

*Feature Review Series - Control of Nematodes in Sheep***Anthelmintic resistance in New Zealand****DM Leathwick*§, WE Pomroy† and ACG Heath‡****Abstract**

Anthelmintic resistance was first confirmed in New Zealand in 1979 and since then has become common-place; more than 50% of sheep farms now have detectable levels of resistance to one or more chemical classes of anthelmintic. Farmer drenching practices have changed little over the last 15–20 years and are clearly exerting a significant level of selection for resistance. In the absence of new chemical classes of anthelmintics, current parasite control practices will be unsustainable in the long-term. Once substantial resistance has developed, significant reversion to susceptibility is unlikely and re-introduction of failed drugs is likely to result in the rapid re-emergence of control problems. The number of anthelmintic treatments applied is not necessarily a reliable indicator of selection pressure and should not be the only factor considered in strategies for minimising the development of resistance. The relative potential of the different anthelmintics now available, particularly the long-acting products, to select for resistance varies with the way they are used and with other epidemiological and management factors; generalisations about their respective roles in the development of resistance are often unreliable. In many cases, literal extrapolation of recommendations for the management of resistance from Australia to New Zealand is unsupportable, given the differences in climate, parasite ecology and farming practices between the 2 countries. In the absence of a refuge for susceptible genotypes, as occurs when anthelmintic treatments are used as a means of generating low-contamination 'safe' pasture for young stock, the rapid development of resistance is likely. Anthelmintic treatments applied to animals with a high level of immunity, or which become immune while the anthelmintic is active, are likely to select for resistance faster than treatments applied to non-immune stock.

KEY WORDS: *Sheep, nematode parasites, anthelmintic resistance, drench, genetics*

Key points

- The management of anthelmintic resistance remains difficult; much is unknown, but knowledge is increasing.
- Widespread resistance to all existing broad-spectrum anthelmintic chemical classes is an inevitable consequence of current drenching practices.
- Once substantial resistance has developed, significant reversion to susceptibility is unlikely and rapid re-emergence of resistance should be expected if a failed drug is re-introduced.
- The number of anthelmintic treatments applied is not necessarily a reliable indicator of selection pressure and should not be the only factor considered in strategies for minimising the development of resistance.
- Literal extrapolation from Australia to New Zealand of some recommendations for the management of resistance is unsupportable given the differences in climate, parasite ecology and farming practices.
- The potential of persistent anthelmintics to select for resistance varies and generalisations about their effect should be interpreted with caution.
- Using drenching as a means to generate low contamination 'safe' pasture for lambs is likely to result in the rapid development of resistance.
- Anthelmintic treatments applied to animals with a high level of immunity, or which become immune while the anthelmintic is active, are likely to select for resistance faster than treatments applied to non-immune stock.

Introduction

Anthelmintic resistance has been the subject of numerous reviews, which have considered the subject from many perspectives (Waller et al 1995; Waller 1997; Sangster 1999). This review focuses on the situation in sheep in New Zealand and on issues and aspects relevant to the New Zealand veterinary practitioner or farmer.

Where data are conflicting or recommendations have been made for another country, an attempt is made to interpret these from a New Zealand perspective and to present factual information that can be used to guide New Zealand farmers and practitioners in making informed practical decisions.

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|----------------|---------------------------------|
| CRC | Controlled release capsule |
| FEC | Faecal nematode egg count |
| L ₃ | Infective third-stage larvae |
| RR | Homozygous resistant genotype |
| RS | Heterozygote genotype |
| SS | Homozygous susceptible genotype |

Anthelmintic resistance was first detected in New Zealand in 1979 (Vlassoff and Kettle 1980) and warnings of possible consequences soon followed. Despite these warnings, farmers have, for the most part, continued to successfully control parasites using anthelmintics. Continued use of anthelmintics to which resistance has developed has been conservatively estimated to result in reduced liveweight gains of lambs by 0.5–2.0 kg over 80–200 day periods (Mulvaney 1995; Macchi et al 2001), but if the presence of resistance continues to be ignored, greater losses are likely. Once resistance has been detected on a property, it has generally been possible to replace the implicated drench with one from a different chemical class that is still fully effective, thus maintaining parasite control. However, the accuracy of the original warnings has been demonstrated on some goat farms in New Zealand and on sheep farms overseas where resistance has now developed to all of the available broad-spectrum anthelmintic classes (benzimidazoles, levamisole, and the macrocyclic lactones), with profound consequences, particularly where more than one parasite species is involved (van Wyk et al 1997; DM Leathwick and WE Pomroy, unpublished data). There is little doubt that farming practices will need to change substantially as the level of anthelmintic resistance increases.

