Consequently, the objective of the current work was to determine the influence of factors effecting egg recovery (flukeFEC method, livestock species, and fluke egg concentration), evaluate the fluke egg detection limits and egg recovery ratios of FF and the Becker method (Becker et al., 2016) at a range of fluke egg levels 'spiked' into faecal samples pooled from uninfected sheep and cattle. Moreover, we also sought to compare the level of agreement between both flukeFECs methods in naturally infected animals.

## 2. Methods

### 2.1. Egg spiking study

#### 2.1.1. Collection of fluke eggs

Adult *Fasciola hepatica* parasites were harvested from condemned sheep livers at a local abattoir (Randall Parker Foods, Powys, UK). Infected livers identified by the meat inspector were placed into a large metallic tray, the main bile ducts were severed and squeezed. Emerging flukes were collected and transferred into pre-warmed phosphate buffered saline (PBS). Each liver lobe was carefully sliced at 1 cm intervals and any adult parasites were added to the warm PBS and maintained at 37 °C in a water bath until transportation back to the laboratory. After 3 h, the adult parasites were carefully removed and the solution containing the eggs was filtered through two sieves at 180 and 35 μm with tap water and washed thoroughly. The filtrate was discarded and the material on the bottom 35 μm sieve was backwashed, collected and allowed to sediment for 30 min. The supernatant was decanted and the sediment was re-suspended in distilled water ($dH_2O$) for a further 30 min. This was repeated until the supernatant became clear. Collected eggs were stored in $dH_2O$ at 4 °C until required.

#### 2.1.2. Fluke egg negative faecal samples

Composite faecal samples were collected from fluke-free animals for the purpose of having a source of fluke-egg negative faeces to use in the egg-spiking study. The faeces came from animals considered not to be shedding fluke eggs due to their age and grazing history, however each homogenised mob sample was tested 10 times using the FF protocol, to confirm the faeces was fluke free.

One hundred four-month old lambs were held in a concrete yard for two hours. A composite sample was made from fresh samples collected from the ground and the whole sample (approximately 1.5 kg) placed into a single sealable sample bag. In addition, a large composite faecal sample was collected from the straw bed of 20 zero-grazed 5-month-old heifers (approximately 3 kg). All samples were stored at 4 °C until required for the egg spiking experiments.

#### 2.1.3. Egg spiking

Harvested *F. hepatica* eggs were counted into batches (as summarised in Supplementary Table S1), to spike into fluke egg-negative faeces at concentrations of 2, 5, 10 and 20 epg. Using a 100 μL pipette, *F. hepatica* eggs were counted onto a standard glass slide, that had a printed grid on the underside, using a low-resolution microscope (mag x 40 Zeiss, West Germany). Enumerated eggs were either directly transferred to the faecal sample or carefully washed into 3 mL Eppendorf tubes using $dH_2O$ and stored in batches at 4 °C for up to 48 h. The number of eggs added to each sample is shown in Supplementary Table S1. For egg concentrations of 2 epg and 5 epg the eggs were transferred directly to the sample, but for 10 epg and 20 epg the eggs were kept in batches. The batches were made to minimise the time the eggs spent outside the fridge, inhibiting unwanted development.

### 2.2. FlukeFEC protocols

#### 2.2.1. Modified Flukefinder®

FF was carried out according to the manufacturer's instructions with an additional step involving a 50 mL Falcon tube and a 4-minute sedimentation. This step was added to ensure all of the backwashed sediment was collected from the FF apparatus. In detail, 2 g of faeces were weighed, and a known number of fluke eggs were added. Approximately 30 mL of water was mixed into the sample to produce a slurry which was poured into an assembled FF and held under a steady flow of water. The FF was firmly tapped to expedite the flow of water through the filters. This was repeated at least 3 times before the top unit was removed and the sediment caught on the bottom filter was carefully backwashed into a 50 mL Falcon tube. Once all the sediment was collected, tap water was added to make a total volume of 45 mL and it was left to sediment for 4 min. The supernatant was siphoned off and the faecal debris was transferred to a 15 mL Falcon tube. This was sedimented for 2 min and repeated four times before one drop of methylene blue stain (1 %) was added. The sample was mixed and poured into a concentric 30 mm diameter lined petri dish. Care was taken to rinse/wash all the faecal debris from the 15 mL Falcon tube into the Petri dish before the entire

