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SHORT COMMUNICATION

Monitoring drug efficacy against gastrointestinal nematodes when faecal egg counts are low: do the analytic sensitivity and the formula matter?

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Abstract The faecal egg count reduction test (FECR) is the recommended technique to monitor anthelmintic drug efficacy in livestock. However, results are often inconclusive due to the low analytic sensitivity of the diagnostic technique or the conflict in results from FECR formulae. A novel experimental set-up was, therefore, used to compare the impact of analytic sensitivity and formulae on FECR results. Four McMaster techniques (analytic sensitivities 50, 33.3, 15 and 10) and a FLOTAC technique (analytic sensitivity~1) were used on faecal samples of 30 calves with a FEC of less than 200 eggs per gram. True drug efficacies of 70%, 80% and 90% were experimentally mimicked by comparing FEC before and after dilution (3:10, 2:10 and 1:10, respectively). The FECR was summarized using group (FECR(1)) and individual (FECR(2)) based formulae. There was a significant increase in precision of FECR when the analytic sensitivity increased ($p<0.0001$). The precision also depended on the formula used, FECR(1) ($p<0.05$) resulting in more precise FECR compared to FECR(2). The accuracy of the FECR differed marginally between the two formulae ($p=0.06$), FECR(1) being more accurate. In conclusion, the present study describes a novel methodology to compare techniques for the precision and the accuracy of their FECR results. The results underscored that techniques with high analytic sensitivity will improve the interpretation of FECR in animal populations where baseline FEC are low. They also point out that the precision of individual-based formulae is affected by the analytic sensitivity.

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Introduction

At present, the McMaster techniques (Ministry of Agriculture, Fisheries and Food 1986) are recommended by the World Association for the Advancement of Veterinary Parasitology to monitor drug efficacy against gastrointestinal nematodes in livestock based on the reduction in faecal egg counts (FECR) (Coles et al. 1992). Although they are simple and user friendly (Levecke et al. 2009), their chief limitation is the high analytic sensitivity (ranging from 10 to 50) that may thwart interpretation of the obtained FECR (El-Abdellati et al. 2010). This is particularly so in small herds, when the parasite population is highly aggregated or when animals are excreting a low number of eggs. With the development of novel faecal egg count (FEC) techniques with an analytic sensitivity of 1, such as the FLOTAC (Cringoli et al. 2010), it has been proposed that their use is likely to result in more precise and accurate estimates of the true FECR. However, to date, there are no studies available comparing FEC techniques for monitoring drug efficacy based on the FECR.

Apart from detection techniques, important differences in FECR have been observed depending on the formula used to calculate the reduction, individual-based formulae resulting in lower FECR compared to group-based formulae (Cabaret and Berrag 2004; Vercruysse et al. 2011). However, it was noted that these differences disappeared when only subjects with high FEC were considered,

Table 1 The total volume in which aliquots are re-suspended, the volume examined and the analytic sensitivity for the McMaster (four variants) and the FLOTAC techniques

Technique	Total volume (ml)	Volume examined (ml)	Analytic sensitivity (= total volume/volume examined
McMaster	15	0.3	50
McMaster	10	0.3	33.3
McMaster	15	1	15
McMaster	10	1	10
FLOTAC	6 (11)[a]	5 (10)[a]	1.2 (1.1)[a]

[a] Due to the large amount of faeces (1 g), which may impede a clear microscopic view, the aliquots representing baseline FEC were initially re-suspended in the flotation solution up to 11 ml and distributed among two chambers (2×5 ml), resulting in a multiplication factor of 1.1 (= 11 ml/10 ml) (Cringoli et al. 2010)

suggesting that the recommended formula to use may depend on the analytic sensitivity and/or the baseline FECs of the subjects under study.

Until now, effects of formulae have been studied by simulation studies (Dobson et al. 2009), as this allows to compare the FECR with a fixed true drug efficacy. However, the results of these studies depend strongly on the assumptions made, particularly for the FEC obtained by the techniques. The observed FEC are modelled according to the Poisson distribution, which implies that techniques with the same analytic sensitivities result in comparable FEC results. Yet, as illustrated by Levecke et al. 2009, this is not always the case.

In the present study, a novel experimental set-up which allowed to determine the true drug efficacy was used to compare detection techniques with different analytic sensitivities for FECR results when baseline FEC are low. In addition, differences in individual- and group-based formulae on FECR were examined.

