

## FAECAL EGG COUNTING

Faecal egg counting is a process where faeces is diluted in a saturated saline solution and examined under microscope for the eggs of helminth parasites ie Worms. When performed carefully, there is a good relationship between the egg numbers and the worm burden present in sheep, horses, dogs and cats. For a number of reasons, cattle faecal egg counting is more complex.

The key to a useful egg count is accuracy – if faeces is accurately weighed from separate well collected and stored samples and diluted in a precisely measured amount of saline, then an evenly mixed aliquot taken and examined by an experienced technician – all will be fine.

### Faecal egg counting in sheep

Faecal egg counting in sheep offers the potential of enormous savings in time and money. Faecal egg counting can prevent the use of unnecessary drenches, detect the need for an unplanned drench prior to production loss or determine the performance of a recent drench. By expanding the last use, faecal egg counting may be used in a drench resistance trial to determine the efficacy of a number of drenches and combinations allowing the development of a drench program for the future.

### Which worms can be seen and what is their relative importance?

Abbreviation, By "group"	Scientific names	Common name	Relative Importance	Comment
Trich/Ost	Trichostrongylus, Ostertagia & Haemonchus	Black Scour Worm, Brown Stomach Worm, Barber's Pole Worm	Great, Moderate, High	SE's greatest problem Occasional but severe
Chab/Oes	Chabertia. Oesophagostomum	Lge Mouthed Bowel W Nodule Worm	None	
Nem	Nematodirus	Thin Necked Intestinal W	Special	
Trichuris	Trichuris	Whipworm	Low	
Monezia	Monezia	Tapeworm	None	Aesthetic – looks bad

### Trichostrongylus, Ostertagia & Haemonchus

This group contains all three of the worm of concern. Unfortunately they cannot be differentiated visually, so it is necessary to resort to further testing to accurately determine their proportions should that be required.

**Trichostrongylus** is the South East's greatest problem, causing considerable ill thrift and wool loss through dags. Untreated cases will often die. It is the most common worm to develop resistance in the SE. (Note that moxidectin does not claim persistence of action against Trichostrongylus). A female Trichostrongylus worm can lay approximately 100 eggs per day.

**Ostertagia** is a lesser problem in the SE, rarely rising to problem levels. Egg laying is similar to Trichostrongylus.

**Haemonchus** is overrated with respect to it's prevalence, but not in it's potential effect. It is a blood feeder, and this rich diet allows the female to lay up to 10,000 eggs per day. This allows explosive outbreaks. Massive egg counts may be seen on faecal egg counting, often swamping all other species. Matched with clinical signs (anaemia,

weakness and occasionally “bottle jaw”) and maybe a post mortem, diagnosis is often simple.

**Chabertia & Oesophagostomum.** Feeding from the faecal matter itself, and with little irritation at their attachment sites, these worms are passengers only. Their eggs can be confused with the above species by inexperienced technicians.

**Nematodirus.** A worm of little pathogenicity (damage potential), Nematodirus has a few interesting features. Nematodirus lays relatively huge eggs – 100 times the volume of the Trich/Ost group – but only about 10 per day. These eggs are supremely resistant to light and heat. The result of this is that Nematodirus egg counts rise in the late Summer and Autumn, when the competing species are at their low ebb.

Nematodirus are alleged to cause the occasional problem under exceptional circumstances –

- Allow sheep with a Nematodirus count of over 300 epg to graze a pasture (never seen)
- Wean lambs into that paddock next autumn

Needless to say – we have never seen it happen

**Trichuris** As per Chab/Ost, except the egg is distinctly different.

**Monezia** The sheep tapeworm is a much maligned creature. It does almost no damage at all, this having been proven over and over. The problem lies with the fact that farmers can see them! Quite large white segments are released into the faeces. Each segment contains thousands of eggs. Faecal egg counting is not useful in assessing the numbers of Monezia, as sampling near one broken segment will reveal thousands of eggs.

## Basic worm life cycle

The various roundworms have a similar life cycle. There is one critical factor to remember – 21 days! It takes 21+/- 1 day for a new infection to reach egg laying. Egg laid, hatches on pasture, infective larvae ingested and develops to adult egg laying worm.