What is Anthelmintic Resistance?

The generally adopted diagnostic definition of anthelmintic resistance in New Zealand, and that of the World Association for the Advancement of Veterinary Parasitology, is a failure to reduce faecal nematode egg counts (FECs) by at least 95% (Coles et al 1992; McKenna 1994). A more technically accurate definition is that resistance is a genetically determined decline in the efficacy of an anthelmintic against a population of parasites that is generally susceptible to that drug (Sangster and Gill 1999). The difference between these two definitions has led to some confusion. Anthelmintics are marketed at dose rates determined to be effective against one or more dose-limiting parasite species, dose rates that can be many times greater than those required to kill 95% of more susceptible parasite species. Also, resistant parasites are initially present in a population at low frequencies and only become abundant after continued selection. Resistance can be developing and anthelmintic efficacy declining on a property, even though this is not clinically apparent and, for all practical purposes, the anthelmintic being used is effective. Experimental work in Australia showed that benzimidazole resistance in both *Trichostrongylus colubriformis* and *Ostertagia circumcincta* could not be unequivocally detected using a FEC-reduction test until the frequency of resistant genotypes in the population approached 50% (Martin et al 1989). Clearly, it is erroneous to assume that resistance is not present on a property simply because it has not been detected using a FEC-reduction test.

History and Prevalence of Resistance

Although no formal surveys have been conducted for 20 years, there is little doubt that anthelmintic resistance is now common in nematodes of sheep and goats in New Zealand. Moreover, the presence of benzimidazole and macrocyclic-lactone resistance in

nematodes of cattle in New Zealand, especially in *Cooperia oncophora*, is well documented (West et al 1994; Vermunt et al 1995; Hosking et al 1996; McKenna 1996). Resistance to benzimidazoles in cyathostomes in horses has also been recorded in New Zealand and there is suspicion of resistance to macrocyclic lactones in this species as well (Islam et al 2001).

Since first confirmed in sheep in New Zealand in 1979 (Vlassoff and Kettle 1980), the prevalence of anthelmintic resistance on New Zealand farms has steadily increased. Many early cases involved benzimidazole resistance in *Nematodirus spathiger* but resistance is now found to both benzimidazoles and levamisole in all common sheep trichostrongylids (McKenna 1995). Amongst parasitological submissions to animal health laboratories in 1993, benzimidazole resistance was evident in 66%, levamisole resistance in 29%, and resistance to combinations of benzimidazole + levamisole was diagnosed in 16% of cases (McKenna et al 1995). Although based on fewer submissions, prevalence of resistance to levamisole and benzimidazole + levamisole appeared to have increased to 42% and 39%, respectively, by 1996–97 (McKenna 1998).

In 1999/2000 the first cases of resistance to the macrocyclic-lactone anthelmintic, ivermectin, in *O. circumcincta* in sheep were detected (Leathwick et al 2000; Mason et al 2001), although ivermectin resistance in this nematode had been detected in goats a decade earlier. The first documented therapeutic failure of the macrocyclic-lactone anthelmintic, moxidectin, also occurred in *O. circumcincta* from goats in New Zealand (Leathwick 1995; Watson et al 1996). Ivermectin resistance in *O. circumcincta* from goats is often associated with resistance to all 3 chemical classes of anthelmintics and, consequently, control of nematodes on affected farms is difficult. Cross infection of multiple-drug resistant strains from goats to sheep has long been considered a significant risk to sheep farmers (Watson 1994), and at least one such occurrence has been documented (DM Leathwick, unpublished data). Ivermectin resistance has also now been confirmed in *T. colubriformis* from goats (Gopal et al 1999) and *Haemonchus contortus* from sheep (Vickers et al 2001).

Genetics of Resistance to Anthelmintics

The genetic mechanisms involved in resistance, in particular the number of genes involved and whether they are dominant or recessive, have important implications for the rate at which resistance develops. Gene dominance, as we use the term here, refers to the ability of the heterozygote (containing 1 R = resistant gene and 1 S = susceptible gene) genotype(s) to survive exposure to a particular drug, usually at the manufacturer's recommended dose rate. If a similarly high percentage of heterozygous (RS) worms are killed by a given dose of drug as are homozygous susceptible (SS) worms, the R gene is said to be recessive, i.e. the resistance character is not expressed. In contrast, if the RS genotype shows an equivalent ability to survive treatment as the homozygous resistant (RR) genotype, then the R gene is said to be dominant. At low gene frequencies, most R genes are present in the population as RS genotypes. If these are recessive (killed by treatment), R-gene survival is poor and resistance is slow to develop. However, if the R gene is dominant, then most RS genotypes will survive treatment and resistance will accumulate more rapidly.