Materials and methods

Study design

Fresh faecal samples were collected from 30 calves excreting a low number of FEC (<200 eggs per gram faeces (EPG), based on FLOTAC). The true drug efficacy (TDE) resulting in faecal egg count reduction (FECR) of 70%, 80% and 90% was experimentally mimicked by comparing baseline FEC with FEC after dilution of the baseline samples (3:10, 2:10 and 1:10). To this end, samples were processed as follows:

For each sample, 10 g was homogenized in tap water (90 ml). The suspension was sieved three times to discard large debris; subsequently, tap water was added to the residue (containing the eggs) up to a volume of 100 ml, resulting in a final concentration of 1 g faeces in 10 ml. For each detection technique, aliquots of 1, 2, 3 and 10 ml of this stock were transferred to centrifuge tubes, representing FEC for a TDE of 90%, 80% and 70%, and baseline FEC. Subsequently, the tubes were centrifuged for 3 min at 170×*g*, and the supernatant was discarded.

Parasitological techniques

Each of the four aliquots was examined with both the McMaster and the FLOTAC counting technique, based on protocols previously described by the Ministry of Agriculture, Fisheries and Food (UK; 1986) and Cringoli et al. (2010), respectively. For the McMaster, four different variants were performed. These variants differed on the total volume in which the aliquot was re-suspended with the flotation solution (10 and 15 ml) and the volume of this suspension examined (0.3 ml [grids only] and 1 ml [entire chambers]), resulting in four different analytic sensitivities (10, 15, 33.3 and 50) (Table 1). For FLOTAC with an analytic sensitivity of 1.2 (= re-suspended in 6 ml/one chamber examined [5 ml]), except for the baseline FEC where the analytic sensitivity was 1.1. Due to the large amount of faeces (1 g), which may impede a clear microscopic view, the aliquots representing baseline FEC were initially re-suspended in the flotation solution up to 11 ml and distributed among two chambers (2×5 ml), resulting in an analytic sensitivity of 1.1 (= 11 ml/10 ml) (Cringoli et al. 2010). For both McMaster and FLOTAC techniques, saturated NaCl (density=1.2) was used as flotation solution.

Statistical analysis

For each dilution (representing the different TDEs), the observed FECR was calculated using two formulae. The first formula was based on the arithmetic mean of the baseline FEC and the FEC of the dilution ignoring the individual variability, whereas the second formula represented the arithmetic mean of the reduction in FEC per subject. For each of the two FECR, 95% confidence intervals were based on bootstrap analysis with 1,000

Table 2 The baseline FEC (mean, minimum [min.], maximum [max.]) and the number of samples with FEC of zero for the different analytic sensitivities

Analytic sensitivity	Mean (EPG)	Min.–max. (EPG)	Number samples with FEC=0
1	61.1	9–160	0/30
10	64.7	0–230	3/30
15	60.5	0–150	4/30
33.3	75.6	0–399.6	8/30
50	71.5	0–350	11/30

iterations (The R Foundation for Statistical Computing, version 2.10.0). Subsequently, the ability of estimating the TDE was assessed on two criteria: (1) the range between the upper and lower limits of the 95% confidence interval (~1/precision) of the observed FECR and (2) the absolute value of the difference between the TDE and the observed FECR (~1/accuracy). The Spearman correlation coefficient was used to check whether an increase in analytic sensitivity resulted in an increase in precision and accuracy of the FECR results. The Wilcoxon signed–rank test was used to test for differences in precision and the absolute bias (accuracy) between the two formulae (SAS 9.1.3, SAS Institute Inc.; Cary, NC, USA). For both tests, the level of significance was set at 0.05.

$$FECR(1) = 100\% \times \frac{\text{arithmetic mean(baseline FEC)} - \text{arithmetic mean(FEC of dilution)}}{\text{arithmetic mean(baseline FEC)}}$$

$$FECR(2) = 100\% \times \text{arithmetic mean}\left(\frac{\text{baseline FEC} - \text{FEC of dilution}}{\text{baseline FEC}}\right)$$

Results

The baseline FEC (mean, minimum and maximum) and the number of samples with a FEC of zero eggs per gram faeces (EPG) for each of the different analytic sensitivities are summarized in Table 2. Overall, the mean baseline FEC was low and comparable for the different analytic sensitivities, ranging from 61.1 EPG (analytic sensitivity of 1) to 75.6 EPG (analytic sensitivity of 33.3). There was a decrease in the number of samples with zero FEC in function of the analytic sensitivity, resulting in 11 (36.7%) false-negative test results for an analytic sensitivity of 50.

