MANAGING REFUGIA

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INTRODUCTION

With an ever expanding world population the ability to meet the demand for resources, whilst maintaining socio-political and economic stability, has become a very real and major issue for our society. These societal pressures reinforce the need to expand plant and animal production and, under these circumstances, any diseases constraining production become relevant for food security issues at both the international and national level. Parasitoses, which are widely acknowledged as one of the most important production limiting diseases of livestock, are currently largely controlled using chemotherapy and chemoprophylaxis. However, the sustainability of this approach has now become threatened by the widespread emergence of resistance against these chemicals (Jabbar *et al.,* 2006). The development of methods to conserve chemical efficacy of our current antiparasitics are, and will become, increasingly important as we seek to maintain and/or expand livestock production. One approach to conserving efficacy that has gained widespread acceptance amongst parasitologists is to maintain parasite populations *in refugia* (i.e. developmental stages unexposed to the anthelmintic) in order to sustain genetic susceptibility (van Wyk, 2001; Soulsby, 2007). However, in that respect parasitologists are not unique since the potential to exploit refugic pathogen populations as a means of maintaining sensitivity to chemical control agents has been considered by researchers in almost every area where resistance has become an issue (Wearing & Hokkanen, 1994; Frutos *et al.*, 1999). The escalating problem of helminth, and in particular nematode, anthelmintic resistance has always been the key factor driving research on novel approaches and for meetings such as NA5. Since resistance first emerged as a major threat to small ruminant livestock production over 30 years ago the focus of research in this area has, as one would expect, slowly shifted from simply recording its occurrence and using surveys to define the extent of the problem towards trying to understand the mechanisms involved and methods by which it may be managed. An understanding of the economic imperatives that have led to the widespread development of resistance in intensive production systems and of the selection and mechanisms supporting that resistance is clearly a pre-requisite for the development of refugia based effective management strategies. When broad spectrum anthelmintics first appeared on the markets, producers were not only able to use them therapeutically but were also able to exploit them in suppressive prophylactic regimes and, for some time at least, to obtain very useful gains in productivity. In the past, producers could use simple cost benefit analyses based around the increases in yield that resulted from the availability and use of effective anthelmintics. It was only later that it became evident that many of these strategies were not sustainable and that the conflict between the goals of maximising productivity and reducing the rate of selection of resistance became apparent.

Refugia

In the case of anthelmintic resistance the population *in refugia* can simply be defined as a population that is either unexposed to or, in certain circumstances, unaffected by an anthelmintic. Since anthelmintic resistance is a heritable trait the expectation is also that the genetic structure of an exposed population will have been altered in some way. Initially anthelmintic resistance management strategies were not specifically directed towards maintaining refugia but understandably, at a time when parasitism appeared to be an omnipresent threat, tended to be largely concerned with achieving effective control with fewer treatments (Dash, 1986), ways of maximising and extending efficacy (Hennessy *et al.*, 1991) and maintaining biosecurity (Dobson *et al.*, 2001). More recently, attention has been given to maintaining a nematode population *in refugia* in an effort to maintain susceptibility within the suprapopulation (van Wyk *et al.*, 2002; Abbott *et al.*, 2004; Pomroy, 2006).

Nematodoses are essentially challenge density dependant diseases and thus the primary aim of all prophylactic control strategies is simply to reduce challenge from pasture to an extent which does not compromise performance. As figure 1 shows, the relationship between the size of the infrapopulation and the suprapopulation is complex and subject to influence by a variety of host, parasite and environmentally dependant factors all of which can affect the risk of disease. Some of these factors, in particular those related to host and pasture management and acquired immunity, can be manipulated to either directly or indirectly affect suprapopulation size and can thus be usefully exploited with regard to providing refugia within nematode control regimes.