Benzimidazole resistance in *H. contortus*, *T. colubriformis* and other strongylid nematodes appears to involve selection for two or more independent genes and is an incompletely-recessive trait (Dobson et al 1996; Roos 1997). In *T. colubriformis*, levamisole resistance is inherited as a sex-linked recessive trait, probably controlled by a single gene or a cluster of tightly linked genes (Martin and McKenzie 1990). Despite being a recessive trait, and because male nematodes have only one X chromosome whereas females have two, resistance is effectively dominant in males. In contrast, levamisole resistance in *H. contortus* is inherited as an autosomal recessive trait that is not sex-linked (Dobson et al 1996). This difference probably accounts for the observation that levamisole resistance is relatively common in *T. colubriformis* but rare in *H. contortus* (McKenna 1995; Sangster 1999).

Relatively few genetic studies have been published on the character of macrocyclic-lactone resistance in nematodes. In *H. contortus*, resistance to ivermectin appears to be inherited as a completely dominant autosomal trait, but its expression is influenced by sex in that efficacy against RS females is lower than against RS males (Dobson et al 1996; Le Jambre et al 2000). Indications are that ivermectin resistance in *O. circumcincta* is also inherited as a completely dominant trait (DM Leathwick, SA Bisset and IA Sutherland, unpublished data). In contrast, ivermectin resistance in *T. colubriformis* appears to be inherited as a partially dominant trait that is probably not under the control of a single gene (Gill and Lacey 1998; Gopal 2000). That ivermectin resistance is inherited as a dominant or partially dominant trait implies that once the gene is present in a population, resistance will emerge quite rapidly when ivermectin is used. This highlights the importance of effective quarantine drenching when transferring stock in order to prevent the spread of this gene between populations.

The above discussion may, however, be a simplistic view of macrocyclic-lactone resistance. Gill and Lacey (1998) categorised macrocyclic-lactone-resistant isolates into 5 types based on results of *in vitro* assays. They speculated that these groupings may reflect more than one set of genetic changes being involved in the expression of macrocyclic-lactone resistance, an hypothesis supported by studies in *Caenorhabditis elegans* (Köhler 2001).

Reversion Toward Susceptibility

The term 'reversion' refers to the return towards susceptibility of a parasite population after it has become resistant to a particular drug. There are few reports in which this phenomenon has been studied in any detail and most involve benzimidazole resistance in *H. contortus*, *O. circumcincta* or *T. colubriformis*. There are potentially two processes by which reversion may occur which, in the field, are not usually separable. One is when a fitness disadvantage is associated with the resistant genotype and the other is 'counter-selection', which occurs as a result of a different set of selection pressures being applied through the use of alternative anthelmintics. As the genetic changes associated with resistance can differ between nematode species, the occurrence of reversion may also differ both between species resistant to the same anthelmintic as well as between populations within species that are resistant to different anthelmintics.

Early laboratory studies showed little evidence for reversion to susceptibility. Hall et al (1982) reported no reversion to susceptibility in benzimidazole-resistant *H. contortus* and *T. colubriformis* over 12 generations and similarly, Martin (1987) found no reversion in benzimidazole resistance after 6 generations in a highly resistant strain of *O. circumcincta*. Simpkin and Coles (1978), however, reported a reduction in the level of benzimidazole resistance if resistant strains were cycled without further exposure to benzimidazole anthelmintics.

Field studies are probably more pertinent, as they also include fitness characteristics of the free-living larval stages. However, as these studies also usually include the use of more than one anthelmintic, they cannot exclude the possibility of counter-selection. Regular use of levamisole to control benzimidazole-resistant trichostrongylids led to a reduction in the level of benzimidazole resistance in *O. circumcincta* after 4 years (Martin 1987; Waller et al 1988) and in *T. colubriformis* after 8 years (Waller et al 1989). However, reversion was not to full susceptibility and re-introduction of benzimidazoles led to the rapid re-emergence of benzimidazole resistance to former levels in all cases. In another study, the use of levamisole for a 15-year period did not affect the level of benzimidazole resistance in *O. circumcincta* (Jackson and Coop 2000). No variation was found in the level of benzimidazole resistance in *H. contortus* after using levamisole for 6 years (Borgesteede and Duyn 1989) or 8 years (Waller et al 1989). Others have studied the possibility that fitness of resistant genotypes increases with the level of resistance, thus influencing the potential for reversion. In one study using *H. contortus*, an increasing level of benzimidazole resistance was associated with increased larval establishment, egg production and longevity (Maingi et al 1990). In another study, there was no difference in establishment rate, egg production or development rate from egg to infective-stage larvae between benzimidazole-resistant and -susceptible genotypes of *O. circumcincta* (Elard and Sauve 1998). Overall, the above findings indicate that a level of reversion may occur in some cases, but that re-emergence of resistance is likely to be rapid following the re-introduction of benzimidazole anthelmintics. For most practical purposes, therefore, once a substantial benzimidazole resistance is established it is effectively permanent.