The egg count procedure in the lab

1. An equal accurately measured dollop of faeces is taken from each of the 10 samples, and placed together.
2. The samples are diluted in a known volume of saturated saline solution, and mixed well to evenly distribute the eggs through the fluid volume. A small volume of isopropyl alcohol is added to reduce bubbles (surface tension effect).
3. While still mixing, a portion of the mixture is pipetted out and placed into a McMaster Faecal Egg counting slide. This slide has graduated chambers of known volume.
4. The slide is allowed to sit, such that the eggs float to the underside of the upper glass layer.
5. The sample is examined under low power, each slide graduation being about one field of view. Eggs seen are counted by “group”.
6. The total count is put through a simple mathematical formula to bring the eggs per chamber back to eggs per gram of the original sample.

### Sample formula

10 x 2 gram samples = 20 grams

Add saline to make 180ml

Count 3 x 0.3ml Chambers = 0.9ml

Count 37 Trich/Ost eggs

20g/180ml = 0.1111g/ml, multiply by 0.9ml = 0.1gm

37 eggs in 0.1gm = 37 eggs/0.1gm = 370 eggs per gram

#### **How accurate is an egg count?**

If the “avoidable” factors are not encountered, a result within 20% or so is achieved. This is well within limits for giving recommendations. Factors affecting egg numbers that are not directly associated with collection and storage are;

1. Time of sampling – sheep empty out overnight, and by morning the eggs are more concentrated.
2. Similarly, any time the sheep have been starved.
3. Other stress factors – more stress, less natural resistance effect, egg laying increases.
4. Natural fluctuation within the mob – not a great problem unless mixed sheep classes present in the mob – eg lambs and ewes. Egg counts are NOT consistent across a mob of cattle – one reason cattle FEC's are less useful.

#### **Avoidable factors affecting egg counts!**

Add any or some of these factors and the egg count becomes unreliable.

1. Bulk sample. 10 samples must be presented separate to allow exactly 2 grams from each sample. Collect a minimum of a teaspoon, preferably more in case further testing is required.
2. Enough samples. 10 are needed from a mob to give a relevant statistical result.
3. Fresh samples. Eggs begin to hatch and once hatched, do not float in the saline solution. Only floating eggs are seen in the count. Hatching is kept to a minimum at low temperatures (but may be damaged by freezing) Keep samples refrigerated and moist. The technician may notice “larvated eggs” indicating a poor sample.
4. Correct sample. In mixed mobs, the samples should be all of one class. This may require yarding and rectal collection, or drafting into component mobs.
5. Containers. Plastic bags are ideal, especially sandwich bags. Egg cartons are poor as they can tip and mix samples, and cardboard egg cartons absorb the water from the sample drying them out.
6. Labelling – obvious really – keep a black texta handy.
7. Solution not saturated – if the density is insufficient, eggs don't float, lots of zero egg counts. Use a hydrometer or just be careful.
8. Poor egg counting technique.
  - Sloppy or inaccurate measuring or mixing
  - Allowing sample to settle before taking aliquot
  - Bubbles
  - Mistaken identity – experience

- Poor maths – make sure the formula is worked to your sample size, dilution and McMaster volume.

#### **Worms but no egg count?**

There are a few situations where the paddock/stock history is vital.

1. Immature worms – worms take 21 days to reach egg laying age, but they can be doing damage from day one. Newly introduced sheep to a highly contaminated pasture will not have an egg count.
2. Too soon after a drench – an ineffective drench may simply make the worms stop laying for a while – post drench egg counts should be 10 – 15 days after the drench.
3. Cattle *Trichostrongylus*. Weaner sheep grazing a pasture heavily contaminated by typically weaner cattle may develop a sterile but very dangerous infection – with no eggs.

#### **When to use an egg count**

1. Routine survey counts – simply to assess worm numbers to predict the need for a drench. Either use as a winter check “just to see how things are going”, or a week or so before a planned management event rather than just drenching anyway. Prior to the second summer drench is a good example of this. There little benefit in counting before an unavoidable drench, yet the most popular count remains that before the first summer drench!
2. Barber's Pole warning – just an extension of the above.
3. Post drench test to check effectiveness of the drench. Take the samples 10-15 days post drenching = any eggs seen are from worms that survived the drench/drenching. Drench failure can be chemical or managerial!
4. Full drench resistance trial – a simple concept often ruined by poor management.
  - Sheep with a Trich/Ost count of over 400 epg, no Barber's Pole
  - Select sheep at random through the mob and treat with one of the test drenches, clearly identifying each sheep with a persistent mark – ear tags are best. Keep going until there are 15 sheep in each group, including an untreated control group.
  - Return sheep to pasture for 10-12 days.
  - Yard sheep just before attempting collection.
  - Collect faecal samples from the rectum of marked sheep into clearly marked containers. 10 samples are needed and you will often need more than 10 sheep to get them – hence the 15 per group.
  - Egg count each group
  - Compare the egg count to the count of the control group to give a percentage

#### **Anything else?**

To give a recommendation all relevant factors should be given.

