Managing anthelmintic resistance – Parasite fitness, drug use strategy and the potential for reversion towards susceptibility

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ABSTRACT

The rotation of different anthelmintic classes, on an approximately annual basis, has been widely promoted and adopted as a strategy to delay the development of anthelmintic resistance in nematode parasites. Part of the rationale for recommending this practice was the expectation that resistant genotype worms have a lower ecological fitness than susceptible worms, at least in the early stages of selection, and so reversion towards susceptibility could be expected in those years when an alternative class of anthelmintic was used.

The routine use of combination anthelmintics might be expected to negate this opportunity for reversion because multiple classes of anthelmintic would be used simultaneously. A simulation model was used to investigate whether the optimal strategy for use of multiple drug classes (i.e. an annual rotation of two classes of anthelmintic or continuous use of two classes in combination) changed with the size of the fitness cost associated with resistance.

Model simulations were run in which the fitness cost associated with each resistance gene was varied from 0% to 15% and the rate at which resistance developed was compared for each of the drug-use strategies. Other factors evaluated were the initial frequency of the resistance genes and the proportion of the population not exposed to treatment (i.e. in refugia).

Increasing the proportion of the population in refugia always slowed the development of resistance, as did using combinations in preference to an annual rotation. As the fitness cost associated with resistance increased, resistance developed more slowly and this was more pronounced when a combination was used compared to a rotation. If the fitness cost was sufficiently high then resistance did not develop (i.e. the resistance gene frequency declined over time) and this occurred at lower fitness costs when a combination was used. The results, therefore, indicate that the optimal drug-use strategy to maximise the benefit of any fitness cost associated with resistance is the use of combinations of different anthelmintic classes.

Manual calculations confirmed that, within the model, the only resistant genotypes capable of surviving treatment with a combination are those carrying multiple resistance genes. These individuals are less fit, resulting in the worm population surviving treatment having a lower overall ecological fitness. This is a previously unreported perspective on the use of combination anthelmintics and strengthens the argument that any new class of anthelmintic, for which resistance genes can be expected to be rare, should be brought to market in combination.

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1. Introduction

In many countries anthelmintic resistance is a significant problem amongst nematode parasites of goats
and sheep (Besier, 2007) and is of growing concern amongst parasites of cattle (Sutherland and Leathwick, 2011) and horses (Kaplan, 2004). Considerable effort has been invested in understanding the processes involved in the selection of anthelmintic resistance (Barnes et al., 1995; Leathwick et al., 2009) so that control strategies can be developed which minimise both the impact of parasites on animal health and welfare and the development of resistance.

Because anthelmintics are an integral component of most worm control programmes, much attention has been given to optimising their use, both in terms of parasite control (Vlassoff and Brusdon, 1981; Barger, 1999) and the management of resistance (Barnes et al., 1995; Leathwick and Hosking, 2009; Dobson et al., 2011). For many years recommendations promoted the use of strategic timing of treatments (Vlassoff and Brusdon, 1981; Barger, 1999) combined with rotation of different anthelmintic classes on an approximately annual basis (Donald et al., 1980; Prichard et al., 1980; Waller et al., 1989; Coles and Roush, 1992). A perceived benefit of a slow rotation of drugs from different classes was that by not exposing individual worms to more than one class of drug, selection for resistance to multiple actives was less likely to occur (Prichard et al., 1980). In addition, if resistant genotypes have a reduced ecological fitness compared to susceptible genotypes, then some reversion towards susceptibility would be expected in those periods when an alternate drug class was used (Prichard et al., 1980; Roush and McKenzie, 1987; Coles and Roush, 1992). The annual rotation of different anthelmintic classes was widely promoted, and the practice was adopted by many farmers, in New Zealand (Kettle et al., 1983; Lawrence et al., 2007) and in other countries (Waller et al., 1989; Sargison et al., 2007; McMahon et al., 2013).