As illustrated in Fig. 1, there was a significant correlation between the analytic sensitivity and the range between the limits of the 95% confidence intervals (95% CI) (~1/precision) (Rs=0.64, p=0.0001). For an analytic sensitivity of 1, the median range was 3%; for an analytic sensitivity of 50, a median range of 33% was observed. The precision also

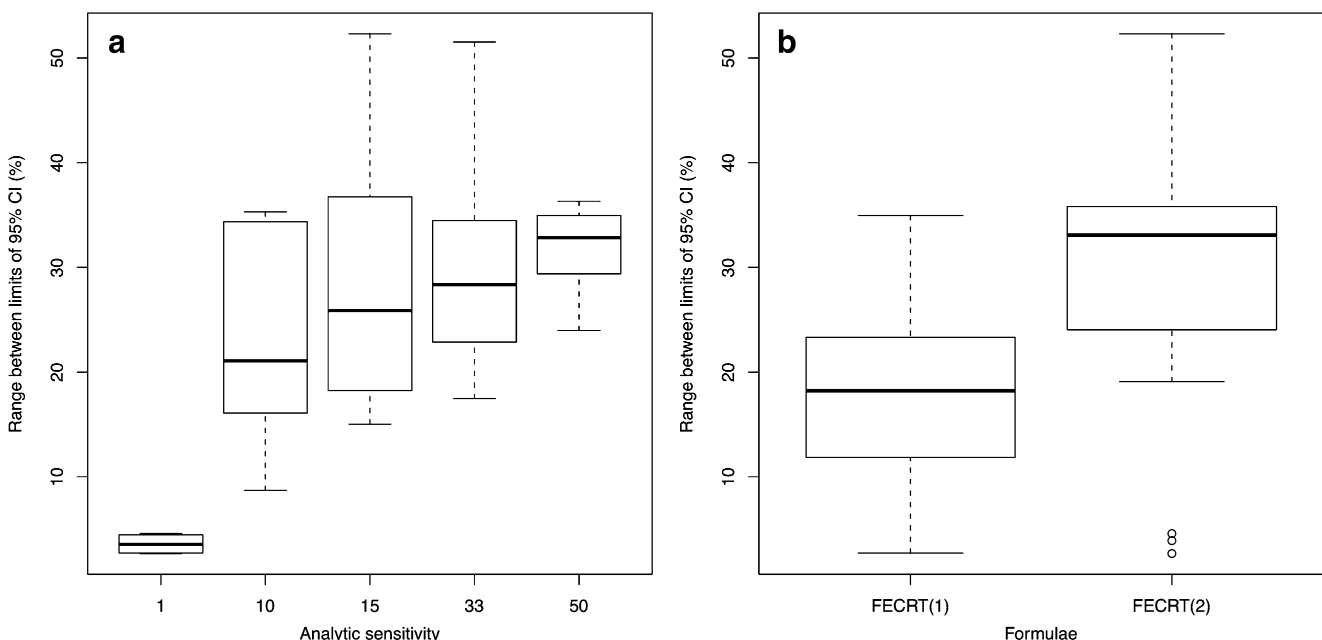

Fig. 1 The range between the limits of the 95% confidence intervals (95% CI) across different analytic sensitivities (**a**) and formulae (**b**), including individual- (FERT(1)) and group (FECRT(2))-based approaches