There have been few studies of reversion of resistance to levamisole or any of the macrocyclic-lactone anthelmintics. Over a 5-year period, the use of thiabendazole was found to select against levamisole resistance in *T. colubriformis*, to a greater extent than natural reversion that occurred in the absence of anthelmintic treatments; however, the level of reversion in both cases was small and in neither case did full susceptibility return (Waller et al 1989). Ivermectin resistance in *O. circumcincta* rapidly re-emerged in goats after a 5-year period during which macrocyclic-lactone anthelmintics were not used (Pomroy et al 1998). In another study, there was no difference in establishment rate, egg production, larval development or larval survival between a goat-derived ivermectin-resistant isolate and 2 susceptible isolates of *T. colubriformis* (Gopal 2000), suggesting that the resistant isolate was equally as fit as the 2 susceptible isolates. These early indications suggest that reversion of resistance to ivermectin is likely to be slow if it occurs at all.

Drenching Frequency

Considerable weight has been given to the importance of drenching frequency in the selection for anthelmintic resistance and resistance management programmes often have the reduction of drenching frequency as one of their stated objectives (e.g. Familton et al 1995). However, not all drenches are equivalent in their ability to select for resistance and selection for resistance is not necessarily proportional to the frequency of treatment. For example, a drench given to lambs in winter is less likely to select for resistance than one given to lambs in late spring or summer because fewer eggs from survivors of the winter treatment are likely to survive on pasture to contribute to subsequent generations of worms. The aim in managing anthelmintic resistance is not to reduce drenching *per se* but to reduce selection pressure for resistance. Unfortunately, the latter is much more obscure and difficult to achieve than the former.

In New Zealand, the main thrust of nematode control has for many years been to minimise numbers of larvae on pastures to which grazing animals are exposed (Brunsdon and Vlassoff 1982). Selection pressure for anthelmintic resistance is likely to be related to the extent to which these low levels are achieved by anthelmintic use compared with other means such as pasture spelling, rotation with other herbivores or use of fodder crops. Achieving low levels of infective larvae on pastures by relying almost exclusively on anthelmintic treatment of stock generally results in high selection pressure for resistance. The issue is further complicated by the fact that when numbers of larvae on pasture are low, anthelmintic treatment of animals results in higher selection pressures than when levels of pasture contamination are high. This has been clearly demonstrated in the hot dry summer areas of Australia, where pasture contamination with parasites is lower than in the more temperate climate of New Zealand, and effective parasite control can be achieved using only 2–3 drenches annually (Barger 1995). Despite a much lower drenching frequency, anthelmintic resistance has developed to high levels in Australia faster than it has in New Zealand. An analogous situation occurs in New Zealand when drenched lambs are moved to 'clean' pasture and eggs passed by worms that survive drenching become the major source of subsequent pasture contamination. Modelling studies by Leathwick et al (1995) indicated that a 'drench and shift' strategy involving only 2 drenches was likely to be as selective for resistance as 5 drenches under a rotational grazing policy. Again, this illustrates the danger of focusing on reducing drenching frequency rather than reducing selection pressure for resistance.

The relationship between treatment frequency and selection for resistance is further complicated by the use of anthelmintics that have persistent activity (over weeks or months) in the animal. While the use of such products can result in fewer treatments being given, this does not equate to reduced selection for resistance; in fact, the reverse is likely. In essence, any anthelmintic treatment has the potential to select for resistance for as many days as it has efficacy against susceptible worms plus the prepatent period of newly established larvae.

Long-acting vs Short-acting drenches

For a long time, broad-spectrum anthelmintics in sheep were only available as oral formulations with little persistent activity

in the animal. However, the last decade has seen an increasing array of anthelmintics registered that have claims for persistent activity. These can be divided into 2 broad types: a) drugs that have high initial activity that subsequently declines logarithmically over time (e.g. moxidectin and closantel) and; b) controlled release capsules (CRCs), which are intraruminal devices that release drug (currently either albendazole or ivermectin) at a constant rate for about 100 days.

