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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, provide refugia and the use of a simulation model were reviewed 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 $100 \times (1 - p)$ (assuming equal treatment group sizes and detection levels, pre- and post-treatment). The LCL is approximated from the cumulative inverse beta distribution by: $95\%\text{LCL} = 100 \times (1 - (\text{BETAINV}(0.975, x+1, n-x+1)))$. This method is simpler than the current method, independent of the number of animals 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:

$95\%\text{UCL} = 100 \times (1 ((\text{BETAINV}(0.025, x+1, n-x+1)))$, 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.

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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 methods for detecting resistance to indicate whether improvements can be made.

Simulations by Dobson et al. (2011a,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 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 et al. (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 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.

Table 1

	Reference
Recommendations to delay anthelmintic resistance	
Annual rotation of unrelated anthelmintics	Prichard et al. (1980), Waller et al. (1989), Coles and Roush (1992), Barnes et al. (1995).
Use of anthelmintics in combination	Dash (1986), Anderson et al. (1988), Smith (1990), Barnes et al. (1995), Dobson et al. (2001), Wolstenholme et al. (2004), Leathwick et al. (2009).
Specific advice for anthelmintic or sheep classes	Leathwick et al. (1995), Dobson et al. (2001), Leathwick et al. (2009), Leathwick and Hosking (2009).
Recommendation to delay pesticide resistance	
Use of pesticides in combination	Comins (1977, 1986), Mani (1985), Roush (1989).

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 and Dobson, 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 and Bath, 2002). On four farms in southern Italy, Cringoli et al. (2009) left 40–60% of dairy sheep untreated 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,