Petri dish was viewed using a light compound microscope (mag x 40 Zeiss, West Germany). Each egg concentration (5, 10 and 20 epg) was repeated 20 times for sheep and 20 times for cattle. The same researcher performed all the flukeFECs. As we were able to detect eggs in 100 % of sheep and cattle samples at 5 epg, we also tested the FF at egg concentration of 2 epg in both species.

#### 2.2.2. Becker method

The Becker method was used as described previously (Becker et al., 2016). Briefly, 5 g of faeces was weighed in a plastic beaker and spiked with *F. hepatica* eggs at densities of 5, 10 and 20 epg. Tap water was added and the sample was mixed using a metal spatula until all clumps had dissipated. The samples were rinsed through a stainless-steel tea strainer with a strong water jet into a 250 mL plastic beaker, filled with tap water and left to sediment for 30 min. The supernatant was siphoned off and the sediment was transferred into a 500 mL conical measure. This was left to sediment for a further 3 min. The supernatant was again discarded, the sample refilled with tap water, and left to sediment for a further 3 min. This step was repeated four times before three drops of methylene blue solution (1 %) were added and the sample was transferred to a standard circular Petri dish (100 mm diameter) before the entire Petri dish was examined for eggs using a compound microscope (mag x 40 Zeiss, West Germany). Each egg concentration (5, 10 and 20 epg) was repeated 20 times for sheep and 20 times for cattle.

### 2.3. Faecal samples from naturally infected animals

Animals identified as high-risk for fascioliasis based on farm history, age and grazing history provided fluke-egg suspect samples for the flukeFECs method agreement analysis. Faecal samples from twenty naturally infected sheep used in this study were collected from ewes on Aberystwyth University farms, Wales, UK. Individual faecal samples were collected in March 2019 from ewes pre-treatment prior to turnout at lambing by placing them in individual pens and waiting for them to defecate. Cattle samples were collected in the summer of 2019 from an upland suckler herd in mid-Wales containing a bull, cows with calves at foot and one in-calf heifer. The cattle were turned out in spring and had been grazing in areas known to harbour the intermediate hosts for fluke. Twenty large fresh pats were collected directly from the pasture, bagged individually, labelled and kept at 4 °C until investigation.

### 2.4. Statistical analysis

Statistical analyses were carried out with R statistical software (R core team 2019) version 3.6.1 and Excel® 365 (Microsoft, Washington). The R code for the generalised linear mixed model (glmm), and raw data can be found in the Supplementary Materials S1, and Supplementary Table S2, respectively.

#### 2.4.1. Generalised linear mixed model for comparative egg recovery

Generalised linear mixed models with binomial response distribution and logistic link function were fitted using the '*glmer*' function (Supplementary Material S1) from the lme4 package (Bates et al., 2015) for R (R core team 2015), such that the estimated coefficients may be interpreted as log-odds ratios (i.e. odds ratios when exponentiated). The number of eggs recovered given the number of eggs present was the response variable, and explanatory variables representing the flukeFEC methods (FF and Becker), livestock species (cattle and sheep) and the egg densities in the samples (2, 5, 10 and 20 epg) were used as fixed effects. An interaction term between method and egg densities was evaluated using a likelihood ratio test. FF, cattle and 10 epg were set as the reference categories within the respective fixed effects. Fixed effects were considered significant when $p < 0.05$. A random effect of sample was also added to the model (Harrison, 2014) to allow for any minor variations in the true egg content of each sample. As this random effect is at the observation level, it is equivalent to fitting an over-dispersed Binomial distribution as the response distribution for the model.