Recently there has been increased interest in the use of combinations of different anthelmintic classes, both as a means of maintaining control in the presence of resistant nematodes (Leathwick et al., 2009), and as a means of slowing the development of resistance (Smith, 1990; Barnes et al., 1995; Dobson et al., 2011; Leathwick, 2012). Combination products are readily available and used extensively in some countries, but not in others (Bartram et al., 2012). A number of modelling studies have indicated that combining anthelmintics from different classes, especially where these still have high efficacy, is likely to result in a significant delaying of resistance development (Dobson et al., 2011; Leathwick, 2012; Learmount et al., 2012). However, use of combinations will naturally reduce the opportunity for rotation, potentially interfering with any reversion towards susceptibility, in the presence of a fitness cost associated with resistance. To date, no modelling studies have fully investigated the interactions between drug-use strategies (i.e., rotation of single active products versus use of multiple actives in combination) and any potential reductions in fitness associated with resistance genes in nematodes.

The purpose of this study was to address this issue by investigating the potential influence of resistance-associated fitness costs, of varying magnitudes, on the best strategy for use of more than one anthelmintic class.

2. Methods

A previously developed simulation model was used to investigate the influence of three variables and two drug-use strategies on the rate at which anthelmintic resistance developed. The variables investigated were the magnitude of the fitness cost associated with resistance, the initial frequency of resistance genes in the population, and the proportion of the worm population which escaped exposure to each treatment (i.e. those in refugia). The impact of these variables under either an annual rotation of two drugs or continuous use of a combination of two drugs was assessed.

2.1. The model

The model has been described previously (Leathwick et al., 1992, 1995) and used to investigate both parasite dynamics and the development of resistance under different management practices (Leathwick and Sutherland, 2002; Leathwick et al., 2008; Leathwick and Hosking, 2009; Leathwick, 2012). Briefly, the model is generic in that it simulates a mixed nematode infection, rather than infection by individual species (Leathwick et al., 1992), and incorporates both lamb and adult ewe flocks. The development and survival of free-living nematode stages on pasture is described for each of a suite of paddocks. When a paddock is being grazed by ewes or lambs it receives inputs of nematode eggs, based on the faecal egg output of an individual multiplied by the stocking rate, and infective stage larvae are removed with ingested herbage. The number of ingested larvae determines subsequent worm burden and faecal nematode egg count (FEC) under the influence of anti-parasite immunity which develops in response to both ingestion of infective larvae and the presence of adult worms.

Importantly, outputs from this model have previously been evaluated and found to be consistent with the results of large-scale field trials with respect to both the development of anthelmintic resistance (Leathwick et al., 2006, 2012) and nematode epidemiology (Leathwick et al., 2008).

2.2. Genetics of resistance

For the purpose of this study the model was used to consider two anthelmintic drugs which had independent modes of action and mechanisms of resistance, and for which the anthelmintic effect was additive (Bartram et al., 2012). Resistance to each drug was assumed to be the result of a single mutation resulting in three genotypes, a homozygous susceptible (SS), a heterozygote (RS) and a homozygous resistant (RR), giving a total of nine genotypes overall (Table 1). It was assumed that resistance was partially recessive under treatment with either drug, leading in each case to mortalities under treatment of 99.9, 50.0 and 5.0% for the SS, RS and RR genotypes, respectively. The initial frequency of resistance genes (q) was varied across sets of simulations in order to assess the effect of this variable on model outcomes.
2.3. Fitness costs

It was assumed that each R gene carried a fitness disadvantage of 0, 1, 2, 5, 10 or 15% compared to the SS genotype, and that the disadvantages were additive as the number of R genes increased (Table 1). This was implemented as a reduction in the fecundity of adult female worms. For example, if the fitness disadvantage was 5%, then an SS SR genotype worm would produce 5% fewer eggs than an SS SS genotype worm, while an SS RR genotype worm would produce 10% fewer. Thus, the more resistance genes an individual carried, the greater its ability to survive anthelmintic treatment but the lower its fitness in the absence of anthelmintic treatment. The complete array of genotype fitness values used is presented in Table 1.

