

FAECAL EGG COUNTING IN SHEEP – BEST PRACTICE

IN sheep, faecal egg counts (FECs) are a mainstay tool of gastrointestinal nematode (GIN) control and should be used throughout the grazing season to inform decisions on grazing management, and appropriate and sustainable use of anthelmintics.

Egg counts may be undertaken by the sheep keeper, in vet practices and agricultural merchants or by specialised diagnostic laboratories. Wherever the testing takes place, important factors need to be right when collecting samples, processing samples and interpreting results to get the most out of this tool.

What are FECs?

FECs are a way of determining the number of internal parasite eggs in faecal samples. This article will focus on the use of FECs for roundworms in sheep, although many of the principles and pitfalls are the same for all livestock and companion animals, and protocols exist for counting a range of different parasites (coccidia and fluke) present in individual or pooled faecal samples.

Limitations exist with regards to what FECs can tell you and an understanding of these will help determine how and when they are used. FECs are also known as faecal worm egg counts, egg counts, worm egg counts and faecal egg counting.

When are FECs useful?

Numerous benefits to conducting FECs exist. Some can be obtained from a single count; others need multiple samples over the season. Testing may be useful for monitoring healthy animals or for diagnosis of disease in sheep with clinical signs consistent with parasitic gastroenteritis. Examples include:

- The early identification of animals affected by parasite challenge. This allows the opportunity to undertake interventions and minimise production loss, and improve efficiency.
- The ability to monitor pasture contamination and help generate farm-specific risk maps for future grazing management decisions. This is really important for GIN-like *Nematodirus*, which can cause

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discuss this process and why it should be used as a tool throughout the grazing season

devastating disease in young lambs.

- Assess anthelmintic treatment efficacy (using pre-treatment and/or post-treatment FECs).
- The optimisation of timing of treatments can help:
 - maintain the effectiveness of anthelmintics now and in the future
 - ensure responsible use of medicines given concerns of the environmental impacts on soil and water fauna and flora

How do you make the most of FECs?

To get the most from FECs, it is essential to minimise the areas where errors can creep in. These can occur at time of sample collection, sample handling, sample processing and interpretation of results.

Faecal sample collection

Collecting samples for FECs need not be difficult – it can be combined with tasks such as moving stock between fields, other management activities or conducted as part of your daily checks:

- Collect sufficient faecal samples – 15 is the optimal number to get a good idea of the parasite situation:
 - When collecting samples for pooling, aim to standardise the amount collected from each animal.
 - Each sample should be at least 3g, which is around the size of a heaped teaspoon.
- Collect from the right animals – typically testing is undertaken in lambs as they have no immunity and are most likely to be clinically affected by GIN.

- Select samples at random (this should include loose as well as solid samples if applicable). This is necessary for general monitoring. When testing to confirm disease, collect samples from animals with clinical signs.
- Ensure samples are fresh and collected as soon as possible – animals do not need to be brought in to do this. Samples can be collected at morning checks, around feed/water troughs or you can gather and hold animals in place for 5 to 10 minutes and collect samples from the ground.
- If testing anthelmintic (Table 1) efficacy, a sample should be collected at the correct time post-treatment:
 - 7 days for levamisole (2-LV)
 - 14 days for the other anthelmintic classes (1-BZ, 3-ML, 4-AD and 5-SI)
 Note if resistance testing against *Nematodirus*, 7 to 10 days post-treatment should be used due to the parasites shorter pre-patent period.

Sample transport, handling and processing

In terms of sample, transport, handling and processing:

- Transport samples without delay. If delay is inevitable, keep samples in a cool box for a short period (a few hours).
- Once in the lab, ensure that fresh samples are kept cool if not processed immediately – refrigeration is best:
 - Do not leave samples on the bench/work surface or in a warm place for any period of time.
 - Eggs can hatch within 24 hours under ideal conditions, making egg counts difficult to interpret.
- It is critical to ensure that samples are thoroughly mixed before taking sub-samples for egg counting:
 - Eggs can be distributed unevenly throughout samples.
 - Shortcuts are often taken in homogenising the samples when pooling and this can lead to lower egg counts.
 Flotation medium (saturated salt solution; specific gravity approximately 1.2; 350g salt and 1000ml of water).