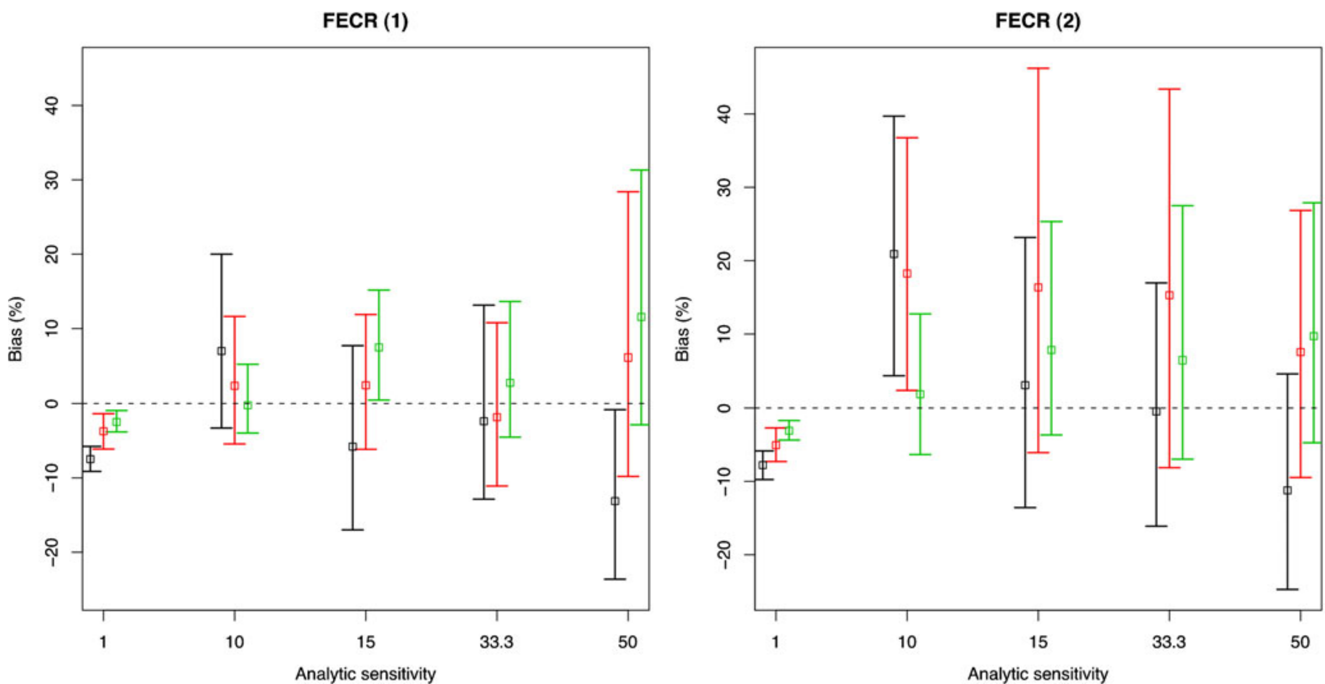

Fig. 2 The bias of the FECR (*empty squares*) and 95% confidence intervals for FLOTAC and four McMaster (McM) techniques for true drug efficacies of 70% (*black*), 80% (*red*) and 90% (*green*) based on including individual (FECR(1))- and group (FECR(2))-based FECR formulae

depended on the formula used, FECR(1) ($p<0.05$) resulting in more precise FECR compared to FECR (2). However, the precision remained unchanged for the highest analytic sensitivity (Fig. 2).

Figure 2 summarizes the bias of the FECR and the 95% confidence intervals for the three dilutions across the different analytic sensitivities and formulae, indicating that 10 out of the 30 FECRT results were biased (95% CI did include the zero). The highest analytic sensitivity (6/10) systematically overestimated the true drug efficacy (bias<0), whereas the remaining analytic sensitivities (4/10) also resulted in underestimations (bias>0).

As shown in Fig. 3, there was no significant correlation between the analytic sensitivity and the absolute bias (~1/accuracy) (Rs=0.21, p=0.26). Yet, the median absolute bias of the analytic sensitivity of 50 (10.5%) was more

