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Preserving new anthelmintics: a simple method for estimating faecal egg count reduction test (FECRT) confidence limits when efficacy and/or nematode aggregation is high.

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Abstract

As it has been 30 years since a new anthelmintic class was released, it is appropriate to review management practices aimed at slowing the development of anthelmintic resistance to all drug classes. Recommendations to delay anthelmintic resistance and provide "refugia" are reviewed and a simulation model used to find optimum treatment strategies that maintain nematode control. Simulated Australian conditions indicated that a common successful low-risk treatment program was a rapid rotation between a "triple-combination" product (benzimidazole + levamisole + abamectin) and a new high-efficacy drug (monepantel). Where *Haemonchus contortus* was a threat, moxidectin was required at critical times because of its persistent activity against this parasite. Leaving up to 4% of adult sheep untreated provided sufficient "refugia" for non-selected worms to reduce the risk of selecting for anthelmintic resistance without compromising nematode control.

For a new anthelmintic, efficacy estimated by faecal egg count reduction (FECR) is likely to be at or close to 100%, however using current methods the 95% confidence limits (CL) for 100% are incorrectly determined as 100%. The fewer eggs counted pre-treatment, the more likely an estimate of 100% will occur, particularly if the true efficacy is >90%. A novel way to determine the lower-CL (LCL) for 100% efficacy is to reframe FECR as a binomial proportion, i.e. define: **n** and **x** as the total number of eggs counted (rather than eggs per gram of faeces) for all pre-treatment and post-treatment animals, respectively; **p** the proportion of resistant eggs is **p**=**x**/**n** and percent efficacy is 100*(1-**p**) (assuming equal treatment group sizes and detection levels, pre- and post-treatment). The LCL is approximated from the cumulative inverse beta distribution by: 95%LCL=100*(1-(BETAINV(0.975,**x**+1,**n**-**x**+1))). This method is simpler than the current method, independent of the number of animals

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tested, and demonstrates that for 100% efficacy at least 37 eggs (not eggs per gram) need to be counted pre-treatment before the LCL can exceed 90%. When nematode aggregation is high, this method can be usefully applied to efficacy estimates lower than 100%, and in this case the 95% upper-CL (UCL) can be estimated by: 95%UCL=100*(1-(BETAINV(0.025,**x**+1,**n**-**x**+1))), with the LCL approximated as described above. A simulation study to estimate the precision and accuracy of this method found that the more conservative 99%CL was optimum; in this case 0.975 and 0.025 are replaced by 0.995 and 0.005 to estimate the LCL and UCL, respectively.

Keywords: Monepantel; Drug resistance; Refugia; Faecal egg count reduction test; Anthelmintic efficacy; Confidence limits; Precision accuracy.

1. Introduction

Broad-spectrum anthelmintics play a major role in the control of nematodes to improve animal health and production, and inevitably this has led to the development of anthelmintic resistance (Besier and Love, 2003; Kaplan, 2004; Jabbar et al., 2006). With the release of a new anthelmintic class (Kaminsky et al., 2008) it is appropriate to review what has been advocated to delay selection for drug resistance, and explore at methods for detecting resistance to indicate whether improvements can be made.

Simulations by Dobson et al. (2011 a, b) to explore how a new anthelmintic could be best integrated with currently available drugs to delay drug resistance to all drug classes while maintaining effective nematode control in Australian sheep farming systems are also reviewed. Finally, problems associated with estimating confidence limits (CL) for the faecal egg count reduction test (FECRT) when apparent drug

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efficacy is high, nematode aggregation is high, or few animals are available to test (as may be the case with horses) were examined. To estimate CL for a FECRT when efficacy was 100%, a different approach from conventional statistical methods was required. This involves a paradigm shift by determining the reliability of an assay from the total number of eggs counted pre-treatment rather than the currently used variables such as the eggs per gram in faeces (epg), group size and variance. The question: "Can this novel approach be more generally applied?" was explored.

1.1 Anthelmintic rotations/alternations or combinations

A review of the literature indicates that few publications have recommended that stockowners use a single anthelmintic in a control program for as long as it remains effective and then change to an alternate drug class. Le Jambre, Southcott and Dash (1977, 1978) initially advocated this approach, but Dash (1986) and Le Jambre (in Dobson et al., 2001) later advocated other strategies to delay resistance.

The problem with the 'use until resistance occurs' approach is that once resistance to an anthelmintic is detected the resistance (R-) allele frequency is fixed in the nematode population at a relatively high level and co-adapted with other fitness traits (Smith, 1998). As a result the possibility of reintroducing the 'used' anthelmintic is generally eliminated. To delay selection for anthelmintic resistance (recommendations summarised in Table 1) the majority of modelling and research studies have concluded that unrelated anthelmintics be alternated annually or used as combinations, or that farm-specific advice is obtained regarding drugs and animal classes. In nematode and other pest species, combination therapy is generally seen as the best option to delay pesticide resistance (Table 1), although the combination

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principle is not universally accepted. Because anthelmintics have different characteristics in terms of potency and persistence the most appropriate approach may be to match an anthelmintic to the particular time and circumstance rather than to advocate only one of the options in Table 1.

1.2 Other methods to delay anthelmintic resistance

Equally important to considering a drug rotation strategy is the management of animals and the timing of anthelmintic treatment. The development of anthelmintic resistance can be substantially delayed by implementing an appropriate treatment regimen (Gettinby et al., 1989; Barnes et al., 1990; Dobson et al., 1996). These authors showed that the use of 'safe' pastures for young stock can sufficiently reduce the number of anthelmintic treatments to slow selection for resistance. One risk associated with this strategy is that if sheep are treated and moved to pasture carrying no worm larvae (low 'refugia'), rapid selection for resistance can occur.

The importance of considering 'refugia' to avoid anthelmintic resistance was highlighted by van Wyk (2001): 'refugium' is the proportion of a parasite population that escapes treatment, but successfully establishes in a host at a later stage and produces viable off-spring. This strategy generally aims at the selective treatment of animals at risk, and the principle has been demonstrated in practice by a number of researchers. Hoste et al. (2002) was able to maintain nematode control in alpine dairy goats in France by treating only animals that were either high milk producers or in their first lactation. Similarly, haemonchosis was successfully controlled by treating only animals exhibiting clinical signs of anaemia (van Wyk et al., 2002). On four farms in southern Italy, Cringoli et al. (2009) left 40-60% of dairy sheep untreated

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without jeopardising the control of mixed infections of mainly *Trichostrongylus*, *Haemonchus* and/or *Nematodirus*. Leathwick et al. (2006a, b) and Waghorn et al. (2008) explored the possibility of leaving some sheep untreated to create refugia and discussed the difficulties of slowing selection for resistance without creating levels of parasitism that would reduce production. Earlier, Leathwick et al. (1995) demonstrated that treating ewes prior to any lamb anthelmintic treatment program will greatly increase selection for anthelmintic resistance. A survey of anthelmintic use in New Zealand found practices that aim to provide a refugia of susceptible worms and that minimise the risk of introduction of resistance through effective quarantine drenching were indeed associated with low levels of ML resistance (Lawrence et al., 2006). More recently, in studies in Western Australia, Besier et al. (2010) found that using a body condition score treatment index, 50% or more of Merino sheep could be left untreated when a flock treatment was given, without a significant reduction in flock productivity.