2.4. Management and refugia

A management scenario typical of sheep farming in New Zealand was used as the basis for all simulations. Ewes and their lambs were set-stocked over all paddocks from pre-lambing until weaning. Lambing occurred in August, weaning in November when lambs were 12 weeks of age, and lamb numbers were progressively reduced thereafter consistent with a farmer selling a proportion of his lambs to slaughter as they reached target weights. Ewes were not treated with anthelmintic at any time. Commencing at weaning, all lambs received a programme of six preventive treatments at 28 day intervals (Vlassoff and Brunsdon, 1981) which is a widely used practice on New Zealand sheep farms (Lawrence et al., 2007). After weaning lambs were rotationally grazed around all paddocks and were followed by a mob of ewes.

In order to simulate different levels of refugia without significantly altering the grazing management, the FEC of the ewes was varied in the model both before and after weaning. Thus three refugia scenarios were created by altering the size of the post-parturient rise in ewe FEC from a maximum value of 250 epg (low refugia) to 1000 epg (medium refugia) and 1750 epg (high refugia). Concurrently, ewe faecal egg count post-weaning was set to constant values of 25 epg (low refugia), 100 epg (medium refugia) and 175 epg (high refugia). This effectively varied the inputs of nematode eggs which had not been exposed to anthelmintic treatment without altering any other parameters which may have affected outputs. The parasitological consequences of these manipulations on model output are outlined in Fig. 1.

2.5. Simulations and outputs

Initially, simulations were run which compared all the assumed fitness costs (i.e. 0, 1, 2, 5, 10 and 15%) together with the three levels of refugia (low, medium and high) at each of three initial gene frequencies (0.01, 0.05 and 0.1). These comparisons were repeated for the two drug-use strategies i.e. an annual rotation of two drugs or the continuous use of two drugs in combination. In order to simplify the presentation of outputs all variables associated with the two anthelmintic actives were set to the same values for any given simulation. For example, if the starting resistance gene frequency for drug 1 was set at 0.1 then the same value was used for drug 2. In all cases the output used to compare simulations was the resistant gene frequency for drug 1 after the model was run for 40 years (i.e. only the q-value for drug 1 in year 40 is presented).

Subsequently, in order to more fully explore the influence of the initial q-values on model output, a second series of simulations was run in which only the medium refugia scenario was used. For these simulations the final q-value for drug 1 was plotted against the initial q-value and a range of assumed fitness costs were compared. Because the parameter values were set the same for both drugs, the resulting final q-values for drugs 1 and 2 were identical for the combination and very similar for the rotation. Because drug 1 was always used in year 1 of the annual rotation it was appropriate to compare this with the combination after 40 years – effectively drug 2 had only been exposed to selection within the rotation for 39 years.

2.6. Calculation of average post-treatment fitness

In order to better understand the mechanism responsible for some of the models outputs, the average fitness of the worm population surviving different drench treatments was calculated manually, as:

\[
\text{y} = \left( \sum_{i=1}^{9} F_i \cdot f_i \cdot s_i \right) / \left( \sum_{i=1}^{9} f_i \cdot s_i \right)
\]

where \(F_i\) = the relative fitness of genotype \(i\), \(f_i\) = the frequency of genotype \(i\) at the time of anthelmintic treatment assuming Hardy–Weinberg equilibrium, and
Fig. 1. Model outputs for faecal nematode egg count of lambs (a) and adult ewes (b) and number of infective stage larvae on herbage (c) when egg outputs of the ewes were varied (see text) to create high, medium and low refugia scenarios.
Table 2

The calculated average fitness of those worms surviving treatment with either a single active anthelmintic or a combination of two actives, when the initial q-value is 0.1, the fitness cost associated with a resistance gene is either 0, 1, 2, 5 or 10%, and the RS genotype is either equal in fitness to the SS (SS > RS > RR), the RR (SS > RS = RR), or intermediate between the two (SS > RS > RR).