Host management based approaches to maintaining refugia

Refugia can be maintained in nematode populations in a number of ways, the most obvious of which fall within the host management category. These include reducing the numbers of treatments by targeting them effectively and/or selectively. However, it is worth noting at this point that the management of refugia always needs to be balanced with the ethical and financial consequences of parasitism. With this in mind, simply reducing the numbers of treatments administered has the potential to reduce contamination with resistant phenotypes whilst extending the interval between treatments should allow a better establishment of susceptible phenotypes

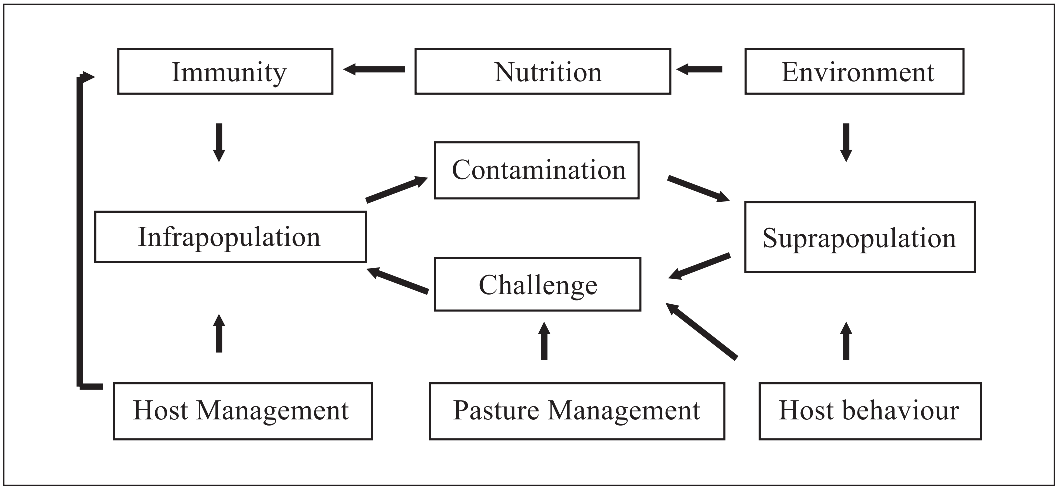

Figure 1. Interactive elements that affect infra and suprapopulation size and thus contribute to the risk of disease.

from pasture. Targeted selective treatments (TSTs) are part flock treatments administered to those animals that need them most either because of their susceptibility to disease and/or their propensity to propagate infection. In managing refugia using a TST approach the need to reduce the risk of nematodoses by limiting pasture contamination must always be taken into consideration. Under these circumstances it may be necessary to trade off the benefit that high egg producers offer in terms of refugia against their impact upon disease risk. However whenever possible, the use of TSTs needs to be promoted simply because this approach offers the prospect of maintaining refugia during periods when treatments are being administered. A full account of the different strategies that have been used by researchers and in the field to better target and/or selectively target treatments has been given by Besier (2008). Whenever anthelmintics are used it is important to consider not only the impact on the infrapopulation but also what is happening at that time to the free living stages. For example anthelmintic treatments that are administered when the suprapopulation size is drastically reduced, such as during dry periods or by the use of reseeded pastures, pose a considerable risk with regard to the selection of resistance. An understanding of this phenomenon has led to a move away from summer treatments in the winter rainfall areas of Australia (Besier & Love, 2003) and recommendations not to dose animals onto clean grazing (Abbott *et al*., 2004).

Other important host, parasite and managemental factors that need to be considered when applying the concept of refugia within worm control are the classes of animal to be treated, the nature of the anthelmintics used in the treatments, the biotic potential of the nematode species exposed to treatment and the treatment regimes being employed. A study in New Zealand (Leathwick *et al*., 2006a) showed that periparturient ewe treatments tended to have a greater impact on the development of resistance than regimes where the ewes were left untreated. This study also suggested the development of anthelmintic resistance can be slowed through the use of a selective treatment strategy in lambs. However, a subsequent study on selective and on demand drenching (Leathwick *et al*., 2006b) showed between parasite species differences in the numbers of unselected worms that accumulated on pasture. On a similar note, sharing of common grazing by groups of animals with different patterns of treatment may provide refugia and has been exploited in the management of resistance in Ethiopia (Sissay *et al.,* 2006) and believed to be an important factor in accounting for the lack of resistance recorded in studies on the Greek mainland (Papadopoulos *et al*., 2001).