#### 2.4.2. Egg detection limits and recovery ratios

The egg detection limit was determined for both flukeFECs methods at egg concentrations of 5, 10 and 20 epg, and at 2 epg only for FF as it was able to detect at least one egg in 100 % of sheep and cattle samples at 5 epg. The egg detection limit is defined as the minimum faecal egg density in which at least one egg was detected in 100 % of the samples. Egg recovery ratios (%) were determined 1) for each egg density by calculating the total number of eggs recovered in the combined 20 repetitions, and 2) for all of the samples (combined egg densities) using the following equation:

$$EggRecoveryRatio(\%) = \frac{total number of eggs recovered over all repetitions}{total number of eggs that went into all the samples} \times 100$$

#### 2.4.3. Agreement between methods in naturally infected samples

The degree of agreement between flukeFEC protocols was determined by Cohens Kappa statistic (κ) using R. The statistical code used to determine κ (with P-values) is provided in Supplementary Materials S2. The cut offs used were: $\kappa < 0$ no agreement, $0 < \kappa < 0.2$ slight agreement, $0.21 < \kappa < 0.40$ fair agreement, $0.41 < \kappa < 0.60$ moderate agreement, $0.61 < \kappa < 0.80$ substantial agreement, $0.81 < \kappa < 1$ is considered almost perfect (Landis and Koch, 1977).

## 3. Results

Sheep and cattle faecal samples were spiked with known numbers of *F. hepatica* eggs at different egg densities. A generalised linear mixed model (glmm) was used explore the influence of different factors (flukeFEC method, species and egg concentration) on egg recovery. Also, the egg detection limits and recovery ratios were determined. Lastly, 40 individual faecal samples from naturally infected sheep ($n = 20$) and cattle ($n = 20$) were used to calculate the agreement between the two flukeFEC techniques.

### 3.1. Generalised linear mixed model for comparative egg recovery

The glmm (controlling for the effect of individual sample by considering it a random effect) indicates that FF recovers significantly more eggs than the Becker sedimentation method in both ruminant species ($p < 0.001$). Both flukeFEC techniques are significantly more likely to recover fluke eggs from cattle faeces than sheep ($p < 0.001$). There was no significant difference in the proportion of eggs recovered at 10 epg compared to 5 nor 20 epg (Table 1). Data were only available for FF at 2 epg and the glmm indicates proportionally more eggs were recovered at 10 epg than 2 epg in both species ($p < 0.001$).

Adding an interaction term between the flukeFEC method and epg, the likelihood ratio test did not significantly improve the model fit (Chi square = P = 0.709), nor did adding an interaction term between flukeFEC method and species ($X^2$ P = 0.157), and epg levels and species ($X^2$ P = 0.113). Consequently, the simpler model with no interaction was retained as the final model. The standard deviation of the random effect of sample (over-dispersion in the response variable) was estimated as 0.144 (95 % CI: 0.000−0.281) - this relatively small estimate indicates that the egg content was experimentally quite well controlled. The glmm output from R is summarised in Table 1.

### 3.2. Egg detection limit and egg recovery ratios from spiked samples

The egg detection limits and the egg recovery ratios of both methods are detailed in Table 2. FF recovered at least one egg in all samples (100 %) at concentrations 5, 10 and 20 epg in both sheep and cattle. FF recovered eggs in 55 % and 75 % of samples at 2 epg of sheep and cattle faeces, respectively (Table 2) and thus we conclude an egg detection limit of 5 epg in both species for this method. By comparison, the Becker

**Table 1**
*F. hepatica* egg recovery odds ratios as determined by a generalised linear mixed model with binomial error distribution. FF, cattle and 10 epg were the fixed factors.