While some of these products clearly have substantial capability to suppress parasite populations, less is known about their ability to select for resistance. Practical experience with other types of pesticides, such as insecticides and herbicides, indicates that in general, long-acting products are likely to select for resistance more strongly than short-acting products (Hughes and McKenzie 1987). However, equivalent data relating to anthelmintics in grazing ruminants are limited and other factors such as treatment frequency require careful consideration. The introduction to, and acceptance by, the market-place of long-acting anthelmintics has preceded the basic knowledge required to gauge the long-term impact of such products on the development of anthelmintic resistance. This has, however, not prevented considerable debate and numerous contradictory claims about which product(s) will most rapidly select for resistance (e.g. Kieran 1994; Rothwell and Rolfe 1994). Given the complexity of the issues involved, it is perhaps not surprising that some confusion exists.

Many factors influence the intensity of selection pressure for resistance. A simplified outline of the selection process for short- and long-acting anthelmintics is presented as follows: Treatment with short-acting drenches, such as oral benzimidazole and levamisole products, remove most but not all of the parasite population in host animals. Worms remaining generally possess some genetic character which makes them less susceptible to the anthelmintic. After treatment, it takes approximately 3 weeks (the prepatent period), in lambs, for new infections to establish and develop to patency. During this time, the resistant surviving worms are the only contributors to pasture contamination by way of eggs output in faeces; thus, they have a reproductive advantage over susceptible genotypes for the duration of the prepatent period. In this way, each treatment increases the frequency of resistant genotypes in the overall parasite population. The selective removal of worms present in the host at the time of each treatment occurs with all drenches and is referred to as 'head' selection (Le Jambre et al 1999).

The use of anthelmintics that have persistent activity in the host has two additional consequences. Firstly, the period of reproductive advantage enjoyed by resistant worms that survive the anthelmintic treatment is much longer than that following the use of short-acting anthelmintics. For example, if a persistent drug provides 4 weeks of protection against the establishment of ingested larvae and the prepatent period is a further 3 weeks, then the reproductive advantage to the survivors of the initial treatment will be 7 weeks. The longer resistant worms are able to pass eggs onto pasture in the absence of susceptible genotypes, the more they will contribute to the overall pool of infective larvae on pasture and hence to subsequent generations of worms (Dobson et al 1996). Secondly, during the period of persistent activity of the drug, ingested larvae of susceptible genotypes cannot establish and develop to patency but, in general, larvae of resistant genotypes can. Thus the drug acts not only on worms present at the time of initial treatment, but continues to screen the parasite population for the period of persistent activity,

allowing only resistant worms to survive or develop, which can only mate with other resistant worms during that period. Jointly, these two processes are referred to as 'tail' selection (Dobson et al 1996).

How then, do these processes interact to determine the overall selection pressure for resistance? The first systematic attempt to address this question was published by Dobson et al (1996), which has become the foundation for most subsequent recommendations made about the use of anthelmintics that have persistent activity and their effects on the development of anthelmintic resistance (Barger 1997a; Sangster 1999). Using data for *T. colubriformis*, Dobson et al (1996) modelled the effects of a theoretical persistent drug, varying drug efficacy and duration of persistence against susceptible and resistant genotypes under conditions simulating those of the Australian 'Wormkill' parasite management programme. Their results showed that, in general, greater persistency of anthelmintic effect was associated with greater selection pressure for resistance. However, if the frequency of treatment with a persistent anthelmintic was reduced and the persistent drug had a high level of efficacy against resident worms at the initial time of treatment, then selection for resistance need not be increased, and could even be reduced. Dobson et al (1996) concluded that 'head' selection, the therapeutic efficacy of the drench against established worms, was the most important factor in determining selection pressure for resistance. The duration of persistent activity was second in importance, as it determines the reproductive advantage afforded to resistant survivors, and the subsequent establishment of resistant larvae during the 'tail' period of persistent activity was of least significance.

However, a subsequent empirical comparison of the development of resistance by *H. contortus* using ivermectin and moxidectin indicated that effects on the establishment of resistant worms during the 'tail' phase was more important than the selection of resident resistant worms at the time of initial treatment, i.e. 'head' selection (Le Jambre et al 1999). The proportion of the worm population subsequently present in animals that was resistant to anthelmintic was greater following the use of the persistent drug (moxidectin) than following the use of the non-persistent drug (ivermectin). Simulations by the same authors using a variant of the *T. colubriformis* model previously described by Dobson et al (1996) indicated that, despite these empirical findings, moxidectin could select for resistance more slowly than ivermectin under some conditions. It appears, then, that the relative importance of 'head' vs 'tail' selection processes varies with operational and epidemiological factors (such as the number of treatments and the use of 'safe' pasture).