Attempting to modify the AR status of the parasite populations either through re-introducing susceptible parasite species or by modifying resistance mechanisms has an intuitive appeal. Although studies in South Africa have shown that it is possible to re-introduce susceptible populations onto irrigated areas (Carmichael, 2002), the practical difficulties of doing this on a large number of farms in temperate climes where a resistant suprapopulation can survive for many months on pasture are challenging. Studies with insects have confirmed the potential for modifying the metabolic mechanisms, such as the cytochrome P450s, which degrade insecticides to restore activity (G. Moore personal communication). In addition, studies using parasitic nematodes have also shown that it is possible to reverse resistance using p glycoprotein (PgP) or cytochrome P 450 (CP450) inhibitors (Xu *et al*., 1998; Kerboeuf *et al*., 1999, 2002; Prichard & Roulet, 2007). However, identification of the most appropriate inhibitors and then producing and registering these combinations represents a formidable scientific and commercial challenge.

The use of bioactive forages as a means of nematode control might be thought to offer in effect a whole range of 'new'anthelmintics in the form of the plant secondary metabolites (PSMs) that confer anthelmintic activity. However, some caution is necessary when considering this managemental intervention since in some cases the active compounds have yet to be identified. It is also possible that the transport and metabolic mechanisms that resistant parasites successfully use to regulate conventional anthelmintics might also operate against these PSMs. If this is indeed the case then there might be some inadvertent selection at resistant loci as a direct result of using these forages. Furthermore, the relative efficiency of non-chemical approaches always needs to be considered if they are to be used in conjunction with anthelmintics since, if they are highly effective in reducing suprapopulation size, there is real risk that an integrated approach might exert a strong selection pressure for resistant genotypes (Leathwick *et al.,* 2006a).

Immunological based approaches and the management of refugia

The various immunological approaches to control can also be used to reduce reliance upon anthelmintics and can thus help to maintain anthelmintic susceptible populations. Immunomanipulation through optimised nutrition, vaccination and/or the selection of resistant or resilient animals usually offers some advantage in reducing the numbers of anthelmintic treatments that animals require. For example, selection studies in Scottish cashmere goats conducted over ten years showed an average reduction in egg counts of over 30%. Modelling studies using this data suggest that it would require between 2-3 treatments each year with a non-persistent anthelmintic to achieve the same level of reduction (Jackson unpublished data). The incorporation of small numbers of resilient animals (which are capable of maintaining their performance whilst under parasite challenge and which often have high egg counts) into flocks could also be used to provide untreated populations and thus manage refugia.

The interaction between the immune status of the host and refugia may, at least in part, account for the differences in the rate of appearance of resistance and its prevalence in different ruminant species (Silvestre *et al*., 2002). Resistance appears to develop most rapidly in goats where, because of their perceived lack of immunity, it is common practice to treat all age classes. The converse is also true, adult cattle generally exhibit a good immunity against GI nematodes and thus through being left untreated adult stock may provide some refugia. In sheep, however, the contribution to refugia is likely to be affected by temporal changes in both the species contributing to parasite challenge within a grazing season and the susceptibility of the host to each of those species. Species prevalence and host susceptibility are two important factors that need to be taken into account when managing refugia particularly when single species resistance is an issue.