| Predictors | | Coefficients | Odds Ratio | 95 % CI* | *P*-value |
|---|---|---|---|---|---|
| Random effects | | | 0.14 | 0.00 – 0.28 | |
| Intercept | | −0.317 | 0.73 | 0.63 – 0.85 | <0.001 |
| Species | | | | | |
| | Cattle | 0 | – | – | – |
| | Sheep | −0.337 | 0.71 | 0.62 – 0.82 | <0.001 |
| Method | | | | | |
| | FF** | 0 | – | – | – |
| | Becker | −2.783 | 0.06 | 0.05 – 0.07 | <0.001 |
| Egg concentration | | | | | |
| | 10 epg | 0 | – | – | – |
| | 5 epg | −0.223 | 0.80 | 0.64 – 1.00 | 0.051 |
| | 20 epg | −0.143 | 0.87 | 0.74 – 1.02 | 0.078 |
| | 2 epg | −0.801 | 0.45 | 0.30 – 0.66 | <0.001 |

* Confidence interval.
** Flukefinder ®.

method recovered *F. hepatica* eggs from 30, 70 and 100 % of the samples at 5, 10 and 20 epg in sheep and 50 %, 100 % and 100 % at 5, 10 and 20 epg in cattle (Table 2). We conclude that the Becker method is unreliable at 5 epg and has a higher egg detection limit of 20 epg in sheep and 10 epg in cattle. The egg recovery ratios vary depending on the species and egg load. FF recovered the highest proportion of eggs at 10 epg in sheep and cattle (33 % and 43 % respectively). The overall egg recovery efficiency in spiked sheep and cattle faecal samples was recorded as 32 % and 38 % for FF and 3 % and 4 % for the Becker sedimentation method (Table 2).

### 3.3. Naturally infected samples

The number of positive naturally infected samples using both flukeFEC techniques were also examined (Table 3 and Supplementary Table S3). The methods were assessed according to the presence or absence of eggs, not the number of eggs recovered. FF recovered eggs from 30 % of sheep and 20 % of cattle samples. In comparison, the Becker method recovered eggs from 10 % of samples in both sheep and cattle, all of which were also positive for the FF (Table 3). In naturally infected samples, the kappa agreement between the flukeFEC techniques indicated a moderate agreement in sheep (0.41, $P < 0.05$) and a substantial agreement in cattle (0.62, $P < 0.05$) (Landis and Koch, 1977).

## 4. Discussion

FlukeFECs are a simple non-invasive tool that can be adapted for pen-side use to diagnose and monitor patent liver fluke infections on farm. The present study compared two of these simple coprological flukeFEC techniques for the recovery of *F. hepatica* eggs from artificially spiked ruminant faecal samples. Using a geglmm we analysed the factors affecting egg recovery from spiked faeces and explored the interactive effects between methods, livestock species and egg density, quantified the egg detection limit and egg recovery ratios, and tested the degree of agreement between the two flukeFEC methods.

Data in this study indicate that FF is more effective than the simple Becker sedimentation method at retrieving fluke eggs in both ruminant species (Tables 1–3). However, the glmm indicates that both flukeFEC methods are significantly more likely to recover eggs from cattle faeces than sheep ($P < 0.001$). The likelihood of recovering more eggs from cattle faeces than sheep is not surprising as variation in coprological test performance between species is not uncommon (Bosco et al., 2018; Cringoli et al., 2010; Paras et al., 2018) and is often attributed to moisture content and faecal composition. Sheep are more inclined to graze closer to the ground. In doing so sheep tend to pick up more soil particles and thus more debris in their faeces. These factors likely affect egg dispersion/aggregation in faeces, the rate at which eggs are able to sediment and are observed under microscopic examination but further investigation is needed.

The glmm was initially run with and without 2 epg because only data from FF were available for this egg concentration. The conclusions based on the fixed effects were the same for both analyses in that no significant difference in egg recovery for 10 epg, compared to 5 epg and 20 epg. However, when 2 epg was included, the model suggested that significantly less eggs are recovered at 2 epg, than 10 epg. The lack of difference between 5, 10 and 20 epg could indicate some kind of plateaux effect but this requires further investigation. That said, when there are less eggs in the sample, losing one in the sedimentation/filtering process or one being overlooked has more of an outsized effect on the final result. So, as the model suggests egg recovery using the FF is much lower at 2 epg. This highlights the challenge of finding individual eggs at the lower end of the detection limits.