Some of the factors which influence selection pressure for resistance and the variability that occurs between long-acting vs short-acting drugs are illustrated in the following discussion using two macrocyclic-lactone anthelmintics, ivermectin and moxidectin as examples. In general, at the manufacturer's recommended dose rates, moxidectin has greater efficacy against gastrointestinal nematodes than ivermectin (Sangster 1995) and has consequently been shown to have greater efficacy against both heterozygote (RS) and homozygous (RR) ivermectin-resistant genotypes of both *H. contortus* (Barnes et al 2001) and *O. circumcincta* (DM Leathwick, SA Bisset and IA Sutherland, unpublished data). This means that, following treatment with moxidectin, fewer resistant worms remain in the host than following treatment with ivermectin, a factor that will tend to

delay the development of resistance in the broader population (Barnes et al 1995). Moxidectin, however, also has persistent activity which affords a reproductive advantage to those worms which do survive the drench (Dobson et al 1996). In addition, for most of its duration, the 'tail' of moxidectin activity does not prevent establishment of resistant worms (Rolfe and Fitzgibbon 1996; Sutherland et al 1997; Le Jambre et al 1999), whether they be RS or RR ivermectin-resistant genotypes (Barnes et al 2001; DM Leathwick, SA Bisset and IA Sutherland, unpublished data). These latter two factors are likely to result in increased selection pressure for resistance (Dobson et al 1996). Results of the modelling work of Dobson et al (1996) and Le Jambre et al (1999) suggest that under the conditions of the Australian 'Wormkill' programme, the selection pressure for resistance that results from the high initial efficacy, but persistent activity, of moxidectin vs the lower initial efficacy, but non-persistence, of ivermectin, is approximately the same. The net result is that no clear or consistent benefit, with respect to selection for resistance, was evident from using one drug in preference to the other. However, differences in selection pressure between these two drugs can occur under some conditions. For example, model simulations presented by Dobson et al (1996) showed that selection for resistance occurred more slowly if lambs were drenched 3 times with a non-persistent drug (e.g. ivermectin) compared with twice using a drug that had a 4-week 'tail' (e.g. moxidectin). In contrast, if the duration of persistent activity was only 2 weeks, the reverse occurred. In further, work Dobson and Barnes (1999) estimated that a drug with a 'tail' 20–35 days long which allows all RS and RR, but no homozygous susceptible (SS) larvae to establish (as is the case with moxidectin) will select for resistance at approximately the same rate as a non-persistent drug which has no efficacy against established RS and RR worms (such as ivermectin). Again, this supports the view that there is no consistent advantage to the use of one of these drugs over the other.

A third member of the macrocyclic-lactone class of anthelmintics, abamectin, might exert less selection pressure for resistance than either ivermectin or moxidectin. Leathwick et al (2000) and Woodgate et al (2001) both demonstrated that abamectin had greater efficacy against ivermectin-resistant isolates of *O. circumcincta* than did ivermectin. Although, not as effective as moxidectin against these isolates, abamectin, like ivermectin, has little persistent activity (Leathwick and Sutherland 2001). By combining high efficacy against resistant genotypes with low persistence, abamectin may exert less overall selection pressure for resistance than either ivermectin or moxidectin, thus delaying the development of resistance to this important class of anthelmintics. Clearly more work in this area is warranted.

Applying These Findings to New Zealand

Would the same net effects and conclusions regarding the relative potentials of the different macrocyclic lactones to select for resistance be likely under New Zealand conditions? In the 'Wormkill' programme, both adult ewes and their lambs are drenched at weaning and the lambs are moved to previously prepared 'safe' pasture and receive two further broad-spectrum drenches during the year (Dobson et al 1996). Thus, at least

some of the drenches are given under conditions of low larval challenge, which logically would reduce the importance of 'tail' selection. In New Zealand, where conditions are much more conducive to the development and survival of larvae on pasture (Barger 1995) and the use of the 'safe' pasture concept is limited (Macchi et al 1999), larval challenge is likely to be higher and hence 'tail' selection more significant than implied by the Australian models. Further, persistent anthelmintics are commonly used in New Zealand as a pre-lamb treatment for adult ewes. In this situation, it is not possible to reduce treatment frequency (at least to the ewes) because a single treatment with a persistent drug is used instead of a single treatment with a non-persistent drug or no treatment at all. Reducing treatment frequency was essential in the Australian studies mentioned above if the rate of development of resistance was not to be accelerated by the introduction of persistent anthelmintics (Dobson et al 1996).