Monitoring the success of refugia based strategies

Given the overwhelming support of the parasitology community for the role of refugia in the selection of resistance it is inevitable that attention is now being directed towards the potential for managing refugia. However, as mentioned previously the management of refugia can never be seen as a discrete issue but must be set in context with the management of animal productivity and nematodoses (Leathwick *et al.,* 2006b). At present the management of refugia almost inevitably operates in an environment where resistant phenotypes predominate within the nematode populations. Under these circumstances the main role of refugia management is to maintain existing levels of susceptibility and to reduce the rate of selection of resistance. However, it is also evident that the development of effective management of refugia strategies, that could be implemented when resistance alleles are still relatively rare within the population, requires the ability to sensitively measure initial resistance allele frequencies and determine the impact of treatments not only upon animal performance and nematode epidemiology but also more importantly on nematode phenotype and genotype. Research on the management of refugia is currently hampered by our limited understanding of the molecular mechanisms of resistance, particularly to the macrocyclic lactones and imidazothiazole families, and the lack of sensitive genotyping tools. It has been suggested that there are several anthelmintic resistance mechanisms that operate at the molecular level (Wolstenholme *et al*., 2004). Firstly, there may be changes at the target site or amplification of target genes which render the drug ineffective. Secondly, there may be changes in the metabolism that affect drug activation or removal. Finally, there may be changes in the distribution of the drug in the organism which limit its access to the site of action. It is only when we understand the relative importance of these mechanisms that we will be able to develop sensitive DNA based technologies using SNPs/mutations and/or gene expression to determine both species and anthelmintic resistance status of field material. Advances being made in diagnostic technologies, such as metabolimics, real time PCR and pyrosequencing, all have the potential to be used with pooled material to provide the sensitive and affordable monitoring capacity that is needed for managing refugia. In terms of understanding the molecular mechanisms of resistance, benzimidazole resistance which, in the case of the economically important gastrointestinal nematodes of small ruminants *Haemonchus, Trichostrongylus* and *Teladorsagia* is largely attributable to changes in the beta tubulin gene, offers the best example of the inherent sensitivity and affordability of these emerging technologies. Pyrosequencing studies at Moredun using the F200Y SNP and examining pooled material from different *Teladorsagia* isolates have shown very different resistance allele frequencies. In an isolate first taken into the laboratory over forty years ago, before the widespread use of the benzimidazoles, the SNP associated with benzimidazole resistance was not present whereas in one taken into the laboratory about 15 years ago and shown by egg hatch assays, faecal egg count reduction tests and controlled efficacy tests to be sensitive to the BZ's the resistance allele frequency was around 10% (Skuce personal communication) However, in a *Teladorsagia* population, which has been shown to be resistant to all three broad spectrum anthelmintics (Bartley *et al*., 2004), the resistance allele frequency was over 70%. Each of the pooled samples examined contained over 1000 L_3 and cost in the region of US$4 to process If, through an enhanced understanding of the key mechanisms of resistance, we can develop similar high throughput low cost approaches for other drug families then we will finally have an improved ability to monitor and manage refugia. The benefit of this would be greatly aided by an improved understanding of the population genetics of GI nematode resistance. Advances currently being made in obtaining parasite genomes are also needed in order to provide some of the genetic tools which are required to investigate resistance gene flows within parasite populations.

The role of modelling

Models of nematodiasis that incorporate an understanding of anthelmintic resistance have in the past provided very useful insights into the likely impact of different treatment strategies (Barnes & Dobson, 1990; Leathwick *et al*., 1995). The development of more sophisticated models incorporating an understanding of the population genetics of resistance and multigenic multiple mechanisms of resistance could and should be used to examine some of the fundamental questions concerning the management of refugia. Of particular interest in this regard are intervention levels following the introduction of a new drug family. For example at what point in the development of resistance to any of the possible new families (Mckellar & Jackson, 2004), including the very recently announced AAD family should we introduce changes in management to provide refugia? Changes that might be contemplated include swapping drug families, the use of bioactive forages, immunomanipulation and the re-introduction of susceptibility.

Different anthelmintic families, including any new ones, could in theory be used to provide refugia for other drugs with different target sites providing that the two anthelmintics do not share any of the transport or metabolic mechanisms that contribute to resistance and that the use of one drug does not impose selection on the genes for resistance to the other drug. Unfortunately, evidence is now accumulating that the BZs and MLs may share common transport mechanisms that are implicated in resistance (Kerboeuf *et al*., 2003) and that selection with ivermectin can also lead to modifications of the beta tubulin genes (Prichard & Roulet, 2007). The relationship between the pharmacological mechanisms involved in anthelmintic resistance and the immunological mechanisms enhanced through vaccination, genetic selection or optimised nutrition should be sufficiently distant to minimise the risk of unwanted interactions.

Until such time that we have developed high throughput and affordable screening technologies, it will only be possible to manage refugia at the farm level by reducing anthelmintic input and to monitor progress using our current relatively insensitive resistance monitoring techniques. The aim of current research is to develop our understanding of the mechanisms implicit in resistance and to exploit developing DNA and modelling technologies so that in the long term we will be able to move away from operating reactively to emerging high levels of resistance and towards taking a much more proactive stance. However, even if the goals of this research are achieved we still face the formidable hurdle of translating good science into acceptable and effective on-farm control strategies (van Wyk *et al.,* 2006).

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