A limitation of egg-spiking studies is discerning the true numbers of parasite eggs within a sample. An attempt was made to mitigate this by

**Table 3**
The agreement between the two flukeFEC methods for recovering *F. hepatica* eggs from naturally infected ovine and bovine faecal samples.

| | FF | | Becker | | | |
|---|---|---|---|---|---|---|
| | positive | negative | positive | negative | κ agreement | P value |
| Sheep | 6 | 14 | 4 | 16 | 0.41 | 0.022 |
| Cattle | 2 | 18 | 2 | 18 | 0.62 | 0.002 |

**Table 2**
Sensitivity and recovery ratios of FF and Becker sedimentation methods for the detection of *F. hepatica* in artificially spiked cattle and sheep faecal samples. Samples were spiked at 2, 5, 10 and 20 epg.

| Species | epg | Sensitivity* (%) | | Egg Recovery Ratio (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | FF | Becker$ | FF | | | Becker$ | | |
| | | | | Eggs in | Eggs out | Eff (%) | Eggs in | Eggs out | Eff (%) |
| Sheep | 2 | 55 | – | 80 | 14 | 18 | – | – | – |
| | 5 | 100 | 30 | 200 | 61 | 31 | 500 | 10 | 2 |
| | 10 | 100 | 70 | 400 | 134 | 34 | 1000 | 20 | 2 |
| | 20 | 100 | 100 | 800 | 262 | 33 | 2000 | 60 | 3 |
| Total efficiency | | | | 1480 | 471 | 32 | 3500 | 90 | 3 |
| Cattle | 2 | 75 | – | 80 | 21 | 26 | – | – | – |
| | 5 | 100 | 50 | 200 | 75 | 38 | 500 | 17 | 9 |
| | 10 | 100 | 100 | 400 | 173 | 43 | 1000 | 54 | 5 |
| | 20 | 100 | 100 | 800 | 292 | 37 | 2000 | 75 | 4 |
| Total efficiency | | | | 1480 | 561 | 38 | 3500 | 146 | 4 |

epg = eggs per gram.
* Sensitivity = The percentage of samples in which at least one egg was recovered.
$ The Becker sedimentation method was not evaluated at 2 epg because the sensitivity at 5 epg was already below 100 %.

counting each individual egg into batches before seeding the samples, but still within sample variation is inherent. Additionally, eggs from 2 and 5 epg egg densities were added directly to the sample, and eggs in 10 epg, and 20 epg samples were added via batches. To overcome the potential source of bias a random effect of sample was included in the final model. The relatively small standard deviation estimate (0.14, 95 CI 0.00 – 0.28) indicates the number of eggs in the samples was experimentally quite well controlled. A further limitation of this study is the same observer carried out all of the sampling, and thus intra- and inter-observer repeatability were not evaluated.

In practice, the confirmation of presence or absence of fluke eggs is commonly used for veterinary interventions. A negative egg count does not definitively confirm the absence of infection, and false negative flukeFECs are often reported in field studies (Anderson et al., 1999; Arifin et al., 2016; Charlier et al., 2008; Mazeri et al., 2016). This failure is a consequence of *Fasciola* spp. eggs being only detectable in faeces at around 8–10 weeks post-infection (Beesley et al., 2017), and they are not shed in a regular fashion via the biliary system (Dorsman, 1956a,b). FlukeFECs often do not correlate well with fluke burden in sheep (Sargison, 2012) or cattle (Brockwell et al., 2013). Composite flukeFECs can be used to detect active liver fluke infection in a group of animals (Daniel et al., 2012; Graham-Brown et al., 2019). However, when animals within a group are shedding low numbers of eggs, or if the fluke population is highly aggregated in just a few animals, the number of eggs in the composite sample will be dispersed (Morgan et al., 2005). Therefore, a flukeFEC test with a low detection limit to recover fluke eggs from samples with low numbers of eggs is of paramount importance. There are only a small number of liver fluke egg-spiking studies using ruminant faeces (Becker et al., 2016; Conceição et al., 2002; Happich and Boray, 1969). One of the earliest reported sedimentation/fine filtration methods was developed by Happich and Boray (1969a). The authors artificially seeded sheep faeces with known numbers of *F. hepatica* eggs and reported a detection limit of 10 epg. In cattle, a modified McMaster sedimentation technique was able to recover *F. hepatica* eggs from 100 % of samples at 2 epg (Conceição et al., 2002). We found that FF is able to consistently detect eggs where concentrations are as low as 5 epg in both ovine and bovine samples representing a significant improvement in low level detection. However, how this relates to egg output in a commercial flock or herd requires further investigation.