In addition, the population dynamics of parasites around the time of lambing differ markedly from those at the time of weaning. The relaxation of immunity in ewes around lambing is well documented (Vlassoff et al 2001); anthelmintic treatment of ewes 2–3 weeks pre-lambing would coincide with the start of this period of relaxed immunity. At this time, ewe worm burdens tend to be low (Brunsdon 1970), coming at the end of a period of high immunity, and so the importance of 'head' selection would tend to be reduced. In contrast, for the period of 4–6 weeks following treatment the ewes' immunity is compromised and more ingested larvae are able to establish. Thus, the opportunity for 'tail' selection (i.e. establishment of only resistant larvae) is increased. Once the ewes' immunity returns to full strength, 2–4 weeks after lambing and further establishment of ingested larvae is minimal (Leathwick et al 1999), any further persistence of anthelmintic activity is likely to be unimportant. Thus, it would seem that as long as a drench is persistent enough to cover the period of relaxed immunity in ewes, any additional duration of persistence would exert little additional selection pressure for resistance. Much of the logic behind this argument is supported by results from a model of nematode infections under New Zealand conditions (Leathwick et al 1995; DM Leathwick and A Vlassoff, unpublished data). While the above argument is speculative, it may be equally or more valid than simply extrapolating conclusions drawn from studies done under Australian conditions and applying them to New Zealand. However, the knowledge and understanding gained from the Australian studies indicates that, under some New Zealand conditions at least (e.g. the pre-lamb treatment of ewes), persistent anthelmintics are likely to select for resistance more strongly than reported in the original studies.

Long-acting Oral, Injectable and Capsule Formulations

Most of the information and debate regarding persistent vs short-acting drenches for sheep has related to the oral formulations of ivermectin and moxidectin, and to a lesser extent, moxidectin-injection. However, the basic principles outlined above apply equally well to all anthelmintics that have persistent activity. The only difference between CRCs and persistent oral or injectable drug formulations is the duration of activity and efficacy against established worms and ingested resistant larvae. Simulation

models developed in New Zealand have shown that CRC use need not increase selection pressure for resistance, provided the continuous action of the capsule makes the RS genotype recessive when this is not so for the same drug given as a single oral dose, and where there are susceptible genotypes *in refugia*, i.e. on pasture or in untreated animals where they are able to reproduce without exposure to anthelmintic (Leathwick et al 1997). There is evidence that the first of these requirements may be met by the albendazole CRC, which has shown greater efficacy against resistant worms than oral formulations of the same drug (Barger 1993), particularly against resistant infective third-stage larvae (L_3). Thus, from a New Zealand perspective, there is no clear indication that use of an albendazole-CRC to replace the standard 5-drench preventive programme in lambs (Vlassoff et al 2001) will accelerate the development of resistance, a similar conclusion to that drawn by Barger (1993).

However, studies with ivermectin CRCs indicate that efficacy against resistant adults of *H. contortus* and *O. circumcincta* is no greater than that of the oral formulation (Barnes et al 2001; IA Sutherland, SA Bisset and DM Leathwick, unpublished data) indicating that the CRC is unlikely to make the RS genotype recessive. For *H. contortus*, this appears to also apply to ingested L_3 for which the efficacy of ivermectin CRCs against RS genotypes was similar to that against RR genotypes (Barnes et al 2001). In contrast, for *O. circumcincta*, establishment of RS L_3 was significantly lower than RR L_3 in lambs treated with ivermectin CRCs (DM Leathwick, SA Bisset and IA Sutherland, unpublished data). Thus, based on currently available evidence, it seems more likely that the development of resistance will be accelerated by the use of ivermectin CRCs.

The oral formulation of moxidectin is highly effective against resident ivermectin-resistant adults of *H. contortus* (Barnes et al 2001), *O. circumcincta* (DM Leathwick, SA Bisset and IA Sutherland, unpublished data) and *T. colubriformis*. Although the injectable formulation was highly effective against the first 2 species, it performed very poorly against ivermectin-resistant *T. colubriformis* (Gopal et al 2001). The ivermectin CRC, however, is no more effective than the oral formulation of ivermectin (see above). Whereas resistance appears to be dominant in the presence of the moxidectin 'tail' for both *H. contortus* and *O. circumcincta*, in the latter species it appears to be incompletely-recessive in the presence of an ivermectin CRC. It appears, then, that these different persistent drug formulations vary in their efficacy against ivermectin-resistant genotypes of different parasite species, making it difficult to draw general conclusions as to which products are likely to result in the most rapid development of resistance.