2. Simulations of control options in Australia with a new anthelmintic

To assess how best to delay selection for resistance to a new anthelmintic and maintain the effectiveness of current anthelmintics, Dobson et al. (2011 a, b) modelled treatment options for self-replacing Merino flocks in four different climates. A simulation model to predict gastro-intestinal nematode populations and the evolution of drug resistance to help identify low- and high-risk treatment programs was used. Simulations were defined by anthelmintic treatment, lambing dates, sheep movements between paddocks and historical weather data. Kojonup (Western Australia, Western Australia, (WA)) and Hamilton (Victoria, Victoria (VIC)) represented winter rainfall zones, with hotter and drier summers in WA. Glen Innes and Armidale (both northern

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New South Wales (NSW) are summer rainfall zones selected for years with medium and high risks for haemonchosis, respectively. In the simulations three anthelmintics; monepantel (MPL), moxidectin (MOX) and a 'triple combination' (COM) of benzimidazole (BZ) + levamisole (LEV) + abamectin (ABA), were used under a variety of combination and rotation programs. A risk-based scoring system was used to rank relative effectiveness of the treatment options on their ability to control nematode populations (productivity) and delay selection for resistance to all drug classes in each worm genus (*Trichostrongylus* at all sites, *Haemonchus* in NSW and *Teladorsagia* in WA and VIC). The application of MPL plus COM (i.e. four active ingredients) for all anthelmintic treatments most effectively delayed selection for anthelmintic resistance but was not always optimal for nematode control. Because of cost constraints or the reluctance of graziers to apply multiple applications, Table 2 illustrates other low-risk programs for the three regions, which can be summarised as a rapid rotation between a triple combination product and the new drug (MPL). In NSW, where *Haemonchus* was a major threat, it was necessary to include MOX at critical times in the drug rotation because of its persistent activity against *Haemonchus*. Additional grazing management planning may also overcome this problem but this was not explored. At the high-risk site for *Haemonchus*, the schedule in Table 2 was insufficient to prevent unacceptable host mortalities, but these were prevented by rotation at each treatment between MOX and MPL (option NSWr). In this, it was assumed the efficacy of MOX against homozygote susceptible (SS), heterozygotes (RS) and homozygote resistant (RR) genotype *Haemonchus* adults were 99.9%, 99.9% and 87.3%, respectively. Persistent activity against incoming *Haemonchus* infective larvae was assumed to last for 32 days and was 95%, 55% and 55% against SS, RS and RR genotypes. From these assumptions it follows that MOX

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171 efficacy remained relatively high (> 85%) even when ML-resistance was high and
172 some but not all ML-resistant incoming larvae were prevented from establishing in
173 the host. Consequently MOX became, in effect, a short-acting drug with moderate
174 efficacy when ML-resistance was high.
175
176 The triple combination played a useful role in delaying anthelmintic resistance despite
177 setting resistance to the component active ingredients at relatively high levels. The
178 initial R-allele frequency was set at 40% for both BZ and LEV, i.e. resistance was
179 assumed to be common. Initial ML R-allele frequency was set at 3% denoting an
180 emerging resistance problem, selected for by the drugs ABA and MOX. The R-allele
181 frequency was set at <0.005% for the new class of drugs (amino-acetonitrile
182 derivatives, represented by MPL) and was purposely set such that resistance would
183 emerge in less than 10 years if MPL was used for all treatments, and R-allele
184 frequency therefore varied slightly between nematode species.
185
186 In addition to the regimens shown in Table 2, Dobson et al. (2011a) explored the
187 importance of nematode populations in refugia at all sites by leaving 0, 1, 4, 7 or 10%
188 of adult sheep untreated. They concluded that leaving up to 4% of adult sheep
189 untreated reduced the risk of selecting for anthelmintic resistance without
190 compromising nematode control. Similar findings were made by Pech et al. (2009)
191 who modelled the optimal economic management of anthelmintic resistance in sheep
192 flocks exposed to *Teladorsagia* under WA conditions with refugia managed by
193 leaving 0, 2, 5 or 10% of sheep untreated. These authors concluded that anthelmintic
194 resistance greatly reduced the profit of sheep enterprises and leaving 2% of the flock
195 untreated optimised profit by allowing the effectiveness of drenches to be prolonged,

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196 providing the best long term benefit. The concept of deliberately leaving some sheep
197 untreated is potentially controversial and farmers may have difficulty in accepting it
198 (discussed by Waghorn et al., 2008). However, as it has been approximately 30 years
199 since the last new anthelmintic class was commercialised, with the release of MPL it
200 is important to promote management practices that will slow the development of
201 anthelmintic resistance to all drug classes.
202
203 **3. Detecting anthelmintic resistance and estimating drug efficacy by FECRT**
204 Only one test is currently widely available for field use for estimating drug efficacy,
205 the faecal worm egg count reduction test (FECRT). Debate has occurred over whether
206 arithmetic (AM) or geometric (GM) means are most appropriate for determining drug
207 efficacy (Dash et al., 1988; Vercruysse et al., 2010). Dobson et al. (2009)
208 demonstrated (by Monte Carlo simulation techniques, used when analytical solutions
209 are intractable) that AMs provide the best estimate of efficacy and clearly
210 demonstrated that GMs often yield extremely biased results. Recently, research has
211 addressed how better to analyse data from a FECRT using Monte Carlo or bootstrap
212 techniques, particularly in relatively difficult situations involving low egg count data
213 such as can occur with horses and cattle (Cabaret et al., 2004; Vidyashankar et al.,
214 2007; Denwood et al., 2010). The problem with these approaches is that they are
215 computationally intensive and require a high level of mathematical sophistication,
216 which is available to researchers but generally not to veterinary consultants who
217 conduct FECRTs.
218
219 In a review of published anthelmintic efficacy data, McKenna (2006) compared
220 efficacy based on controlled slaughter trials, which are the 'gold standard', with four

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methods for calculating efficacy from FECRT. McKenna (2006) found 61 data sets with both worm count and FECRT data and from these he estimated *sensitivity* (Se): the proportion of anthelmintic resistance cases correctly diagnosed as 'resistant' by the FECRT methods, and *specificity* (Sp): the proportion of susceptible nematode isolates correctly diagnosed as 'susceptible' by the FECRT methods. The four FECRT percent efficacy estimates were defined by McKenna (2006) as:

FECRT1 = 100 x *(1–[T2/T1]* x *[C1/C2]);*

FECRT2 = 100 x *(1–[T2/C2]);*

FECRT3 = 100 x *(1–[T2/T1]);*

FECRT4 = 100 x *(1–[T2/C1]);*

where T1 and T2 represented the mean pre- and post-treatment faecal nematode egg counts of a treated group, and C1 and C2 represented the mean pre- and post-treatment counts of an untreated control group, respectively.

For all estimation methods Sp was 1, i.e. 100% or no false positives (i.e. 1-Sp) while the false negative rate (1-Se) ranged from 8% (for FECRT1-2) to 4% (for FECRT3-4). Because there was no significant difference between the four estimates of Se (0.92 vs. 0.96), McKenna (2006) concluded that it was simplest to use either FECRT2 or FECRT4 as these required the minimal cost or labour. However, McKenna (2006) was concerned with sheep, where the data is generally less constrained by low egg counts and group sizes are determined by cost considerations. For horses, common practice to estimate efficacy using pre- and post-treatment counts from the same animals (FECRT3) (Denwood et al., 2010), in particular because of estimation problems associated with relatively low counts, high variability and small group sizes.

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246 However, for the McKenna (2006) results it is important to note that using the post-
247 treatment control group or correcting efficacy to account for changes in control group
248 (Presidente, 1985) between the pre- and post-treatment counts (FECRT 1and 2)
249 caused 4% more cases to be incorrectly diagnosed as susceptible, noting all estimates
250 were based on the same data. This implies that the egg counts measured at the time of
251 treatment (C1 or T1) are the most appropriate for estimating efficacy and the
252 correction method of Presidente (1985) does not improve efficacy estimation. The
253 correction is based on the assumption that parallel changes in egg counts occur in the
254 treated group similar to those observed in the control group, however, this assumption
255 is rarely justified and does not stand up to scrutiny, as demonstrated by McKenna
256 (2006). In cattle, density-dependent control of fecundity, particularly for *Ostertagia*
257 *ostertagi* (Smith et al., 1987), may well further exacerbate this problem. For example,
258 counts of untreated control animals may decline because of density-dependent
259 constraints on fecundity exacerbated by any incoming infection between pre- and
260 post-treatment counts, while in treated groups the worms surviving treatment may
261 have increased fecundity because of reduced worm populations or competition. In this
262 situation correcting for changes in control egg count (as epg) or relying on post-
263 treatment control egg count (FECRT1 or 2) will cause efficacy to be underestimated.
264
265 The four methods for estimating efficacy (FECRT 1- 4) are dependent on determining
266 a minimum of two mean egg counts and the variance of the means are components in
267 the CL calculations:
268 95% lower CL=100*(1-(T2/C2)exp(+2.048√V)), where V = variance of
269 reduction on log scale, which is a function of variance of the control and treated group
270 mean egg count (for details, see Anderson et al., 1991; Coles et al., 1992). This is the

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simplest form for estimating CL and can be used for FECRT 2 - 4. Lyndal-Murphy et al. (2010) provide a more complete expansion of this formula for estimating efficacy CL when corrections for changes in control counts are involved (i.e. FECRT 1).