- recent mob history – drenches, shifted to new pasture etc
- planned mob management – onto stubble next month etc

- details – sex, age, pregnancy, scour percentage
- environmental factors – irrigated pastures etc

**Associated tools**

- Larval Culture – faeces is cultured over 10– 14 days allowing the eggs to hatch. A technician then identifies the larvae by species – splitting up the Trich/Ost group for instance.
- Pasture Larval Count – larvae from about 1kg of pasture tops (not roots) are washed off and filtered out, then counted. Gives a very good indication of pasture contamination, but is relatively expensive – maybe. Compare it to a worm disaster in 500 weaners!
- Drenchrute Test – an in lab drench resistance trial for \$220 is accurate but only tests the basic drenches at single dose strength.

**Basic recommendations**

It is impossible to give generic hard figure recommendations that will suit everyone every year. Still, here are some basics. All counts refer to the Trich/Ost group alone. All recommendations should be related to management plans, class of stock and environmental conditions.

Early Summer	Late Summer	Autumn	Winter/Spring
Drench mid November to Mid December	Drench Late January to February	Egg count prior to crutching etc	Survey Count
Any egg count over 0	Over 20 epg	100 epg+	200 epg+
	Wethers over 40 epg		

**A few philosophies and facts**

Worm population in a paddock

	Winter Spring	Summer
On the pasture	95 - 99%	0 – 5%
In the sheep	1 – 5%	95 – 100%

- Drench in early summer, kill 99% of the worms, drench in winter, kill 1%. All drench programs revolve around an effective first summer drench, and years of testing show us that this first drench should be given mid November to mid December.
- Egg counting has been promoted for years, but it appears that there is a maximum of 20% of farms in any district in Australia takes it up.
- There is NO new drench group in development in the world at this stage.

### Timing of the First Summer Drench

The timing of the first summer drench is the single most important concern in the entire years drenching program, and is the most common error seen.

Worm larvae DO NOT die of heat and desiccation. Worm larvae do not have the capability of eating, and are born with a fixed amount of energy – if used before they are eaten by a sheep, they die. They use this energy when moving. In warm MOIST conditions, they burn up this energy quickly and die. If they dry out, they stay in “suspended animation” until the next rain/heavy dew etc.

Therefore, the best summer drench is given while there is still moisture around. Once it has fully dried off, it is too late.

Mid November to Mid December is the optimal time in this district.

### Opinions/Developments currently being passed around.

- “First summer drenches are dangerous and should be avoided” – It is true that a failed summer drench causes more resistance development than many failed winter drenches. To not summer drench will result in many more drenches through the next year however. Make sure an effective compound is used.
- “Drenching should not be done if the egg count is under eg 500”. First, check what region the advice has originated from. Find what worm is the most concern in that district. If it is not the same as in your district, ignore it! Also find if the advice is given for winter, summer or other.
- Refugia – this is a term referring to worms that at the time of drenching are not exposed to the drench. These can be in undrenched sheep or simply on the pasture. Such worms are available to dilute the resistant worms left in sheep after a drench. They can therefore reduce the rate of resistance development. If the refugia is too large, worm control will be ineffective. Note that a well timed first summer drench will leave sufficient refugia in most cases. Drenching onto a truly clean pasture or stubble will not, and should be avoided at all costs.
- That there are any compounds at all that have no resistance, or are 100% effective on any property. Any and every drench will leave some survivors – they may well be at a level undetectable to faecal egg counting, but they are there, and they represent the beginnings of clinical resistance.
- Combination drenches. There is clear evidence that the use of combination drenches slows the development of resistance compared to using the components in rotation. The effect is improved the earlier the use of combinations is commenced. The more compounds used in combination in the drench, the better the effect.
- Resistance figures should always be presented with worm species differentiation. Ostertagia or Haemonchus resistance is less important here than Trichostrongylus resistance.

### Of all the things we can do, what are the MOST important?

1. Always test to check the effectiveness of strategic drenches.
2. Consider the paddock as much as the sheep in the paddock.
3. Never, ever, drench onto stubbles. Yes, you read it right!
4. Don't leave your summer drench too late – in this district that's Mid December.
5. Ignore advice given that is not specific to your own area. This includes this document if you are reading it elsewhere!
6. Regard sponsored information as suspect