It is important to appreciate that in the absence of a refuge for susceptible genotypes, modelling studies indicate that assumptions or uncertainties about gene dominance and drench efficacy become irrelevant. When there is no refuge of susceptible genotypes, such as when drenches are used to produce pasture with minimal parasite contamination (i.e. 'safe' pasture), the rapid development of resistance is inevitable. In New Zealand, reduction in pasture contamination is seen as a major benefit of using persistent anthelmintics in adult sheep (Gogolewski et al 1997). Clearly, generating pasture with very low numbers of drench-susceptible larvae gives an enormous advantage to any resistant parasites able to survive the anthelmintic and so it is not surprising that using anthelmintics in this way will result in the rapid development of resistance.

Host Immunity

It is now well accepted that drenching adult, fully immune, sheep results in greater selection pressure for resistance than drenching lambs in which immunity has not yet fully developed (Dash et al 1985; Leathwick et al 1995; Le Jambre et al 1999; Smith et al 1999). Selection pressure for resistance is minimised when treatment is followed by the ingestion and establishment of larvae whose genotypic makeup resembles that within the host at the time of treatment (Smith et al 1999), i.e. the reproductive advantage afforded the drench survivors is minimised. An immune host, such as an adult ewe, is able to prevent all but a few ingested larvae from establishing (Leathwick et al 1999), so the diluting effect of incoming larvae from pasture is reduced and selection pressure for resistance is increased. In a similar manner, an anthelmintic that had persistent activity that killed all incoming larvae, regardless of genotype, for a period after treatment was highly selective for resistance (Dobson et al 1996). This finding regarding ewe drenching was not, however, supported by Barger (1997b), who showed that using the Australian *T. colubriformis* model (Barnes and Dobson 1990), drenching ewes soon after lambing did not select strongly for resistance. However, Barger (1997b) also pointed out that there was a significant difference between the two models with respect to the level of host immunity in the ewes at the times compared and that this was the likely cause of the different results. The Australian model, which is based on Merino sheep, assumes a complete loss of immunity over lactation, which means that the ewes behave similarly to lambs; the New Zealand model, which is based on Romney sheep, assumes a more modest and shorter duration decrease in immunity. In both cases, results predicted by the models are consistent with empirical data from their respective countries and the breeds of sheep concerned (Leathwick et al 1999).

Recent work has indicated an additional interaction of host immunity with the results of anthelmintic use affecting selection pressure for anthelmintic resistance. Sutherland et al (2000) measured the level of resistance of parasite eggs passed by sheep that had been either untreated or treated with CRCs at 7 months of age. While the capsules were active, the animals were challenged with a mix of resistant and susceptible parasites. In the CRC-treated animals, only the resistant genotypes established and these produced eggs that also showed a high level of resistance (approximately 100x higher than those from the non-capsule-treated animals). After the capsules expired on day 100, challenge was switched to susceptible larvae only, with the expectation that once parasites were again able to establish in the hosts the susceptible parasites would rapidly dilute out the resistant worms which had established while the capsules were active. This, however, did not happen, as almost all of the sheep had developed a substantial level of immunity and virtually none of the susceptible larvae established. For 5–7 weeks after the capsules had expired the level of resistance of eggs passed from the CRC-treated animals remained as high as when the capsules were active. Thus, Sutherland et al (2000) demonstrated that CRCs can combine with developing host immunity to produce an extremely long period (>149 days) of reproductive advantage for highly resistant worms that either survived the initial treatment or subsequently established during the period of CRC activity.

The Future?

Since 1980, the prevalence of anthelmintic resistance in New Zealand has increased to the point where it is now common and occurs for all of the currently available broad-spectrum anthelmintic classes. Despite this, the frequency of anthelmintic treatments applied by New Zealand sheep farmers has remained essentially unchanged (Brunsdon et al 1983; Macchi et al 1999). However, given the routine use in recent times of persistent anthelmintics, it is likely that parasite exposure to anthelmintics is greater now than it ever was before. It seems unlikely that this will change in the immediate future since most farmers appear to be making little effort to reduce drench usage (Macchi et al 1999). It is clear that current patterns of anthelmintic use are applying significant selection pressure for resistance, and in the absence of any major change, it is inevitable that resistance levels will continue to increase. We must conclude, therefore, that unless new chemical classes of anthelmintics become available, current chemical-based parasite control practices will be unsustainable in the long term.

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