4. A novel way to estimate confidence limits for FECRT

The FECRT essentially relies on the ratio of two means. However, to accurately estimate the mean egg count when the variance is high, a large number of animals is required (Morgan et al., 2005). Rather that attempting to estimate the mean it can be demonstrated (Section 4.5) that effort would be better directed to counting a large number of eggs pre-treatment from high shedding animals (e.g. the four animals in the group with the highest counts) and then counting the same animals with the same degree of sensitivity post-treatment, thus avoiding the low or zero egg producing animals. These animals contribute greatly to the variance but provide little useful information to the estimate of efficacy, and need be included only if the flock mean is to be estimated (e.g. with regard to pasture contamination). The required paradigm shift is to ignore the animal as the experimental unit, regarding the egg as the unit of interest. This can be defined as either susceptible or resistant, thus creating a binomial variable; the host thus becomes a vessel for supply of nematode eggs, which is the case for *in vitro* assays. The animal can be thought of as an *in vivo* equivalent of an *in vitro* culture system used to support the growth of micro-organisms for similar *in vitro* drug assays and replicate animals are equivalent to replicate assays. The advantage is that binomial CLs are defined for a single animal, based on how many eggs were counted, which provides an estimate of reliability for the assay (i.e. how accurately the drug efficacy was determined in one animal). When multiple animals are tested then the drug efficacy for the group or farm can be estimated. This focuses attention

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296 on how many eggs are observed, rather than the number of animals in each group or
297 the egg detection level.
298
299 *4.1 When the efficacy estimate is 100%*
300 The World Association for the Advancement of Veterinary Parasitology (WAAVP)
301 guidelines for estimating efficacy by FECRT (Coles et al., 1992) provide
302 straightforward methods for estimating the 95% CL that can be easily applied to
303 routine field data. While these estimates are robust, a problem with the CL method
304 occurs if the efficacy is 100% (i.e. no eggs are counted in the post-treatment samples)
305 as the upper (UCL) and lower (LCL) limits are then both 100%. This problem also
306 occurs when using the Lyndal-Murphy et al. (2010) formula for estimating CL. As a
307 result, CL for 100% efficacy are not reported (eg, Soutello et al., 2007; Lyndal-
308 Murphy et al., 2010). No CL or CL of 100-100% implies that the 100% efficacy
309 estimate is equally reliable whether for example a total of 10 or 300 eggs from all
310 animals were counted pre-treatment; this is not reasonable if the true efficacy was
311 98% as the expected total count from all animals post-treatment would then be 0.2 and
312 6 eggs, respectively in this example. Assuming a Poisson distribution of sample
313 counts approximately 81% and 0.2% of trials will provide an efficacy of 100% if the
314 expected total count is 0.2 and 6 eggs, respectively for the above example, (i.e. if the
315 mean count/chamber was 0.2 eggs and 100 chambers were counted, then 19 chambers
316 would contain 1 or more eggs and the remainder would be zero, assuming that sample
317 counts have a Poisson distribution.) It is clear that the fewer eggs counted pre-
318 treatment, the more likely an estimate of 100% will occur particularly if the true
319 efficacy is greater than say 90%. (This discussion is not referring to 10, 300, 0.2 or 6

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as the number of eggs per gram but as the total eggs counted over all animals in a test group pre- or post-treatment.)

A proposed approach to solve this problem is to estimate the LCL for a binomial proportion using methods described by Brown et al. (2001), who coined the name 'Jeffreys interval' to describe a confidence interval (CI) derived from Bayesian procedures assuming non-informative priors. The Jeffreys interval is simple to calculate as the uncertainty about the binomial proportion is described by the beta distribution, which is a standard function in the Excel spreadsheet (as is the inverse-beta function). The Jeffreys interval can be reframed in terms of a FECRT by defining: **n** as the total number of eggs counted pre-treatment, **x** the total number of eggs counted post-treatment, **p** the proportion of resistant eggs is **p**=**x**/**n** and percent efficacy is 100*(1-**p**). The LCL can be approximated from the cumulative inverse beta distribution as:

$$(1-\alpha)\%LCL = 100*(1-(BETAINV(1-\alpha/2, \mathbf{x}+1, \mathbf{n}-\mathbf{x}+1))) \quad \text{equation (1)}$$

$$(1-\alpha)\%UCL = 100*(1-(BETAINV(\alpha/2, \mathbf{x}+1, \mathbf{n}-\mathbf{x}+1))) \quad \text{equation (2),}$$

where α is the significance level used to define the CI and the percent CI is 100*(1-α), e.g. α=0.05 and 0.01 for 95% and 99%CL, respectively. BETAINV is the cumulative distribution function for the beta distribution specified by the latter two parameters (i.e. **x**+1and **n**-**x**+1) of the three mandatory Excel parameters in the equations above. The Jeffreys interval defined by Brown et al. (2001) uses 0.5 (e.g. **x**+0.5) rather than 1 (e.g. **x**+1) as the non-informative prior, as the 0.5 prior provides a better estimate of the UCL for small **n**, however a prior of 1 was chosen as it provides a more conservative LCL, particularly for **n**<40 eggs; there is little difference between

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the results from the two priors for larger **n**. Figure 1 shows the LCL for 100% efficacy using equation (1) for varying numbers of eggs counted pre-treatment.

A minimum requirement to estimate efficacy with reasonable accuracy (95%LCL>95% efficacy) is that no fewer than a total of 70 eggs should be counted in the pre-treatment counts (Figure 1). If 90% efficacy were an acceptable 95%LCL then at least 37 eggs need to be counted pre-treatment (i.e. a total 37 eggs counted from all animals and egg count chambers). Figure 1 provides a useful guide to the precision of a FECRT when the observed efficacy is 100% unlike the current methods (Coles et al., 1992; Lyndal-Murphy et al., 2010) that provide no estimate of precision when efficacy is 100%.

4.2 When the efficacy estimate is less than 100%

To more generally apply this method for efficacy <100% (i.e. for **p**>0) it is required that the samples are drawn from the same animals pre- and post-treatment, and are counted at the same level of sensitivity (i.e. the same number of chambers per animal are counted at the same detection level pre- and post-treatment). This requirement was relaxed for 100% efficacy as rough approximation of precision is better than no approximation of test reliability, which is the current situation. An example; if a total of 100 eggs (**n**) and 10 eggs (**x**) were counted pre- and post-treatment, respectively from the same animals then the efficacy is 90% and for α=0.05 the CL are computed in Excel using equations (1) and (2) by:

95%LCL = 100*(1-(BETAINV(0.975, 11, 91))) = 83%

95%UCL = 100*(1-(BETAINV(0.025, 11, 91))) = 94%

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Figure 2 shows the Jeffreys intervals for 95% and 90% efficacy, and can be used as a guide to required FECRT precision. For example, approximately 140 eggs are required to estimate 95% efficacy with CLs 90-98%, this equates to a mean epg of 700 or 350 for a detection factor of 50 or 25, respectively assuming 10 animals per group. If the basic requirements for a FECRT are considered then from Figures 1 and 2, a minimum total of 70 eggs counted pre-treatment would be adequate to provide reasonable precision. While this is subjective and the purpose of the FECRT may influence the level of precision required, Figures 1 and 2 provide a useful guide. The Jeffreys interval is not defined when efficacy is less than zero, i.e. when the post-treatment count is greater than the pre-treatment count. However, in this situation the drug is clearly useless and the LCL and UCL were arbitrarily set to zero.