<table>
<thead>
<tr>
<th>Initial R gene frequency</th>
<th>Fitness cost (%)</th>
<th>Mean fitness relative to SS SS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Single drug</td>
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<tr>
<td>SS &gt; RS &gt; RR</td>
<td></td>
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</tr>
<tr>
<td>0.1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>0.1</td>
<td>1</td>
<td>0.9878</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>0.9757</td>
</tr>
<tr>
<td>0.1</td>
<td>5</td>
<td>0.9393</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>0.8787</td>
</tr>
<tr>
<td>SS &gt; RS = RR</td>
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<td>0.1</td>
<td>0</td>
<td>1</td>
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<tr>
<td>0.1</td>
<td>1</td>
<td>0.9889</td>
</tr>
<tr>
<td>0.1</td>
<td>2</td>
<td>0.9777</td>
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<tr>
<td>0.1</td>
<td>5</td>
<td>0.9442</td>
</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>0.8885</td>
</tr>
<tr>
<td>SS &gt; RS ≥ RR</td>
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</tr>
<tr>
<td>0.1</td>
<td>10</td>
<td>0.9902</td>
</tr>
</tbody>
</table>

$s_i$ is the proportion of genotype $i$ individuals surviving the anthelmintic treatment. This was set up in a spread sheet format (Microsoft Excel) so that for any defined q-value and fitness cost the proportion of individuals of each genotype surviving a treatment (with both a single active and a combination), and the average fitness of the population surviving treatment, was calculated. By changing two numbers in the spread sheet (i.e. the initial q-value and the fitness cost), the calculation corresponding to any of the model simulations could be reproduced. Examples of output are presented in Table 2.

2.7. Fitness cost in relation to genotype

There are several different ways in which a fitness cost could potentially be linked to resistant genotypes. Here, it has been assumed that each resistance gene carries a fitness cost and that these are additive (i.e. SS is more fit than RS which is more fit than RR; represented as SS > RS > RR). This might reasonably approximate the situation where resistance is intermediate in dominance under treatment and the SS, RS and RR genotypes are all phenotypically different in the presence of drug. Another possibility is that the SS and RS are equal in terms of ecological fitness, which would be consistent with the resistance gene being completely recessive i.e. the resistance mechanism is not expressed in the heterozygote. Alternatively, the RS and RR may be equal, consistent with the resistance mechanism being fully dominant. These options can be represented as SS = RS > RR or SS > RS = RR. To ensure that these alternative possibilities would not negate the effects seen in model output, manual calculations of average post-treatment fitness were carried out for all three options, under a range of initial q-values and fitness costs.

3. Results

Increasing FEC of the ewes slowed the development of resistance regardless of the fitness cost of resistance, the initial q-value or the drug-use strategy (Fig. 2). Thus, in all cases the larger the proportion of the adult, reproductive, worm population which was not exposed to treatment (i.e. those in refugia) the slower the development of resistance.

Furthermore, resistance always developed more slowly when a combination was used compared to an annual rotation (Figs. 2 and 3) and this occurred regardless of any fitness cost associated with resistance. If resistant genotypes were less fit than susceptibles then resistance developed more slowly, and the greater the fitness cost the more resistance development was slowed (Figs. 2 and 3). Indeed if the fitness cost was sufficiently large then resistance did not develop at all, with the q-value declining over time despite routine anthelmintic treatments (Figs. 2 and 3). This occurred under both drug-use strategies, although it was more pronounced when a combination was used than under an annual rotation i.e. when a combination was used resistance failed to develop if the fitness cost was 10% or 15% and in some simulations when the fitness cost was 5% (Figs. 2 and 3). If the fitness cost for each resistance gene was 5%, resistance only developed under use of a combination if the initial q-value was greater than about 0.17. In contrast, under an annual rotation, resistance always developed unless the fitness cost per resistance gene was 15% and there was at least a medium level of refugia i.e. under low refugia resistance always developed even when the fitness cost per resistance gene was 15%.