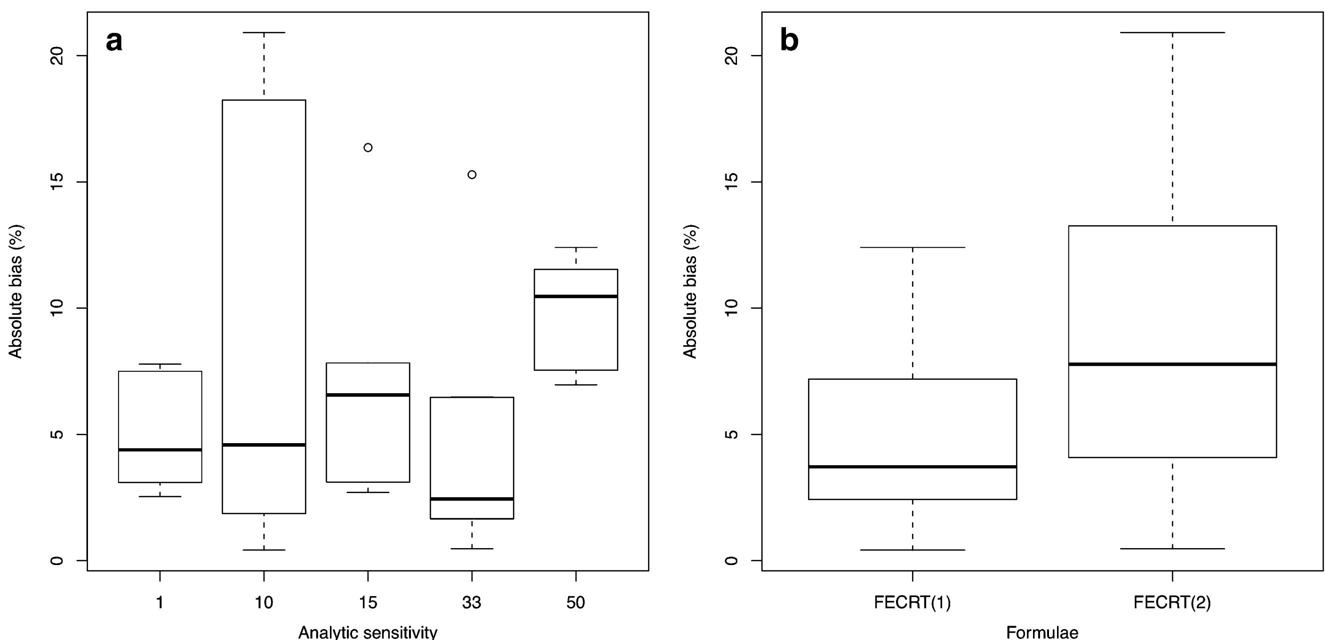

Fig. 3 The absolute bias across different analytic sensitivities (**a**) and formulae (**b**), including individual (FERT(1))- and group (FECRT(2))-based approaches

pronounced than that of the remaining analytic sensitivities, ranging from 2.5% to 6.7%. There was a marginal difference in accuracy between formulae ($p=0.06$), FECR(1) resulting in a smaller absolute bias. However, in analogy with precision, the accuracy of the highest analytic sensitivity remained unchanged across the formulae (Fig. 2).

Discussion

The present study assessed the impact of the analytic sensitivity of the technique and the formula on the precision and accuracy for FECR results when baseline FEC are low.

The results indicated that an increase in analytic sensitivity and a group-based formula (FECR(1)) results in more precise FECR, improving the final interpretation. The effect of analytic sensitivity is not surprising, since a difference in one egg count after treatment can have a considerable impact on the FECR for techniques with a high analytic sensitivity. The difference in formulae is caused by the large number of animals with a FEC of zero at baseline, resulting in an increase in variation for FECR(2), where these animals ought to be excluded, but not for FECR(1). This also clarifies why the formulae do not affect the precision of the lowest analytic sensitivity (~1), because for this analytic sensitivity, baseline FEC were available for all animals.

For the accuracy of the FECR results, only a marginal difference was found between the two formulae, the group-based formula (FECR(1)) resulting in more accurate FECR (approached a bias of zero) compared to the individually based formula (FECR(2)). Yet, this finding was more pronounced for low analytic sensitivities, which can also be explained by the two reasons mentioned above. The discrepancy in formulae confirms previous studies involving sheep (Cabaret and Berrag 2004) and humans (Vercruysse et al. 2011) where FECR(2) based on the McMaster technique (multiplication 5–50) yielded lower drug efficacies compared to FECR(1), and indicate that the use of FECR(2) to summarize FECR is not recommended for techniques with low analytic sensitivities when baseline FEC are low.

For the analytic sensitivity of 1, there was a systematic decrease in bias with increasing TDE. This observation is unexpected but may be due to the experimental set-up. Because the ratio of the volume of faeces to volume of flotation solution increased with decreasing drug efficacy, the density of the flotation solution may have been lower in samples where low drug efficacy was mimicked, resulting in lower FEC and rendering a positive bias.

This study focused on the impact of formula and analytic sensitivity; however, other factors inherent to the study design (e.g. inclusion of an untreated control group and sample size) or host–parasite interaction resulting in aggregated FEC both across and within hosts should not be ignored. For instance, the amount of faeces homogenized prior to the examination (McMaster, 3–4 g; FLOTAC, 10 g) may already thwart FEC, as the eggs are not equally distributed among faecal samples.

In conclusion, we used a novel methodology to assess the precision and the accuracy of FECR results based on different analytic sensitivities and formulae. The comparison indicated that techniques with a high analytic sensitivity are preferred for monitoring drug efficacy in populations with low baseline FEC. They also point out that the interpretation of individual-based formulae is affected by the analytic sensitivity of the technique. Finally, further studies are needed to improve the interpretation of FEC considering other factors such as the inclusion of an untreated control group, sample size and aggregation of the FEC across and within hosts.

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