The Jeffreys interval approach was compared with the current statistical method (Coles et al. 1992) using unpublished nematode characterisation data covering a wide range of drug efficacies (supplied by Novartis Animal Health Australasia Pty Limited). The test protocol consisted of three animals in each treated and control group, for 52 data sets including 35 and 17 susceptible and resistant cases, respectively. Nine data sets were from cattle and the remainder from sheep, covering 10 nematode species and the ML, BZ and LEV drug groups, including some tests with BZ+LEV combinations. Of these cases 24, 11, 4, 4 and 9 had efficacies of 100%, <100 to 95%, <95 to 90%, <90 to 60% and <60%, respectively, determined from controlled slaughter studies. Egg counts were also performed pre- and post-treatment on all groups. Detection levels for cattle were 20 for both worm and egg counts, for sheep these were 20 and 50 for worm and egg counts, respectively. A mean of 128 eggs was observed pre-treatment, however, in 35% of the data sets fewer than 25 eggs

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394 were counted pre-treatment for some species (e.g. *Nematodirus*, *Cooperia*,
395 *Trichostrongylus axei*).
396
397 These data were used to estimate specificity (Sp) and sensitivity (Se) of FECRT 2 and
398 3 as defined by per McKenna (2006; see Section 3 above). In addition, the error rate
399 (accuracy) and precision of using the 99 and 95% Jeffreys CI was compared with the
400 95% RESO CI (Coles et al., 1992). Precision was estimated by the average width of
401 the FECRT CI and error rate was defined as the percent of times the FECRT CI did
402 not include the efficacy determined from worm count data.
403
404 Table 3 gives the results of this analysis and show that Sp and Se were very similar to
405 those estimated by McKenna (2006). The average width of the 99% Jeffreys FECRT
406 3 CI was 7% less than the 95% RESO FECRT 2 CI and the error rate for FECRT3
407 was 10% less that for FECRT2. The mean 95% Jeffreys CI was 5% less than the mean
408 99% Jeffreys CI, but this did not change the FECRT3 values for Se, Sp or error rate
409 (data not shown). One important difference was observed for error rate: for FECRT 2
410 11 of the 13 errors occurred when efficacy was in the range <100 to 95% (in all 11
411 cases UCL=LCL=100% as determined by RESO), while seven of the eight errors for
412 FECRT 3 occurred when efficacy was below 60% (in five of these seven cases the
413 efficacy was <0% and thus UCL=LCL=0% for Jeffreys CL).
414
415 In summary, FECRT 3 (comparisons of pre- and post-treatment counts without
416 controls) better estimated efficacy than FECRT 2 (post treatment counts only with a
417 control) when efficacy was moderate to high (above 60%), though neither falsely
418 declared resistance when a strain was susceptible (Sp=100%). FECRT 2 more

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accurately estimated efficacy than FECRT 3 when efficacy was below 60%. When the efficacy was between 80 and 95%, FECRT 2 and FECRT3 mis-diagnosed resistant isolates in two and one test, respectively. The high Sp and Se observed here for FECRT were based on experimental infections and probably represent the best possible values, and in the field lower values could be expected.

4.3 Comparison of Jeffreys interval with Monte Carlo techniques from cattle data

A survey for cattle anthelmintic resistance using FECRT was conducted by El-Abdellati et al. (2010). Preference was given to sampling as many farms as possible by sampling 10 cattle per treatment group at a detection level of 50 epg. The same groups of animals were tested pre- and post-treatment but the same 10 animals were not necessarily sampled (a mix of FECRT3-4 as defined in Section 3). The authors were concerned with falsely declaring reduced anthelmintic efficacy (the opposite issue to that discussed in Section 4.1). Using Monte Carlo simulation techniques they generated the distribution of results for their sampling protocol for a drug with 95% efficacy (10,000 iterations per treatment group). If the observed efficacy was in the lowest 5% of the simulated distribution then reduced efficacy was assumed.

Even though the same animals were not tested pre- and post-treatment the UCL for their observed efficacies was approximated by:

UCL=100*(1-(BETAINV(**c**,**x**+1,**n**-**x**+1))), where **c** was set at 0.005, 0.025 and 0.05 for 99%, 95% and '1 tail 95%' UCL, respectively; total eggs counted pre-treatment (**n**) and total eggs found post-treatment (**x**) for each farm were estimated by: **n**=epg*10/50 and **x**= **n***(%efficacy/100). The criteria using were: if the observed efficacy's UCL was less than 95%, then the farm had a significant reduction in drug

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efficacy. Of the 33 farms with mean efficacy <95%, the 99% and 95% Jeffreys interval classified 32 and 31 of the farms, respectively, which was the number classified by Monte Carlo simulation. Given that the latter required 10,000 iterations to obtain a single result the Jeffreys method provided a simple rapid estimate of CL for this data, despite pre- and post-treatment counts coming from different animals. The Jeffreys method should not be used to estimate CL if control or pre-treatment animals are different individuals to the treated animals (i.e. FECRT 2 and 4). The mean total eggs counted pre-treatment by El-Abdellati et al. (2010) for these farms was 25, which generally provides low precision for an assay as can be seen from Figure 2. In 58% of the data sets fewer than 25 eggs were counted pre-treatment and on only three occasions were more than 40 eggs counted pre-treatment. This further demonstrates that it is as important to establish a minimum number of eggs to count in an assay as it is to set a minimum group size, particularly for cattle.

4.4 Use of Jeffreys interval with cattle and horse field data

4.4.1 Cattle data

Unpublished results from one farm in a survey of anthelmintic resistance in Western Australian cattle conducted by the Department of Agriculture and Food WA in 2010 were made available to the authors for comparisons of FECRT methods, including the Jeffreys interval. The particular farm was selected because it contained multiple species and reduced efficacy for some drugs. The survey adopted a relatively high precision protocol by sampling a minimum of 15 animals per group (up to 20) and a detection level of 12.5 epg, using a modified McMaster egg count procedure and counting two chambers per animal. Egg counts were conducted pre- and post-treatment for treated and untreated control animals. Faecal culture and larval

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differentiation were carried out on all groups pre- and post-treatment. At the pre-treatment count five animals with the highest counts were identified and six additional chambers were counted for these animals. At the post-treatment counts these animals were counted as usual plus six additional chambers were counted. This process increased the effective detection level to approximately 3 epg for the animals with high egg counts. The aim here was to compare three procedures to estimate the efficacy and 95%CL:

Method-1, the standard estimate (FECRT 2, defined above) and analysing the results using RESO as per Coles *et al* (1992);

Method-2, FECRT 3, but using the Jeffreys interval;

Method-3, determining efficacy and 95%CL only from the five animals with the highest counts and additional chambers counted and using the Jeffreys interval to estimate the 95%CL (i.e. additional chambers counted to increase the total number of eggs observed to improve the FECRT precision).

