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Are multiple pre-treatment groups necessary or unwarranted in faecal egg count reduction tests in sheep?

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ABSTRACT

Previously conducted faecal egg count reduction tests (FECRTs) in sheep involving a number of different anthelmintic treatments, were used to examine the effects of comparing post-treatment faecal egg counts (FECs) with pre-treatment counts from either the same treatment groups (matched FECRs) or with those from other treatment groups (unmatched FECRs). Each of these unmatched FECRs were considered to be analogous to those that might otherwise have been obtained by the use of a randomly selected group of animals to provide a single pre-treatment baseline for comparing all post-treatment results. An examination of these comparisons showed that the use of either procedure was likely to result in similar estimates of anthelmintic efficacy and the detection of a comparable number of cases of anthelmintic-resistance. Only on 1.1% of occasions did the FECRs from any of the unmatched groups fall outside the 95% confidence limits of the FECRs of their corresponding matched counterparts and in just 9.8% (54/553) of instances were there any disagreements between the number of cases categorised as either resistant or susceptible on the basis of a < or ≥95% FECR. These findings suggest that any improvements in accuracy and reliability that might supposedly be achieved by the use of multiple pre- and post-treatment FECs from the same treatment groups as opposed to those likely to be provided by the use of a single randomly selected representative pre-treatment group, may be largely illusory.

1. Introduction

There are several methods for the detection of anthelmintic resistance in sheep but the faecal egg count reduction test (FECRT), with its ability to provide a measure of the performance of a number of different anthelmintics at a time, is the one most widely used for on-farm assessments of drench efficacy. Four variants of this procedure have been described as follows:

$$\text{FECRT1} = 100 \times \left(1 - \frac{T2}{T1} \times \frac{C1}{C2}\right)$$

$$\text{FECRT2} = 100 \times \left(1 - \frac{T2}{C2}\right)$$

$$\text{FECRT3} = 100 \times \left(1 - \frac{T2}{T1}\right)$$

$$\text{FECRT4} = 100 \times \left(1 - \frac{T2}{C1}\right)$$

where T1 and T2 represent the mean pre- and post-treatment FECs of each treated group, and C1 and C2 represent the mean pre- and post-treatment FECs of an untreated control group, respectively (McKenna, 2006a). A comparison of the results obtained using each of these four alternatives found a correlation coefficient of 0.93 between FECRT1 and FECRT2 and a correspondingly good correlation of 0.95 between FECRT3 and FECRT4. The

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correlations between either FECRT1 or FECRT2 on the one hand, and FECRT3 or FECRT4 on the other, were not quite as good, ranging from 0.81 to 0.88 (McKenna, 2006a). A subsequent study (McKenna, 2006b) also found a similar dichotomy relating to the sensitivities of these four tests with false negative rates of 8% and 4% being recorded in FECRT1/FECRT2 and FECRT3/FECRT4 respectively, although there were no significant differences between them.

The closer relationship of the results observed within these two pairs of tests rather than between them is not surprising given the greater similarities of their methodologies. Thus neither FECRT3 nor FECRT4 include any allowances for changes in untreated controls and are based solely on comparisons between the pre- and post-treatment faecal egg counts (FECs) of treated groups. Both FECRT1 and FECRT2, on the other hand, are very reliant on fluctuations in the FECs of an untreated control group with FECRT2 representing a simplified version of FECRT1 using post-treatment samples only. The rationale for including such untreated controls in the latter two tests appears to be largely based on the proposition that their inclusion would enable any changes in the FECs of the treated groups to be corrected for those that may naturally occur in untreated animals and thus provide a more accurate assessment of efficacy as a result (Presidente, 1985). However, while such corrections might well be reflected in those changes induced by a largely ineffective anthelmintic, they are less likely to be paralleled in those instances where the anthelmintic is highly effective and very few eggs remain following treatment. Notwithstanding this, it is also evident that if the FECs of the untreated control group go up over the test period and all other things remain equal, then there is a tendency for the efficacy of the anthelmintic to seemingly improve. Likewise, if the FECs of the untreated control group go down, then there is a similar propensity for anthelmintic performance to ostensibly decline. Because of this, it seems more rational that measurements of anthelmintic efficacy should be determined by the changes in the FECs of only those animals that are actually treated rather than being influenced by changes in those that are not. Some support for this suggestion has recently been provided by Dobson et al. (2012), who found that there was no evidence that correcting FECRT data for changes in control FECs improved the estimate of anthelmintic efficacy and that efficacy estimates based on pre- and post-treatment counts only were likely to be more reliable.

While FECRT3 and FECRT4 are both tests that are solely dependent on changes in the FECs of only treated animals there are obvious differences between them. Thus where a multiplicity of drench types is involved, the former test necessitates comparing the FECs of groups of animals sampled at the time of anthelmintic treatment (pre-treatment) with the same animals sampled several days later (post-treatment) whereas the latter represents an abbreviated version of FECRT3 and uses only a single and common pre-treatment group as a baseline for comparing all post-treatment results. Although FECRT4 is consequently simpler and less expensive than the FECRT3 procedure, previous studies have shown that the FECR values produced by either one of them is likely to result in similar estimates of anthelmintic efficacy (McKenna, 2006a,b). Given this, and given that FECRs are frequently accompanied by wide confidence intervals, it is questionable as to whether or not the use of the more elaborate and expensive of these two tests is justified. The present study was therefore undertaken to try to help clarify this matter.

2. Materials and methods

The study was based on an examination of case submissions to Gribbles Veterinary Laboratory, Palmerston North, New Zealand, for FECRTs in sheep between 2009 and 2012. Only those cases involving the testing of more than one anthelmintic at a time with at least 10 animals per treatment group supported by individual FECs and undertaken according to the FECRT3 procedure were examined. For obvious reasons, instances where the FECR was 100% were excluded from consideration, as were those in which the arithmetic group mean pre-treatment counts were less than 200 eggs per gram (epg) of faeces. In each case, the percentage reductions determined from using the pre- and post-treatment egg counts of the same treatment group (matched FECRs) were compared to those obtained using the pre-treatment FECs of each of the other treatment groups (unmatched FECRs) as illustrated in Table 1. These former calculations were each considered to be representative of those FECRs that would be obtained using the FECRT3 methodology, the latter those that might otherwise have been achieved by using a randomly selected group of animals to provide a single pre-treatment baseline for comparing all post-treatment results as in the FECRT4 procedure.

In all instances these FECRs and their upper and lower 95% confidence limits were calculated using a RESO computer programme (Wurston and Martin, 1990) according to the arithmetic group means of their undifferentiated FECs. Initially, all results were categorised as indicating either anthelmintic-resistance or anthelmintic-susceptibility on the basis of a < or ≥95% FECR (McKenna, 2006b) but some were subsequently re-classified as signifying confirmed resistance (where the FECR was <95% and the lower confidence limit was <90%), suspect resistance (where only one of these two criteria was met), and susceptible (where neither was achieved) (Coles et al., 1992).

3. Results

A total of 39 FECR3 cases, involving the testing of between 2 and 7 anthelmintics on each occasion, provided 553 unmatched FECRs that could be compared to their matched counterparts. An examination of these comparisons (Fig. 1) showed that there was a strong and statistically significant correlation ($r = 0.9427$, $p < 0.001$) between them. It was also found that only in 6/553 (1.1%) of instances did the unmatched FECRs fall outside the 95% confidence limits of their corresponding matched FECRs and then only marginally so.

Cross tabulation of these unmatched and matched FECRs showed that 499/553 (90.2%) were similarly classified as drench-resistant or drench-susceptible on the basis of < or ≥95% FECR with disagreement between them