The true number of eggs in a naturally infected faecal sample will always remain unknown (Paras et al., 2018). Nevertheless, by using spiked faeces we can estimate the recovery ratio as an indication of the proportion of eggs lost in the flukeFEC procedure, providing a parameter to compare methods. In total 32 % of the total number of *F. hepatica* eggs added were recovered from sheep faeces (Table 2), which agrees with previous findings from Happich and Boray (1969) who also reported a consistent recovery rate of around one third across all tested egg densities. The combined egg recovery efficiency using the Becker method in this study across all epg levels was 3% in sheep and 4% in cattle (Table 2). This lower egg recovery ratio is potentially reduced as a consequence of the Becker technique using larger vessels, and increased volumes of water. In a 500 mL conical measure *F. hepatica* eggs are required to sediment across a distance of approximately 230 mm to settle at the bottom of a vessel. Happich and Boray estimated a fluke egg will sink at a rate of 100 mm per minute (Happich and Boray, 1969). At this rate an egg would require 2.5 min to reach the bottom if the water was still. Given that the vessel is also larger, filling it with the volume of water needed causes a significant level of water turbulence that takes over a minute to subside. These factors suggest eggs could be lost when the supernatant is decanted and the vessel refilled. The recommended sedimentation time is 3 min which is only 1 min longer than for FF (2 min), but the FF sample is held within a 15 mL falcon tube.

Due to well documented reasons, quantitative flukeFECs can be misleading as egg excretion of *Fasciola* spp eggs in faeces from naturally infected animals is sporadic, aggregated and over-dispersed (Dorsman, 1956a,b; Gonzalez-Lanza et al., 1989; Zárate-Rendón et al., 2019). For the naturally infected sample, we mimicked what occurs in practice, in that samples were regarded as fluke positive or negative. In spite of the significant difference in egg recovery, detection limits and recovery ratios, we report moderate agreement between both FF and the Becker sedimentation in sheep and a substantial agreement in cattle. Our model indicates eggs are more likely to be recovered in cattle samples than sheep in either flukeFECs method and this could explain the improved agreement. That said, only 20 samples of each species were included so a much more thorough investigation is necessary to explore this issue further. In conclusion, data in this study indicates *F. hepatica* eggs are recovered more readily from cattle faeces than sheep using both FF and the Becker sedimentation method. Furthermore, given the recognition of the importance of low fluke egg detection limits, we report that FF is consistent at recovering at least 1 egg in 100 % of artificially spiked sheep and cattle samples at 5 epg indicating it has great potential for the future of on-farm fluke diagnostics.

## Data availability

None of the data were deposited in an official repository. However, all data is available upon request from the corresponding author.

## Ethics approval

Not applicable.

## CRediT authorship contribution statement

**Claire Reigate:** Conceptualisation, Methodology, Validation, Formal analysis, Investigation, Visualisation, Writing - original draft, Writing - review & editing, Project administration. **Hefin W. Williams:** Conceptualisation, Methodology, Software, Formal analysis, Visualisation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Matthew J. Denwood:** Formal analysis, Software, Data curation, Writing - review & editing. **Russell M. Morphew:** Conceptualisation, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition. **Eurion R. Thomas:** Conceptualisation, Writing - review & editing, Supervision, Project administration, Funding acquisition. **Peter M. Brophy:** Conceptualisation, Methodology, Writing - original draft, Writing - review & editing, Supervision, Funding acquisition.

## Declaration of Competing Interest

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the

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