The efficacy results for the three methods are shown in Table 4, using the criteria defined by Coles et al. (1992): "*Resistance is present if (i) the percentage reduction in egg count is less than 95% and (ii) the 95% confidence level is less than 90%. If only one of the two criteria is met resistance is suspected*." For undifferentiated total egg count, only Method-3 estimated an efficacy of <100% for ivermectin (IVM) injectable; fenbendazole (FBZ) was categorised as 'suspect resistant' by Method-1 but not by Method-2 or 3; LEV was considered 'resistant' by Method-1 and Method- 2 and 'suspect' by Method-3. Methods-1 and 2 categorised *Ostertagia* eggs 'FBZ-resistant' while Method-3 found the *Ostertagia* population to be 'susceptible'

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493 (efficacy 95.8% and 95%LCL 90.5%). Only Method-3 found an efficacy of <100%
494 for IVM injectable against *Cooperia* eggs.
495
496 For the control group a total of 120 eggs was recovered from 18 animals; when
497 converted to mean eggs per gram (83 epg) to determine efficacy, as required for
498 Method-1, efficacy would be overestimated for IVM injectable with mean 65 epg and
499 underestimated for FBZ, LEV and IVM pour-on with mean epg of 108, 138 and 155,
500 respectively. Methods-2 and 3 both resolve this problem.
501
502 Low numbers of *Oesophagostomum* were found on this farm (mean 7% pre-treatment
503 and 0% post-treatment), indicating that all drugs were 100% effective against this
504 parasite and a LCL of 100% for Method-1. However it would not be appropriate to
505 report this result to the stock owner, as Method-2 indicated a 95%LCL <60% for FBZ
506 and IVM injectable, 84% for LEV and 90% for IVM pour-on. Method-3 provides
507 slightly more confidence in the results with a 95%LCL of the four treatments of 84%,
508 81%, 93% and 95%, respectively. It would be reasonable to report a result of 100%
509 efficacy if the Jeffreys 95%LCL was above 90%, but otherwise a result of
510 'insufficient data or worm eggs' should be reported.
511
512 Under this protocol, if the three Methods were used in isolation then Method-1 would
513 require the least work, i.e. 200 chambers counted (2-chambers x 5-groups x 20-
514 animals x 1-time-post-treatment). Method-2 requires 320 chambers to be counted (2-
515 chambers x 4-groups x 20-animals x 2-times) or 60% more work than Method-1.
516 Method-3 requires 440 chambers be counted (2-chambers x 4-groups x 20-animals x
517 1-time-pre-treatment + 6-chambers x 4-groups x 5-animals x 1-time-pre-treatment +

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8-chambers x 4-groups x 5-animals x 1-time-post-treatment), i.e. 2.2 times more effort than Method-1 and 38% more than Method-2. To determine if the additional work provided more information, the total eggs counted by each method can be compared after correction to 20 animals/group (e.g. for the control group, 120 eggs were counted from 18 animals; if 20 animals were counted this would then increase to approximately 133 eggs). The mean number of pre-treatment eggs counted for the five Method-3 animals was 439/group, indicating that 2.2 times more work than Method-1 yielded 3.3 times more eggs. For Method-2, the mean number of eggs counted pre-treatment was 186 (after correction to 20 animals), so that Method-3 yielded 2.4 times more eggs for 38% more work. Conditions which may justify the additional work to increase the precision of an assay are discussed below (Section 4.5.3 and Conclusion).

4.4.2 Horse data

On many properties, relatively few horses are present, making the routine FECRT (McKenna 2006) difficult to conduct. Even for farms where large numbers of horses are present, the numbers in homogeneous groups suitable for FECRT (i.e. similar age, blood line and grazing and treatment history) are usually relatively small compared with sheep and cattle. In this situation Jeffreys interval can provide estimates of test precision. Table 5 shows nematode FECRT data from seven horse owners in the Perth region of WA; because the drug efficacy was generally 100% only the LCL are shown.

As discussed in Section 4.1 the standard method (RESO; Coles *et al* 1992) cannot provide helpful CI when efficacy is 100%. On the seven farms LCL were able to be determined by Jeffreys interval but only on one farm could RESO estimate a LCL.

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543

544 *4.5 Precision and error rate for Jeffreys interval and standard statistical methods*

545 4.5.1 Method

546 For the purpose of comparing Jeffreys interval with the standard statistical approach
547 (Coles et al., 1992),'precision' was defined as the width of the CI and 'error rate' the
548 percentage of results where the true efficacy lies outside the CL of the estimated
549 efficacy. As CI=UCL-LCL, the wider the CI, the chance of error is reduced but
550 precision is sacrificed. Comparing the error rate and precision of Methods-1, 2 and 3
551 (Section 4.4) requires Monte Carlo simulations where the true efficacy, sampling
552 variability, mean egg count and distribution of counts in the host population are
553 defined. Variability in simulation results follow from random selection of 10 hosts
554 from the population with known *k* for the Negative Binomial Distribution (NBD) and
555 laboratory sampling variability associated with the Poisson distribution of egg counts
556 (Dobson et al., 2009). Four scenarios were simulated: *k* for the NBD either set at 0.5
557 or 2, both with low and high sensitivity for detecting nematode eggs. For *low*
558 *sensitivity* the mean epg was set at 250 with a detection factor of 50, i.e. the expected
559 count per animal was five eggs or a total of 50 eggs observed pre-treatment if 10
560 animals were used. For *high sensitivity* the mean epg was set at 300 with a detection
561 factor of 25, i.e. the expected count per animal was 12 eggs or a total of 120 eggs
562 observed pre-treatment if 10 animals were used. The Monte Carlo methods of Dobson
563 et al. (2009) were used to generate 10,000 results for each simulation scenario;
564 efficacy and CL were estimated at each iteration by the three methods. For Method-2,
565 that requires pre- and post-treatment counts from the same animals (FECRT 3), CL
566 were estimated by both the Jeffreys and RESO (Coles et al., 1992) methods. For
567 Method-3, three additional counts of the two, three, four or five of the highest egg

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568 shedding animals from the 10 randomly selected animals was simulated by drawing
569 additional Poisson samples at the appropriate detection level. This process changed
570 the detection factors of 50 and 25 to 12.5 and 6.25 epg, respectively for the high egg
571 shedding animals. For Jeffreys CI both 99% and 95%CI were determined ($\alpha = 0.01$
572 and 0.05, respectively), for RESO only 95%CI were calculated. As noted, Jeffreys
573 interval can only be used when the same animals are counted pre- and post-treatment,
574 and the calculations therefore cannot be applied to Method-1 data. In the simulations,
575 true efficacy was set at 95% as it is preferable to detect resistance while efficacy is
576 relatively high rather than when it declines to low levels that risk parasitism and
577 production losses.
578
579 4.5.2 Results
580 Table 6 shows the mean width of the CI and error rate for each scenario and method.
581 The error rate for the 95% Jeffreys CI was about 6% and was reasonably consistent
582 across scenarios and methods. This was approximately half the error rate for the
583 standard method (RESO; Coles et al., 1992) at low assay sensitivity but was generally
584 higher than the RESO error rate at high assay sensitivity (see Table 6). The precision
585 of the 95% Jeffreys CI, measured by the width of the CI, was approximately 50%
586 better (smaller) than the RESO 95%CI. By determining the 99% Jeffreys CI, the
587 width of the CI only increased by about 2-4% on average (minimal loss of precision)
588 but was still substantially smaller than the RESO 95%CI. The error rate associated
589 with the 99% Jeffreys CI was about 1.4% and was lower than the RESO error rate for
590 all scenarios and methods except for the high sensitivity pre- and post-treatment
591 (Method-2) with k=2, which had the same low error rate as the Jeffreys results. By
592 restricting the Jeffreys estimates to data only from either the two, three, four or five