Manual calculations were used to elucidate the mechanism underpinning the difference between the drug-use strategies. These calculations indicated that, except for the case where there was no fitness cost associated with resistance, the average fitness of those parasites surviving treatment with a single active was always higher than that following use of a combination (some examples are presented in Table 2). This reflects the greater efficacy of the combination against genotypes carrying low numbers of resistance genes, and which were therefore, more fit. For example, using the values outlined above, efficacy of a single active against an SS SR genotype would be 50% for one of the drugs, but the combination would remove 99.95% of these individuals every treatment. These calculations demonstrate that the combination would not only leave fewer resistant individuals surviving treatment but these would, on average, be less fit than the resistant individuals surviving treatment with a single drug.

What is more, this outcome occurred regardless of whether the RS genotype was equal in fitness to the SS (i.e. SS = RS > RR), was equal in fitness to the RR (i.e. SS > RS = RR) or was intermediate in fitness (i.e. SS > RS > RR) (Table 2).

Although the magnitude of differences varied between these scenarios (Table 2), in all cases the average fitness of those worms which survived treatment remained lower after use of a combination than a single active, indicating that the overall results of the modelling are unlikely to be influenced by the assumption of additive fitness costs.
4. Discussion

Numerous modelling studies have indicated that in most situations the development of anthelmintic resistance will be delayed by the use of combinations compared to any single drug-use strategy (Smith, 1990; Barnes et al., 1995; Dobson et al., 2011; Leathwick, 2012; Learmount et al., 2012) and recently this conclusion has been validated in the field (Leathwick et al., 2012). The mechanism by which combinations are expected to delay the development of resistance involves an increased efficacy against resistant genotypes, principally those only carrying genes for resistance to one of the actives in the combination. The result is fewer resistant survivors of treatment and a consequently greater dilution of those survivors by susceptible genotypes in ‘refugia’ (Smith, 1990).

However, none of these earlier modelling studies fully investigated the potential influence of different fitness costs associated with resistance mechanisms on the development of resistance under different drug use strategies. The results of the current study suggest that if there is a fitness cost associated with resistance the use of combinations is likely to be even more advantageous, compared to an annual rotation, than when there is no fitness cost.

The basis for this is that where each resistance mechanism (i.e. to each anthelmintic class) has an associated fitness cost, the use of combinations is likely to reduce the average fitness of those worms surviving treatment,
because the only individuals which can survive are those which possess multiple resistance genes and are therefore the least fit. This is a previously unreported perspective on the use of combination anthelmintics.

A comprehensive review of selection for anthelmintic resistance and the relationship with ecological fitness of resistant genotypes can be found in Prichard (1990). Essentially, experiments to demonstrate a fitness cost associated with anthelmintic resistance have provided variable and sometimes contradictory results (Donald et al., 1980; Maclean et al., 1987; Waller et al., 1989). However, it is difficult to interpret these findings as in many cases the parasites compared have been isolated independently from the field and so may differ in aspects of their epidemiology as a result of adaptation to host and microclimatic factors (Kelly et al., 1978). Despite this, it is reasonable to assume that when an anthelmintic is first introduced, resistance genes, should they exist in a population, will be rare or else the anthelmintic would not be effective. Individuals carrying these genes are unlikely to be more fit, in the absence of anthelmintic selection, than individuals carrying equivalent genes for susceptibility or else resistant genes would be more common in the population, and their rarity therefore suggests some degree of fitness disadvantage (Martin, 1987; Roush and McKenzie, 1987; Prichard, 1990). It has been proposed that in a fully susceptible (naïve) population susceptible genotypes have a fitness advantage over those carrying resistance mechanisms (Martin et al., 1988; Prichard, 1990). Following the introduction of a new anthelmintic class the resistant genotypes are selected for, but, in the absence of the drug, they retain a lower biological fitness. However, as selection continues the resistance genes re-associate (co-adapt) with other fitness characteristics and so the fitness differential between resistant and susceptible genotypes becomes smaller over time. Hence, once the resistance genes have become common in the population, and resistance is detectable based on efficacy of treatment, then any fitness disadvantage may be reduced or negligible (Martin et al., 1988; Prichard, 1990). This very reasonable hypothesis would be consistent with the initial rarity of resistance genes but the variability of demonstrating a fitness cost in resistant isolates (Donald et al., 1980; Waller et al., 1989).