Saturated salt solution (sodium chloride) allows eggs to float to the top of the counting chamber, away from faecal debris for easier visualisation:

- Ensure that your flotation medium is saturated. If you are unsure, add more salt and agitate. When no more salt dissolves it is saturated. Some salt crystals can be left in the bottom of containers.
- If using a McMaster slide, agitate rather than stir the faeces/salt solution mixture before loading to prevent centrifugal forces affecting distribution eggs in the mixture.
- Load the McMaster slide immediately after the sample is agitated – do not allow the sample to rest.

Microscope

With regards to the microscope:

- Ensure that the microscope is set up appropriately – for example, correctly focused, proper magnification, clean eyepieces and/or objectives, and that the power is switched on.
- A rule of thumb when focusing on a sample originally is to focus on the grid lines or air bubbles. These lie at the correct focal plane as any eggs (Figure 1).
- Leave the loaded McMaster slide for two minutes to allow eggs to float and any debris to sink.

Interpretation of sample results

Monitoring

Count the eggs that can be differentiated morphologically (Figure 2) and that are significant in terms of pathogenicity, such as:

Class	Symbol	Group names
1-BZ (white)	1-BZ	Pro-benzimidazoles Benzimidazoles
2-LV (yellow)	2-LV	Imidazothiazoles
3-ML (clear)	3-ML	Avermectins Milbemycins
4-AD (orange)	4-AD	Amino-acetonitrole derivatives
5-SI (purple)	5-SI	Spiroindoles (+ avermectin)

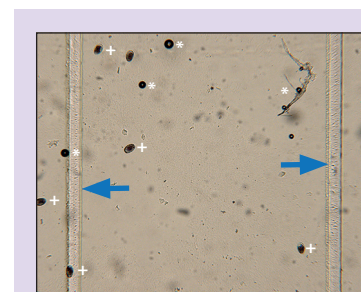


Figure 1. An image of a McMaster slide with grid lines (arrows), air bubbles (*) and eggs (+) highlighted.

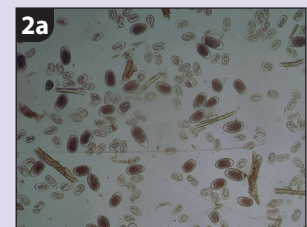


Figure 2. Examples of parasite eggs found in sheep faeces. **2a.** Strongyloides and trichostrongyle-type eggs. **2b.** Mixed field infection, including a *Trichuris* and *Nematodirus* egg.