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593 animals with the highest egg counts (Method-3) there was little impact on the error
594 rate while the precision improved slightly as more animals were included in the
595 FECRT. Figure 3 shows the distribution of Monte Carlo efficacy results for each
596 method at the high sensitivity FECRT level and for k=0.5, the frequency bar at 81%
597 efficacy includes efficacy results less than 81%. Figure 3 shows that efficacies based
598 on pre- and post-treatment counts are more closely distributed about the true efficacy
599 (95%) than results obtained from control and treated groups. Additional improvement
600 in the distribution was obtained by Method-3. The equivalent distributions for k=2
601 (data not shown) shows no difference between the two pre- and post-treatment
602 methods (Methods-2 and 3) but both were better than Method-1.
603
604 4.5.3 Discussion
605 Morgan et al. (2005) found k for the NBD to range from 2.3 to 0.18 in 14 flocks of
606 commercially managed sheep. In four lamb flocks k was >1 with mean 1.8. In the
607 remaining three ewe flocks and seven lamb flocks the range for k was 0.63 to 0.18
608 with mean 0.38, with these low values for k associated with low egg counts (<400
609 epg). For sheep, it is therefore not unreasonable to expect to encounter some groups of
610 animals that have k<0.6, and the situation in cattle, where egg counts are generally
611 lower, would expected to be associated with similar or lower k values. In the FECRT
612 simulations the choice of k=2 represents a optimal situation where within group egg
613 counts would be relatively homogenous, while setting k=0.5 represents a more
614 difficult situation where some animals with zero epg are likely to be found in the pre-
615 treatment egg counts (Dobson et al., 2009). In the field it is not possible to know what
616 k for the NBD is associated with a particular FECRT, however if some animals have
617 very low or zero counts pre-treatment then k is likely to be low. In this situation pre-

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618 and post-treatment counts on the same animals are essential to estimate efficacy with
619 reasonable accuracy (Figure 3) and the accuracy can be further improved by using
620 Jeffreys method to determine the 99%CI as it provides smaller error rates and CI
621 when efficacy is moderate to high (above 60%). Restricting sampling to the animals
622 with the highest counts and counting these animals at a higher level of sensitivity will
623 improve precision. It is futile to retain in a FECRT animals that have low or zero
624 counts pre-treatment as they contribute very little or nothing to the estimation of
625 efficacy. Expanding the RESO CI from 95 to 99% would have reduced its error rate
626 but increased the size of the CI, which were already substantially larger than the
627 Jeffreys intervals. On the other hand, if counts are high and with relatively low
628 variation between animals then any of the methods recommended by McKenna (2006)
629 would yield a suitable result. However, a method that only relies on post-treatment
630 counts (FECRT 2) would fail to identify the difficult tests where *k* is low, thus
631 remedial action, as suggested above, can not be taken to avoid a low precision test.
632

633 **5. Conclusion**

634 The onset of anthelmintic resistance to a new highly effective drug (MPL) and the
635 currently available drugs was delayed by applying all four drug classes for all
636 anthelmintic treatments. A rapid rotation between MPL and a triple combination
637 (BZ+LEV+ABA) generally was the next best option. However, in areas where
638 *Haemonchus* was a high risk, inclusion of a persistent drug (MOX) in the rotation was
639 necessary to reduce production losses and additional grazing management may also be
640 beneficial in this situation. Even though resistance to BZ and LEV was assumed to be
641 relatively high they were still useful in helping to delay anthelmintic resistance to
642 other drugs when used as components of a triple combination. Leaving up to 4% of

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adult sheep untreated generally helped delay selection for resistance without compromising nematode control. A strategy that provides a source of refugia is essential when a combination treatment is used.

In FECRT where efficacy is estimated to be 100% the current statistical methods fail to provide CL. In this case the Jeffreys interval provides a simple way to estimate the precision of the test. There is no evidence that correcting FECRT data for changes in control egg counts improves the estimate of drug efficacy, and efficacy based on pre-treatment counts are likely to be more reliable. For FECRT involving pre- and post-treatment counts of the same animals the 99% Jeffreys interval generally had higher precision with the least error rate by comparison with the current statistical methods (RESO; Coles et al., 1992). When conducting a FECRT it is reasonable to assume that *k* for the NBD is low when there are zero counts in the pre-treatment animals. In this situation restricting the test to animals with high counts, increasing the sensitivity of the counting procedure and using Jeffreys interval to estimate the 99% CL will improve the precision and accuracy of the FECRT.

Conflict of interest

The authors declare there is no conflict of interest.

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Table 1.

Recommendations to delay anthelmintic resistance	*Reference*
Annual rotation of unrelated anthelmintics	Prichard et al., 1980; Waller et al., 1989; Coles et al., 1992; Barnes et al., 1995.
Use of anthelmintics in combination	Dash, 1986; Anderson et al., 1989; Smith, 1990; Barnes et al., 1995; Dobson et al., 2001; Wolstenholme et al., 2004; Leathwick et al., 2009a.
Specific advice for anthelmintic or sheep classes	Leathwick et al., 1995; Dobson et al., 2001; Leathwick et al., 2009, 2009a.
Recommendation to delay pesticide resistance	*Reference*
Use of anthelmintics in combination	Comins, 1977, 1986; Mani, 1985; Roush, 1989.

Table 2. Summary examples of low-risk anthelmintic treatment[a] programs in key sheep farming areas of Australia

Zone	Class	Oct	Nov	Dec	Feb	Mar	Apr	May	Jun	Jul	Aug
WA[b]	Ewe						COM		MPL		
VIC	Ewe		COM		MPL					COM	
NSW	Ewe			MPL	MOX	COM	MPL				MOX
NSWr	Ewe			MPL	MOX		MPL				MOX
WA	Lamb	MPL		COM					MPL		
VIC	Lamb		COM		MPL			COM			
NSW	Lamb	COM		MPL	MOX	COM	MPL		MOX		
NSWr	Lamb	MOX		MPL	MOX	MPL	MOX				

[a] Treatments are scheduled to occur at various time points within the months; MPL = monepantel; COM = triple combination (BZ+LEV+ABA); MOX = moxidectin.
[b] WA = Western Australia; VIC = Victoria; NSW = northern New South Wales (low *Haemonchus* risk); NSWr = northern New South Wales (high *Haemonchus* risk).

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Table 3.
Comparison of diagnostic parameters for FECRT2 (efficacy from post-treatment counts of treated and control animals with 95% confidence interval (CI) estimated by RESO as per Coles et al. (1992) and FECRT3 (efficacy from pre- and post-treatment counts from the same animals using the 99% Jeffreys interval, see text). FECRT parameters were tested against efficacy based on worm count data, regarded as the 'gold standard' and drug resistance was defined as per Coles et al. (1992), ie, "*Resistance is present if (i) the percentage reduction in egg count is less than 95% and (ii) the 95% confidence level is less than 90%.*

Parameter	post/control FECRT2	post/pre FECRT3
[a]Sensitivity	88%	94%
[b]Specificity	100%	100%
[c]CI width	28%	21%
[d]Error rate	25%	15%

[a] The percent of drug resistant cases, based on worm count data, correctly diagnosed as resistant by FECRT2 and FECRT3.
[b] The percent of drug susceptible cases, based on worm count data, correctly diagnosed as susceptible by FECRT2 and FECRT3.
[c] The average width of the CI (precision).
[d] The percent of times the CI does not include the efficacy determined from worm count data (accuracy).

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Table 4.
Example cattle results from an efficacy study on one farm. Efficacy and 95% confidence limits (CL) estimated by Method-1 (post/control all animals with RESO CL), Method-2 (post/pre all animals with Jeffreys CL) and Method-3 (post/pre 5 highest animals with Jeffreys CL) (see Section 4.4). Also shown is: the number of animals per group; total eggs counted (not epg) pre- and post-treatment; percentage of *Ostertagia* and *Cooperia* pre- and post-treatment. Results in bold font were resistant for criteria (i) efficacy<95% or (ii) LCL<90% (see Section 4.4.1). Results in italic font highlight difference between the three methods.

