Evaluation of the FECPAK Sheep Method (Moredun Institute, Scotland)

General Observations

The microscope included in the kit had a "floating stage"; samples would rapidly fade out of focus making counting difficult and could lead to some inaccuracies if the users were unaware that they were not focussed on the correct focal plane.

The saturated sodium chloride container was unlabelled which might lead to some confusion during the course of the procedure.

The plastic cylinder provided appeared to underestimate the volume of the sample by around 5% which would lead to an incorrect estimation of EPG. A catheter tipped 50 ml syringe might be used to provide a more accurate means of dispensing relatively small volumes.

No details are given of the volume examined under each grid which is critical in determining multiplication factors. The crude measurements below suggest that about 1 ml is examined under the two grids.

Size of grid examined: $1.15 \times 1.8 \times 0.25 \text{ cm} = 0.5175 \text{ cm}^3$
Duplicate count: $2 \times 0.5175 = 1.035 \text{ cm}^3$ = approximately 1ml of liquid examined.

For sheep samples, 30ml tapwater is added to 10 grams faecal material. If 1 gm faeces has a volume of 1 ml then 30ml of faecal suspension contains 7.5 gm faeces. Thirty mls of suspension is made up to a total volume of 230ml in saturated salt (i.e 1 gram of faeces in 30.66 ml saturated salt). If approximately 1ml of suspension is examined the multiplication factor should be around 30.

Comparison between FECPAK and alternative methods of nematode ova enumeration (Salt flotation technique & McMaster technique).

Methodologies

Faecal material from a monospecifically infected Haemonchus contortus donor was homogenised, aliquotted and examined using one of three techniques for determining faecal egg counts; FECPAK, Salt flotation technique & McMaster technique.
Fecpak technique

Materials:

- Sample bag
- Balance
- Measuring cylinder
- Saturated sodium chloride
- Cylinder and plastic bung
- Filter apparatus
- Stirrer
- Pipette
- Counting chamber
- Monocular microscope
- Count sheet

Procedure:

1. Tare empty sample bag
2. Weigh sample
3. Add water (lookup table)
4. Remove air
5. Crush and mix
6. Measure out 30ml (jar)
7. Add saline up to 230ml mark
8. Fit lid and shake
9. Wet filter
10. Run mixed sample through filter
11. Randomly stir liquid
12. Take sub sample (pipette)
13. Run onto slide (chamber 1)
14. Randomly stir liquid
15. Run onto slide (chamber 2)
16. Examine chamber 1
17. Fill out count sheet
18. Examine chamber 2
19. Fill out count sheet
20. Summarise count sheet (look-up table)
Salt Flotation technique

Materials:

- Stomacher
- 10ml Finnpipette
- 250ml plastic beaker
- 1mm sieve
- Beckman polyallomer centrifuge tubes
- Benchtop centrifuge
- Artery forceps
- 4ml disposable micro cuvettes and cuvette lids
- Compound microscope (x40 magnification) with Miller square graticule
- Cuvette carrier for microscope

Procedure:

1. Weigh faeces and add tapwater at 10ml per gramme of faeces contains 1 gram faeces, emulsify using stomacher.
2. Pipette a 11ml subsample (containing 1 gram of sample) over 1mm sieve into beaker, wash the retentate with a further 5mls tapwater.
3. Squeeze retentate on sieve to remove excess fluid and discard retentate.
4. Pour filtrate into centrifuge tube and spin for 2 minutes at approximately 1000rpm.
5. Remove the supernatant using a vacuum line, add approximately 10ml saturated sodium chloride solution and gently resuspend pellet (vigorous mixing creates air bubbles, obscuring eggs).
6. Centrifuge at approx. 1000 rpm for 2 minutes.
7. Using artery forceps clamp the tube just below the meniscus and pour the contents of the upper chamber into a cuvette, rinse the upper chamber of tube using approximately 1ml saturated sodium chloride solution and add to the cuvette.
8. Gently invert the cuvette several times and fill with saturated sodium chloride until there is a small positive meniscus. Carefully slide a cuvette lid onto the cuvette.
9. Place cuvette for reading onto carrier and count eggs present on upper surface of cuvette. For smaller numbers of eggs count those present in the larger graticule square over two complete traverses of the cuvette and multiply the total by 3 or for larger numbers use two complete traverses of the cuvette counting eggs present in the smaller square and multiply the total by 9. For samples containing very few eggs the entire cuvette should
be counted and no multiplication factor used since this represents all of the eggs recoverable from 1 gram of faeces.

**McMaster technique**

**Materials:**

- Shaker jar containing approx. 45 glass balls
- Saturated Sodium Chloride
- Polythene bag
- McMaster counting chamber
- 0.15mm wire mesh screen
- Beaker
- Pasteur pipette

**Procedure:**

1. Place 45 glass balls in a shaker jar and add 42ml saturated Sodium Chloride.
2. Weigh 3 gm of faeces from a rectal sample, put in the jar and fit the stopper.
3. Shake the jar until the faecal matter is thoroughly dispersed.
4. Pour the faecal suspension through a wire mesh screen with an aperture of 0.15mm and collect the filtrate in a beaker. Discard the faecal debris left on the screen.
5. Re-suspend the filtrate, draw some of the filtrate into a pasteur pipette and carefully run the fluid into one counting chamber of a McMaster slide. Repeat the process and fill the second chamber of the slide.
6. All the eggs under the two grids are counted. Since 3g of faeces is in 45 ml of suspension (1g per 15ml suspension) and the volume of suspension examined is 0.3ml
7. (0.15ml under each grid of the counting chamber) the number of eggs per gram of faeces is obtained by multiplying the total number of eggs under the two grids by 50.

**Statistical Analysis**

Analysis of variance was conducted on log10 transformed faecal egg count data (count + 1).
Results

Arithmetic mean egg counts for each of the three techniques are shown in Table 1. The FECPAK data was significantly higher than that generated by the salt flotation technique (p>0.0034) but not significantly higher than the McMaster data.

Table 1. Comparison of average egg counts recorded from a single sample of sheep faeces using three techniques FECPAK, salt flotation and McMaster, (n = 20 per assay).

<table>
<thead>
<tr>
<th>Technique</th>
<th>Mean faecal egg count (S.E.M.)</th>
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</thead>
<tbody>
<tr>
<td>FECPAK</td>
<td>1840 (70)*</td>
</tr>
<tr>
<td>Salt Flotation</td>
<td>1493 (43)*</td>
</tr>
<tr>
<td>McMaster</td>
<td>1598 (147)</td>
</tr>
</tbody>
</table>

Significant difference p<0.0034

Comments

The FECPAK kit provided a simple reproducible means of determining faecal egg counts. The kit was well documented and contained all of the materials and equipment needed to monitor faecal egg counts. Between sample variability was relatively low (similar to that recorded with the salt flotation technique employed in our laboratory) and less than that of the McMaster technique. However using the recommended FECPAK multiplication factor for sheep faeces of x 30 produced egg counts that were significantly higher (23.2% p>0.0034 flotation technique) and 15.1% higher than those of the McMaster technique. The difference between the FECPACK and the flotation technique may be attributable to losses incurred during the flotation process.

The differences in yield between the techniques may only be important if users are using a single individual or mob sample and treating at a pre-determined threshold rather than a series of egg counts and treating in response to dynamic changes in the pattern of faecal egg output as would often be the case.