October 22, 2024

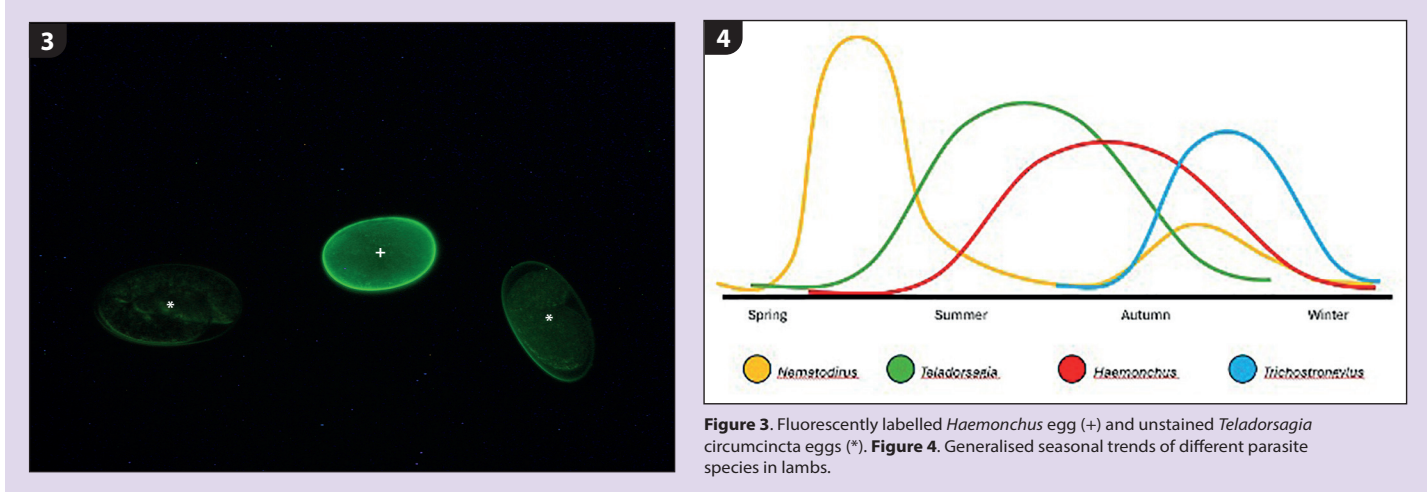


Figure 3. Fluorescently labelled *Haemonchus* egg (+) and unstained *Teladorsagia circumcincta* eggs (*). **Figure 4.** Generalised seasonal trends of different parasite species in lambs.

- Trichostrongyle-type eggs (which comprises a number of different species varying in significance, but including *Teladorsagia circumcincta*, *Haemonchus contortus*, *Trichostrongylus* species and others).
 - *Nematodirus battus* (large parallel-sided eggs).
- Other worm eggs may be present that are easily identified (for example, *Strongyloides*, *Trichuris*, tapeworm) though these should not be included in the trichostrongyle count because they are not usually clinically significant.
- Nematodirus* can cause the most damage in the early pre-patent phase of development in the animal.

Detection of eggs can be useful for future risk predictions, but less useful for treatment timing and choice early in the season.

Haemonchus eggs cannot be reliably distinguished morphologically from other trichostrongyle-type eggs, although *Haemonchus* infections can result in very high egg counts, in which case an additional test is needed using special staining (peanut agglutinin) to distinguish and provide results as a proportion of total FEC.

The fluorescently labelled lectins specifically bind to the carbohydrates found on *Haemonchus* eggs, as seen in **Figure 3**.

FEC is affected by the GIN species present and some seasonal trends of when different GINs predominate (**Figure 4**), although this varies between farms and is different each year, depending on weather and grazing patterns.

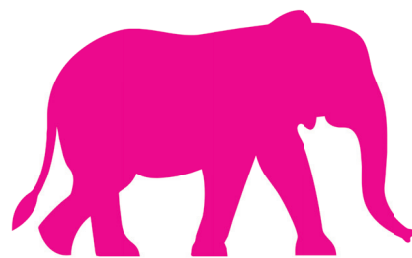
Different worm species produce variable numbers of eggs, so it can be difficult to provide defined low/moderate/high interpretation. This is where assessing trends in serial FECs can be helpful. FEC results should always be interpreted in light of the condition of the animals and changes in grazing. The worms producing eggs that are detected

were ingested as larvae two to three weeks earlier. A rule of thumb for low, medium and high egg counts can be found in **Table 2**.

FECs in ewes

FECs are typically used in lambs, although situations exist when they may be used in ewes. Adult ewes in good condition with no concurrent infections will generally have a good immunity to roundworms having had previous exposure.

Immunity helps modulate the egg counts and, therefore, FECs correlate less well with worm burdens in these sheep. ⇒ **page 16**



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Table 2. Exemplars of low, medium and high egg counts for trichostrongyles (general roundworms) and *Nematodirus battus*

Worm species	Faecal worm egg count (eggs per gram)		
	Low	Medium	High
Trichostrongyles	Less than 250	250-750	Greater than 750
<i>Nematodirus battus</i>	50-150	150-300	Greater than 300

⇒ continued from page 15

FEC may be useful:

- To check mob samples to manage timing of spring rise targeted treatment to reduce pasture contamination from ewes under physiological stress in the peri-parturient period.
- To monitor for *Haemonchus contortus* eggs and determine if narrow-spectrum anthelmintics may be appropriate for treatment. When closantel is used to treat haemonchosis in sheep the appropriate interval after treatment for collection of samples to check efficacy is 14 days.

FEC reduction test

The FEC reduction test assesses the reduction in FECs at the time of treatment and at a set time post-treatment. The test can be completed using individual faecal samples or appropriately pooled samples. Individual samples are costly to do commercially and, as such, pooling is often preferred, allowing testing to be undertaken each time a wormer is used and at different times of year so that a clearer picture of efficacy can be developed on a farm over a few years.