All eggs		[a] IVM Inj	FBZ	LEV	IVM PO
Method-1	%Efficacy	100	96.5	**88.5**	99.3
RESO	95%UCL	100	99.1	95.9	99.9
CL.	95%LCL	*100*	**86.6**	**68.2**	93.4
Method-2	%Efficacy	100	97.3	**92.9**	99.6
Jeffreys	95%UCL	100	98.9	95.8	99.9
CL.	95%LCL	*96.2*	*93.2*	**88.3**	97.8
[b] Number of animals		18	17	17	20
Total eggs	Pre-treat.	94	147	184	248
counted.	Post-treat.	0	4	13	1
Method-3	%Efficacy	*99.1*	98.8	**94.1**	99.8
Jeffreys	95%UCL	*99.7*	99.5	95.9	100
CL.	95%LCL	*97.4*	*97.3*	*91.6*	99.0
[c] Number of animals		5	5	5	5
Total eggs	Pre-treat.	335	422	472	528
counted.	Post-treat.	3	5	28	1
[d]*Ostertagia*					
Method-1	%Efficacy	100	**93.6**	**79.1**	99.1
RESO	95%UCL	100	98.3	92.5	99.9
CL.	95%LCL	*100*	**75.7**	**42.2**	92.0
Method-2	%Efficacy	100	**90.3**	**55.8**	99.4
Jeffreys	95%UCL	100	96.0	72.1	99.9
CL.	95%LCL	*91.1*	**77.5**	**38.2**	96.0
Total eggs	Pre-treat.	39	41	29	122
counted.	Post-treat.	0	4	13	1
Method-3	%Efficacy	100	*95.8*	**62.9**	99.7
Jeffreys	95%UCL	100	98.1	73.0	99.9
CL.	95%LCL	*97.4*	*90.5*	**51.6**	98.1
Total eggs	Pre-treat.	137	118	76	259
counted.	Post-treat.	0	5	28	1
% *Ostertagia*	Pre-treat.	41%	28%	16%	49%
% *Ostertagia*	Post-treat.	0%	100%	100%	67%
[d]*Cooperia oncophora*					
Method-1	%Efficacy	100	100	100	99.3
RESO	95%UCL	100	100	100	99.9
CL.	95%LCL	*100*	*100*	*100*	94.2

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Method-2	%Efficacy	100	100	100	99.5
Jeffreys	95%UCL	100	100	100	99.9
CL.	95%LCL	*92.7*	*96.4*	*97.2*	94.0
Total eggs	Pre-treat.	48	98	129	69
counted.	Post-treat.	0	0	0	0
Method-3	%Efficacy	*98.2*	100	100	99.8
Jeffreys	95%UCL	*99.4*	100	100	100
CL.	95%LCL	*95.0*	*98.7*	*98.9*	97.1
Total eggs	Pre-treat.	171	283	330	148
counted.	Post-treat.	3	0	0	0
%*Cooperia*	Pre-treat.	51%	67%	70%	28%
%*Cooperia*	Post-treat.	100%	0%	0%	33%

[a] Anthelmintics used: IVM Inj = ivermectin subcutaneous injectable 10 mg/mL; FBZ = fenbendazole 100 mg/L); LEV = levamisole hydrochloride 80 gm/L; IVM PO- = ivermectin pour-on 5 mg/mL. Administered doses were as per label recommendations for each anthelmintic.

[b] The same number of animals shown per anthelmintic treatment group applies to each nematode species and Methods-1 and 2.

[c] For Method-3 there were five animals per group for all groups and nematode species.

[d] Total eggs counted for individual worm species were estimated from the % L3 recovered in larval cultures.

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Table 5.
Individual horse pre-treatment epg and the number eggs counted pre- and post-treatment (detection factor 12.5 epg). Efficacy and lower confidence limits (LCL) were determined for each farm and drug. For RESO (Coles et al., 1992) the 95%LCL was estimated, for Jeffreys interval (see text) the 95% and 99%LCL were estimated.

						Lower Confidence Limit		
	Pre-treatment		Drug	Post-treatment		Jeffreys	Jeffreys	RESO
[a] Farm	epg	eggs	[b] used	eggs	efficacy	95%	99%	95%
1	1250	100	ABA	0				
1	2375	190	ABA	0				
1	2410	193	ABA	0	100	99.7	99.5	100
1	2630	210	ABA	0				
1	2013	161	ABA	0				
1	2575	206	ABA	0				
2	525	42	MOX	0	100	91.8	88.4	[c] NA
3	1150	92	MOX	0				
3	488	39	MOX	0				
3	400	32	MOX	0	100	98.8	98.3	100
3	1350	108	MOX	0				
3	500	40	MOX	0				
3	875	70	IVM	0	100	94.9	92.8	NA
4	50	4	ABA	0				
4	2600	208	ABA	0	100	98.4	97.7	100
4	225	18	ABA	0				
5	438	35	OX+PY	0				
5	538	43	OX+PY	1	98.7	93.1	91.0	89
6	450	36	IVM	0				
6	275	22	IVM	0	100	93.9	91.4	100
7	1438	115	OX+PY	0	100	96.9	95.5	NA
7	0	0	OX+PY	0				
7	0	0	OX+PY	0				

[a] Farms 1-3 have over 30 horses, farms 4-7 have less than four horses.
[b] Anthelmintics used: ABA = abamectin; MOX = moxidectin; OX+PY = Oxfendazole plus pyrantel; IVM = ivermectin.
[c] NA indicates RESO LCL unable to be estimated because only one animal has a positive count.

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Table 6.
Comparison of precision and error rate for various methods of estimating efficacy under different conditions and detection sensitivities. Mean width of the confidence interval (CI) (precision) and mean error rate (percentage of results where the true efficacy was not enclose by the CI) for 10,000 Monte Carlo iterations of each scenario. In each scenario true efficacy was 95%. Method-1 was separate control and treated groups both counted post-treatment. Method-2 was pre- and post-treatment counts of the same animals. Method-3 required three additional counts pre- and post-treatment at the prescribed detection level for two to five animals with the highest nematode egg counts. For RESO (Coles et al., 1992) the 95%CI was estimated, for Jeffreys interval (see text) the 95% and 99%CI were estimated.

[a] **k for NBD 0.5**		[b] Low Sensitivity 250/50		High Sensitivity 300/25	
Method-	n animals	CI width %	Error %	CI width %	Error %
1 RESO 95[c]	10/group	32	16.5	25	7.5
2 RESO 95	10 post/pre	29	13.5	21	2.2
2 Jeffreys 95[c]	10 post/pre	14	5.7	9	6.6
3 Jeffreys 95	2 highest	10	5.4	7	5.8
3 Jeffreys 95	3 highest	9	5.5	6	6.0
3 Jeffreys 95	4 highest	9	5.8	5	6.0
3 Jeffreys 95	5 highest	8	6.1	5	6.0
2 Jeffreys 99[d]	10 post/pre	19	1.5	12	1.5
3 Jeffreys 99	2 highest	14	1.4	9	1.4
3 Jeffreys 99	3 highest	12	1.3	8	1.4
3 Jeffreys 99	4 highest	11	1.4	7	1.5
3 Jeffreys 99	5 highest	11	1.5	7	1.4
[a] **k for NBD 2.0**					
1 RESO 95[c]	10/group	22	12.5	14	4.0
2 RESO 95	10 post/pre	21	11.2	13	1.4
2 Jeffreys 95[c]	10 post/pre	13	5.8	8	6.6
3 Jeffreys 95	2 highest	12	4.6	7	5.7
3 Jeffreys 95	3 highest	10	5.0	6	5.6
3 Jeffreys 95	4 highest	9	5.4	6	5.8
3 Jeffreys 95	5 highest	8	5.6	5	5.9
2 Jeffreys 99[d]	10 post/pre	18	1.4	11	1.4
3 Jeffreys 99	2 highest	16	0.9	10	1.3
3 Jeffreys 99	3 highest	13	1.1	8	1.3
3 Jeffreys 99	4 highest	12	1.0	8	1.3
3 Jeffreys 99	5 highest	11	1.1	7	1.3

[a] k for the Negative Binomial distribution (NBD) was set at 0.5 or 2.
[b] Assay sensitivity was either low by setting mean eggs/g faeces (epg) at 250 with a detection level of 50 or high by setting mean epg at 300 with a detection level of 25.
[c] 95 indicates results shown are for 95%CI.
[d] 99 indicates results shown are for 99%CI.

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Figure 1.

