The comparison of FLOTAC, FECPAK and McMaster techniques for nematode egg counts in cattle

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Abstract
Three methods, FLOTAC, FECPAK and McMaster were compared for accuracy and sensitivity for counting numbers of nematode eggs in faeces of naturally infected cattle with high or low nematode egg counts. Only FLOTAC gave positive results for 12 replicates from pooled samples with low egg counts making it more sensitive than FECPAK (67%) and McMaster (41.7%). FLOTAC resulted in generally higher egg counts and lower coefficients of variation than the other two methods used. The reliability of FECPAK and McMaster is depended on the area under the slide counted. All three methods can be used for making decisions whether to treat but FLOTAC or Mini-FLOTAC should be used for faecal egg count reduction tests when lower egg counts are present.

Keywords
FecPak, McMaster, FLOTAC, nematode egg count, cattle

Introduction
Anthelmintic resistance is an increasing problem in nematodes of cattle in several parts of the world (Sutherland and Leathwick 2011). If the guidelines for detection of anthelmintic resistance are used (Coles et al. 1992) egg counts should be sufficiently high to ensure that meaningful results are obtained. This is depending to the sensitivity of the method used (e.g. McMaster technique may be also up to 50 eggs per gram (EPG)). However, if more sensitive techniques are used lower egg counts can be included in studies (Levecke et al. 2011, 2012). At the same time to detect levels of resistance, the methods used for egg counts should have a variance as low as possible.

The most widely used method for faecal egg counts is the McMaster technique, developed in Australia and originally used for nematode egg counts in sheep where egg counts can be relatively high (Gordon and Whitlock, 1939). The method lacks sensitivity particularly at low egg counts (Mes, 2003). FECPAK is essentially a larger version of the McMaster slide (www.fecpak.com) and it was developed in New Zealand to provide a simple on farm method of egg counting for making decisions on whether to treat or for the determination of anthelmintic efficacy. Because it starts with a larger faecal aliquot (20 g) rather than the 3 g often used for McMaster, it gives much more reliable results with equine faecal samples where eggs are not evenly distributed (Presland et al. 2005). FLOTAC is a multivalent Faecal Egg Count (FEC) technique based on the centrifugal flotation of the sample and the subsequent translation of the top layer of the floating suspension (Cringoli et al. 2010). This makes counting of the sample much easier as most of the colour and debris are no longer in the light path. Due to its high accuracy and sensitivity, FLOTAC has previously been recommended for monitoring anthelmintic drug efficacy in cattle (Levecke et al. 2011, 2012).

The present study was undertaken to compare the three techniques using faeces from naturally infected cattle with low and high egg counts of gastrointestinal (GI) strongyles to determine which is the optimum technique.

Materials and Methods
Fresh faecal samples were collected from 20 cattle excreting 2–10 EPG (mean 8.1 EPG) (based on FLOTAC Basic Technique)

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and from 20 cattle excreting 800–1200 EPG (mean 1112 EPG) (based on FLOTAC Basic Technique) and from these individual samples, two composite samples were prepared using 5 grams per animal, one composite having a low and one having a high GI strongyle EPG level. From each pooled sample, after a good homogenization, 30 g were taken and homogenized in tap water (270 ml; dilution ratio 1:10, total volume 300 ml). The suspension was sieved (wire mesh aperture = 250 mm) to remove large debris. Thirty-six conic tubes were filled with 6 ml of filtered suspension to provide 12 replicates for each of the three methods. Each aliquot was centrifuged for 3 min at 170 x g, the supernatant was poured off and discarded, leaving only a pellet in the bottom of tube. Then, tubes were randomly assigned to the three techniques, FLOTAC (Cringoli et al. 2010), FECPAK (www.fecpak.com) and McMaster (Ministry of Agriculture, Fisheries and Food, 1986). For the three techniques a saturated NaCl flotation solution (FS2; density= 1.2) was used.

For the FLOTAC the aliquot was re-suspended in FS2 up to 6 ml level and one chamber was examined. Thus, a single flotation chamber of the FLOTAC was utilized for each replicate (analytic sensitivity = 2 EPG). The apparatus was then centrifuged at 120 x g for 5 min and translated. For the FECPAK, the pellet was re-suspended in FS2 up to 6 ml level and the slides were examined. For each slide, the GI strongyles counts were performed under each of four reading levels (one grid, two grids, one chamber, two chambers), resulting in an analytic sensitivity of 20 EPG (one grid), 10 EPG (two grids), 7.1 EPG (one chamber), and 3.6 EPG (two chambers). For the McMaster, the pellet was re-suspended in FS2 up to 6 ml level and the slides were examined. For each slide, the GI strongyles counts were performed under each reading level, resulting in an analytic sensitivity of 66.6 EPG (one grid), 33.3 EPG (two grids), 20 EPG (one chamber), 10 EPG (two chambers).

GI strongyle eggs were counted at X 100 magnification.

For all replicates of the composite sample with low levels of GI strongyle eggs, the arithmetic mean EPG values (derived from the 12 replicates), the standard deviation (SD), and the Coefficient of Variation (CV) were calculated for each technique and each reading level (one grid, both grids, one chamber and both chambers). The CV was calculated dividing the standard deviation by the arithmetic mean EPG, i.e. CV = (SD/mean EPG) x 100. The same statistics were calculated for the composite sample with high EPG.

**Statistical analysis**

The arithmetic mean EPG were calculated for each technique. Differences between techniques and reading area were analysed using one-way ANOVA with post hoc Fisher’s least significant difference (Bonferroni). All statistical analyses

![Fig.1. The comparison of FLOTAC, FECPAK, and McMaster techniques for GI strongyle faecal egg counts in cattle for composite samples with low and high burden of eggs: no. positives and sensitivity, mean EPG and accuracy (CV) of the three methods. P < 0.05; significant differences for different letters.](image-url)