Pooling of samples is detailed further on. The reduction is expressed as a percentage; efficacies less than 90 per cent are indicative of potential resistance. The optimal time for re-sampling of treated animals is 7 days for levamisole (2-LV) and 14 days post-treatment for the 1-BZ, 3-ML (ivermectin), 4-AD and 5-SI. Levamisole has no label claim against inhibited worms and, therefore, re-sampling

needs to be conducted before maturation of surviving immature stages occurs. *Nematodirus* counts may reappear sooner than the 14 days due to shorter time to patency than other roundworms.

The efficacy is estimated using the standard formula where T1 and T2 are the FEC of animals pre-treatment and post-treatment, respectively: $(1 - [T2/T1]) \times 100$ using mean counts.

When assessing effectiveness of treatment, it is imperative to ensure that weighing and drenching of sheep has been undertaken correctly or FEC results will not be sufficiently robust to make judgements.

It should be noted that treatment efficacy can change over season – for example, 1-BZ treatments may be useful for treating *Nematodirus* earlier in the season, but may be less effective when other GIN species predominate, such as *Teladorsagia* where resistance is more common.

Post-drench efficacy check

An adaptation of the FECRT, the post-drench efficacy check looks at FECs at a set time post-treatment. If eggs are observed it may indicate that a treatment has not worked. Obviously, a zero count may occur if the animals don't have a patent egg count at the time of treatment.

FEC is straightforward to do and when used throughout the grazing season, provides important evidence for dynamic flock health planning.

Getting this message out to farmers is key, but it can be easy to forget that all the steps along the way need to be done correctly and accurately to end up with a reliable and meaningful

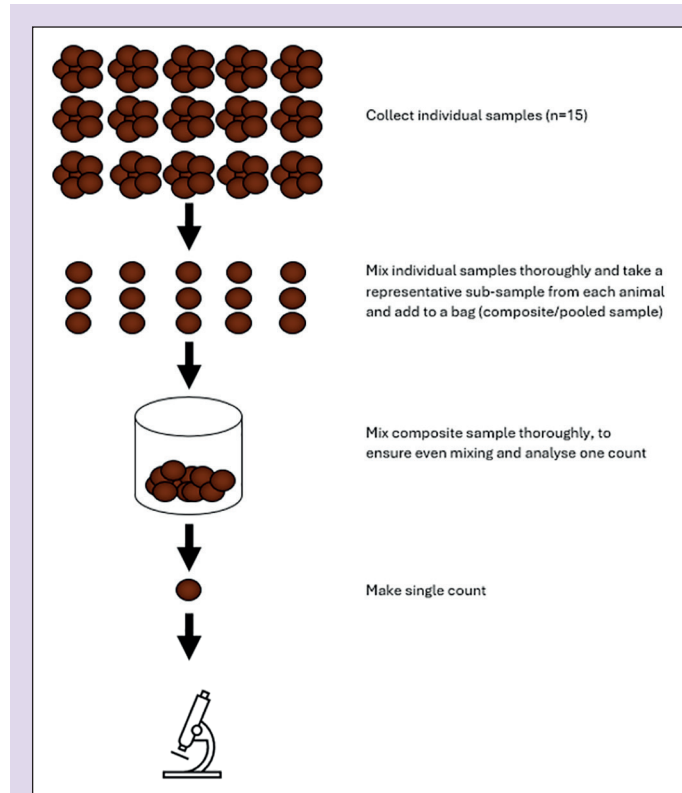


Figure 5. How to ideally make composite faecal samples for egg counting.

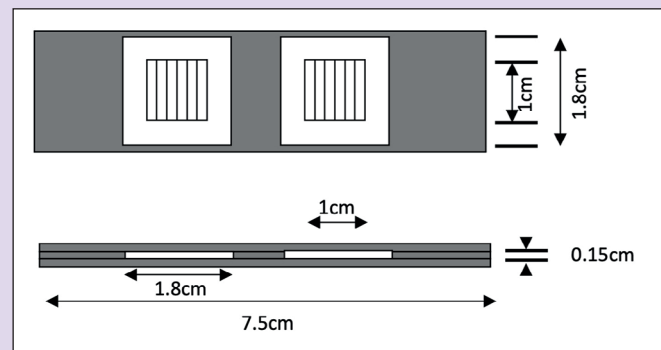


Figure 6. The McMaster slide (Ministry of Agriculture, Fisheries and Food, 1986).

result. The method used – whether at the veterinary practice, agricultural merchant or commercial laboratories – is perhaps less critical than all the other steps in obtaining and preparing the samples before a single egg is counted.