Estimated 95% and 99% lower confidence limits (LCL) for a FECRT based on: **n** the total number of eggs counted pre-treatment, **x** the total eggs observed post-treatment and efficacy of 100% (i.e. **x**=0). In Excel the 95%LCL was determined by the function: 95%LCL=100*(1-(BETAINV(0.975,**x**+1,**n**-**x**+1))), to estimate the 99%LCL 0.995 replaces 0.975 in the inverse beta function (BETAINV) above. Note if less than a total of 30 eggs were observed pre-treatment then the efficacy estimate was unreliable as the LCL was below 90% and rapidly declines as **n** declines. Upper confidence limits (UCL) are 100% and not shown.

Figure 2.

Estimated 95% and 99% upper (UCL) and lower (LCL) confidence limits for a FECRT based on: **n** the total number of eggs counted pre-treatment, **x** the total eggs observed post-treatment for an efficacy of 90% (**x**=**n**/10) or 95%(**x**=**n**/20). In Excel the LCL was estimated as shown in Figure 1, the 95%UCL was determined by the function: 95%UCL=100*(1-(BETAINV(0.025,**x**+1,**n**-**x**+1))), to estimate the 99%UCL 0.005 replaces 0.025 in the inverse beta function (BETAINV).

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Figure 3.

Distribution of 10,000 Monte Carlo efficacy results for three FECRT estimation methods. In this simulation: k=0.5 for the NBD, efficacy was 95%, ten animals per group, a high sensitivity FECRT with mean epg 300 and a detection factor of 25. Method-1 was separate control and treated groups counted post-treatment. Method-2 was pre- and post-treatment counts from the same animals. Method-3 was pre- and post-treatment counts from the two animals with the highest counts, counted at a detection level of 6 epg. Note the bar at 81% efficacy includes all results equal to or less than 81%.

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Figure 1.

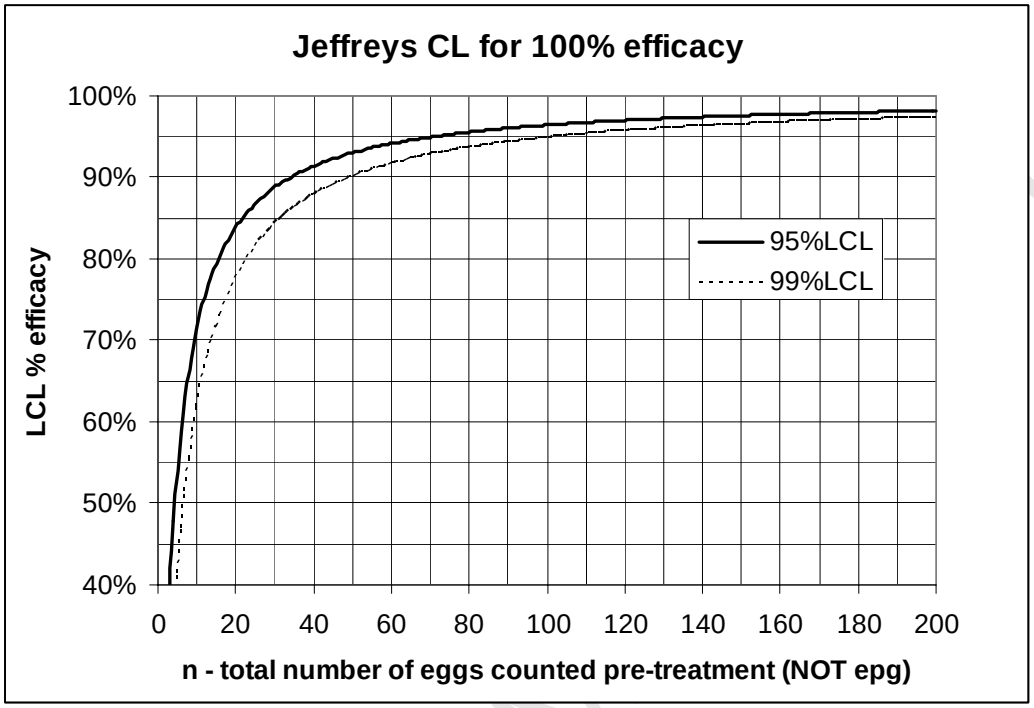

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Figure 2.

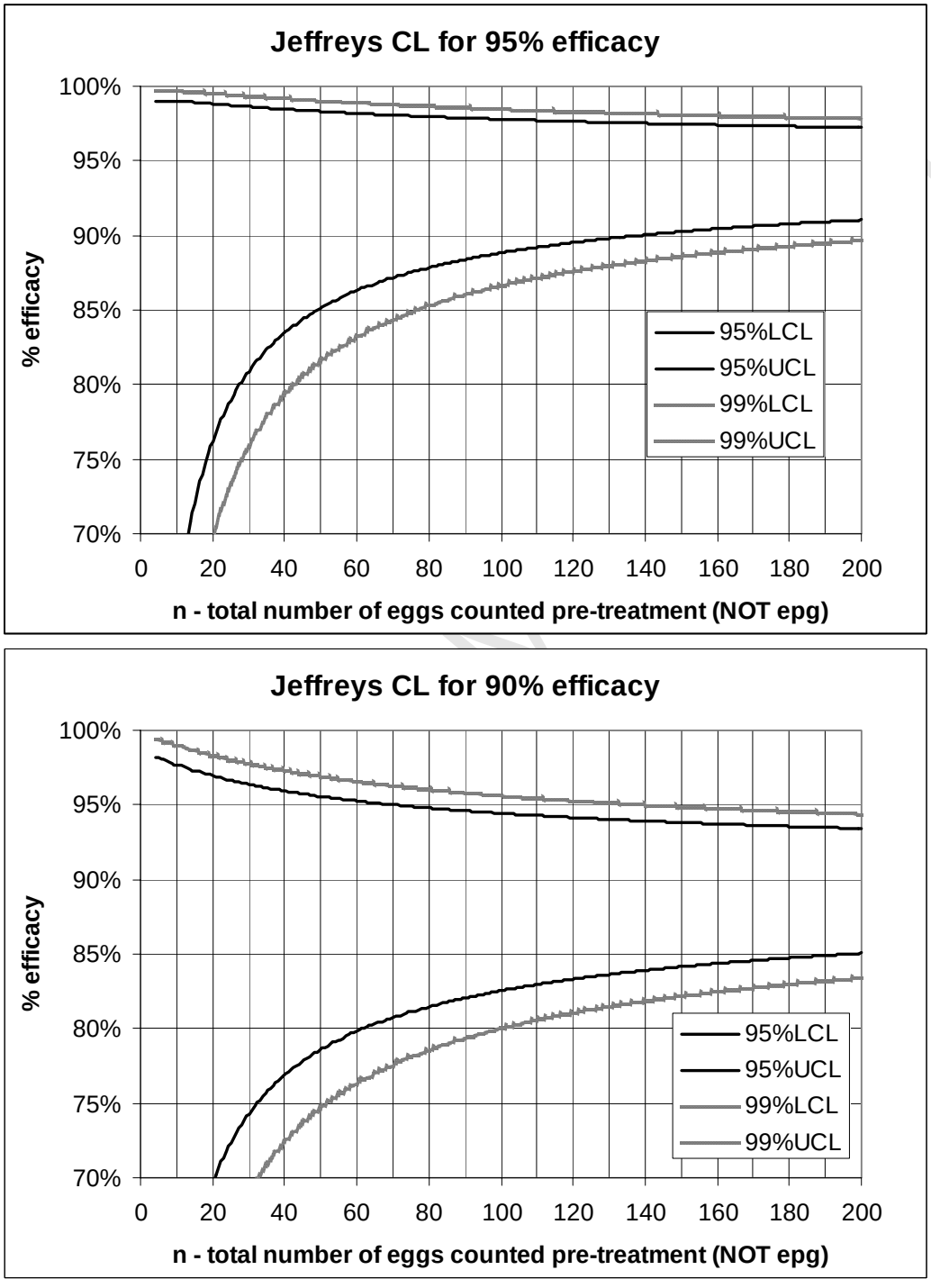

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Figure 3.