Support for this concept was given by several studies (Kelly et al., 1978; Maingi et al., 1990) in which strains of Haemonchus contortus showing moderate levels of resistance to benzimidazole anthelmintics were subjected to further selection in the laboratory. In both cases, further selection resulted not only in increasing levels of resistance, but also to increasing ecological fitness, indicating that fitness of the parasites can indeed increase with ongoing selection for resistance (Kelly et al., 1978; Maingi et al., 1990). While, it is likely that this co-adaptation of resistance and fitness traits occurred more rapidly under laboratory selection than is likely in the field, because of the very low refugia of susceptibility (i.e. the opportunity for resistance and fitness genes to align was high) these studies do demonstrate that co-adaptation can occur. It also follows that, in the field, refugia will be important in the rate of co-adaptation of resistance and fitness-modifying genes, in that co-adaptation is likely to occur more rapidly under low-refugia conditions. This again emphasises the importance of maintaining a sizeable pool of unselected genotypes in order to slow the development of resistance.

A fitness cost associated with resistance is therefore a reasonable assumption, even if it only occurs in the early stages of selection (i.e. when resistance genes are still rare) (Martin et al., 1988). In some studies, the magnitude of the fitness costs associated with resistance has been quite large. For example, a benzimidazole resistant isolate of Trichostrongylus colubriformis showed a 49% reduction in establishment of infective larvae in gerbils, compared to a susceptible isolate (Maclean et al., 1987), while a benzimidazole resistant H. contortus showed a 44% reduction in establishment rate and a 45% lower egg viability than a susceptible isolate (Maingi et al., 1990). In another study, the proportion of eggs from a resistant isolate which developed into infective stage larvae was fewer than half the number from a susceptible isolate (Scott and Armour, 1991). It must be recognised, however, that while some of these differences are large, they are differences in just one
variable. Many variables contribute to the overall fitness of an organism, which is best represented as the Basic Reproductive Rate i.e. the number of eggs produced by a single female parasite that themselves survive to reproduce in the absence of density-dependence (Anderson and May, 1982; Maingi et al., 1990). Hence, extrapolating from differences in a single variable to an overall fitness cost may be misleading. However, given that some of these measured differences are considerably larger than the fitness costs assumed in the current study, the values used here are probably not unreasonable.

If each resistance mechanism has an associated fitness cost, then worms carrying multiple resistance mechanisms are likely to be even less fit. In the current study, all simulations assumed that the fitness costs associated with being simultaneously resistant to multiple anthelmintics are additive. In all cases the model outputs indicate that reduced fitness associated with resistance mechanisms is likely to slow the development of resistance, and in some cases may prevent resistance from developing at all. This will undoubtedly be a balance between the magnitude of the fitness cost, and the intensity of the selection pressure for resistance. This can be seen in the model outputs where the development of resistance is slowed by both the fitness cost of being resistant and the proportion of the population which is not exposed to treatment (which influences selection pressure) (Fig. 2).

The issue of drug-use strategy has risen in importance with the recent release of two new classes of anthelmintics for use in sheep. One of these new actives, monepantel, has been released as a single active product (Kaminsky et al., 2008) while the other, derquantel, is only available in combination with abamectin (Little et al., 2011). Presumably, genes for resistance to both these new actives are currently rare and so if there is any fitness cost associated with resistance to them it will be at its maximum prior to any significant selection. Clearly, the optimum time to take advantage of any such cost is immediately after a new active comes onto the market and before resistance has the opportunity to develop. The results of the current study indicate that the optimum approach to using any new actives sustainably is to formulate them as combinations. Not only are combinations likely to minimise the survival of resistant genotypes thereby slowing the development of resistance (Barnes et al., 1995; Leathwick, 2012; Learmount et al., 2012) but, based on the current analysis, the use of combinations will also maximise the benefit of any fitness disadvantage associated with resistance.

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References