A full discussion of interpretation of FEC is beyond the scope of this article and the Sustainable Control of Parasites in Sheep (SCOPS) website has a detailed technical manual, videos, podcasts and other downloadable resources, which the authors would recommend for further information.

Pooling faecal samples

By pooling, it is possible to assess the infection

level of a group of co-grazing sheep while only paying for one faecal egg count (**Figure 5**). Pooling samples:

- reduces cost of monitoring
- can be an accurate estimation of the group mean egg count
- is useful for regular wormer efficacy checks

To pool faecal samples for a more accurate FEC assessment:

- Collect fresh faecal samples from 15 sheep in individual bags.
- Your FEC provider will mix a representative amount from each sample (**Figure 5**).
- One sample is then processed from the pooled faeces, giving a single composite or pooled sample result.

McMaster method Kit required

To carry out the McMaster method, the following kit is required: a polythene bag, electronic balance, saturated sodium chloride solution, measuring cylinder, beaker, tea strainer/1mm mesh, Pasteur pipette and bulb, McMaster counting slide and compound microscope.



Image: FMSTUDIO / Adobe Stock

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Protocol

The protocol steps are as follows:

1. Mix the sample and take a 3g sub-sample of the faecal sample into a polythene bag. Add 42ml of saturated salt solution and rub bag thoroughly between fingers and thumb to macerate the material.
2. Pour the mixture over a 1mm sieve into a beaker and discard the retentate/debris.
3. Thoroughly agitate the filtrate and immediately draw around 1.5ml into a Pasteur pipette.
4. Carefully run suspension into one side of the counting chamber of a McMaster counting chamber. Repeat step 3 and fill the second chamber.
5. Count all the eggs within the two grids/chambers (see further on), under a compound microscope at x100 magnification. Multiply the total number of eggs observed by the appropriate figure (depending on the area counted; **Table 2**) to calculate the number of eggs per gram (EPG).
The McMaster technique assumes

that the sample is fully homogenised prior to dispensation of the filtrate into the McMaster slide. Egg counts can be estimated using either one grid (0.15ml), two grids (0.3ml), one chamber (0.5ml) or both chambers (1ml) of the McMaster slide depending on egg density within a particular sample. The chosen counting method influences the eventual multiplication factor and subsequent calculations (**Table 3**).

The dimensions of a standard McMaster slide are detailed in **Figure 6** and dictate the multiplication factors used in interpreting FEC results. Outlined below are the calculations required, depending on the area of the slide examined. For example, 3g faeces (approximately equivalent to 3ml) + 42ml of saturated sodium chloride solution = 45ml in total.

Interpretation of results

The following example is provided to assist you in accurately calculating results.

If a faecal sample has 300 EPG, a 3g sub-sample will therefore contain a total of 900 eggs. Following the aforementioned instructions and using the calculations, 45ml of filtrate will contain 900 eggs.

According to the volume examined, such as number of grids/chambers examined, the expected numbers of eggs are highlighted in the shaded column of the table.

If the sample is allowed to sit prior to loading the McMaster slide, the eggs will float to the surface of the saturated sodium chloride solution.

Removing only the surface layer will result in a concentrated sample being examined, therefore generating inaccurate results. Using the aforementioned example, up to 900 eggs could be loaded into the McMaster slide resulting in an over-estimation of the actual egg count (estimated counts of 13,500 EPG to 90,000 EPG, depending on counting method).

Several other methods of performing FECs exist. Whichever is used, it is

important that all steps are followed diligently and quality control is used to check accuracy of sample preparation and counts between staff carrying out FECs.

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- For the purpose of open access, the author has applied a Creative Commons Attribution CC-BY licence to any Author Accepted Manuscript version arising from this submission.

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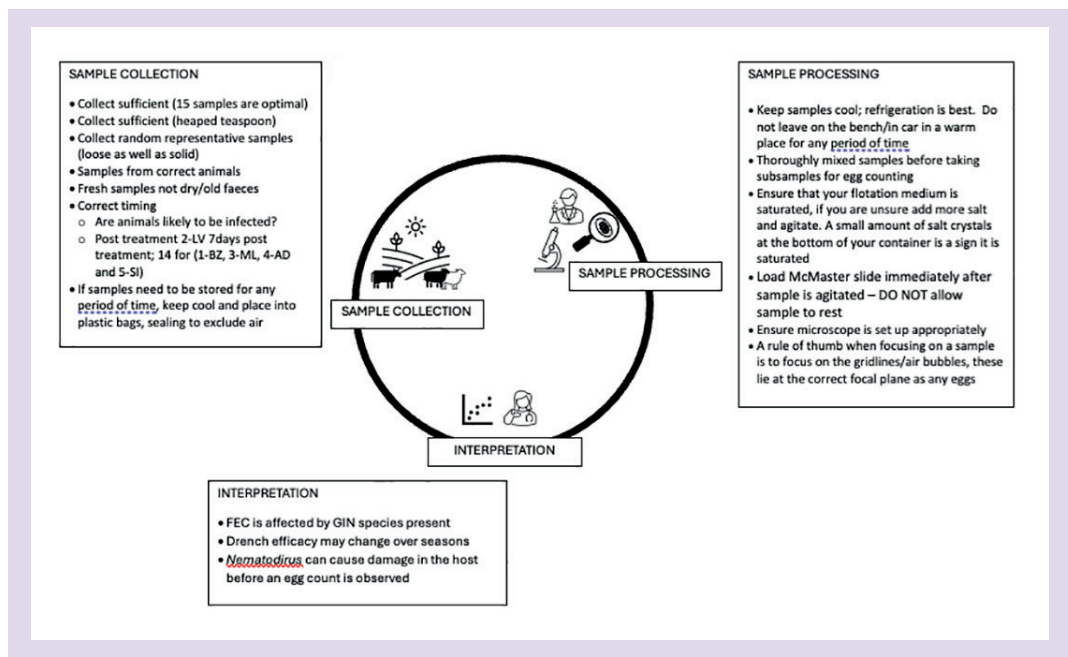
Table 3. Information on faecal egg count calculations using the McMaster slide, based on counting grids or chambers

Area type	Number examined	Volume examined (ml)	Volume calculation*	Multiplication factor	Multiplication factor calculation#
Grid	1	0.15	(1x1x0.15)	100	(45/0.15)/3
	2	0.3	(1x1x0.15) x 2	50	(45/0.3)/3
Chamber	1	0.5	(1.8x1.8x0.15) x 1	30	(45/0.5)/3
	2	1.0	(1.8x1.8x0.15) x 2	15	(45/1.0)/3

*Volume calculation = (length (cm) x width (cm) x depth of area (cm)) x number of grids/chambers examined.

Table 4.

Area type	Number of areas examined	Volume examined (ml)	Multiplication factor	Total number of eggs expected to be observed	Estimated eggs per gram
Grid	1	0.15	100	3	300
	2	0.3	50	6	300
Chamber	1	0.5	30	10	300
	2	1.0	15	20	300



DAVE BARTLEY is a principal scientist at Moredun Research Institute with almost 30 years of experience investigating various aspects of sustainable parasite control in livestock. He has a keen interest in all facets of anthelmintic resistance research, particularly in areas around the detection and management of anthelmintic resistance in roundworms of livestock, and also in stakeholder engagement.



REBECCA MEARNS is senior veterinary advisor at Biobest Laboratories and former president of the Sheep Veterinary Society. She has more than 25 years' veterinary experience and has had her own small flock of sheep for more than 20 years. Rebecca is enthusiastic about the importance of diagnostic tests and how they are used in the management of internal and external parasites.



PATRICIA VAN VEEN is the national veterinary manager for the ruminant VPS products with Zoetis UK. She joined Zoetis in 2020 after 16 years in farm practice. Patricia is passionate about animal health and welfare, focusing on ruminant parasites and sustainability, prescriber training, and technical support.

