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# The role of targeted selective treatments in the development of refugia-based approaches to the control of gastrointestinal nematodes of small ruminants

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## ABSTRACT

Anthelmintic resistance is recognised as a major problem affecting small ruminant production worldwide and now threatens the sustainability of many of these systems. One method that has been proposed to prolong the efficacy of our current anthelmintics is the maintenance of a parasite population in refugia (unexposed to a drug) which will maintain the genes for susceptibility within the parasite population. Management strategies that employ refugia-based methods include targeted or strategically timed whole flock treatments, targeted selective treatments (TST), whereby only a proportion of the flock is treated at any one time, and the dilution of resistant with susceptible parasites. The ability to effectively target anthelmintic use relies on the identification of those animals that will most benefit from treatment. This review explains the concept of refugia, describes the role of refugia-based approaches to the management of anthelmintic resistance and reviews the markers that have been studied as indicators for TSTs as well as the implementation of refugia-based strategies. Recent results suggest that targeting anthelmintic treatment on the basis of anaemia, milk production and liveweight gain may offer a means of reducing anthelmintic usage whilst still maintaining animal performance.

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## 1. Introduction

Anthelmintic resistance in gastrointestinal (GI) nematode populations is now widely recognised as a major

problem in small ruminants (Jackson and Coop, 2000; Wolstenholme et al., 2004; Kaplan, 2004) and threatens the sustainability of many production systems throughout the world. Although it appears to be less of a problem in cattle, there have been reports of resistance from England (Stafford and Coles, 1999), New Zealand (Pomroy, 2006) and South America (Soutello et al., 2007; Suarez and Cristel, 2007) in cattle herds, and thus it is likely to become

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more important in other regions in the future. The way in which GI parasitic diseases are controlled has to be changed: since it is highly unlikely that GI parasites can ever be eradicated (Le Jambre, 2006) animal producers must learn to 'live with worms' (Coles, 2002). The two key approaches to deal with anthelmintic resistance currently used by producers are firstly to prevent the introduction of resistant worms onto farms and secondly to slow the development of resistance. With respect to the former, stringent quarantine measures can be used to limit the risk of introducing resistant worms (Dobson et al., 2001). However, in terms of the latter, the need to conserve the efficacy of current drugs and any anthelmintic families that may be developed in the future must be balanced with the ability to maintain high levels of animal production and welfare. One approach which has been the focus of recent research and debate is to maintain a parasite population in refugia (unexposed to drug), in order to maintain both phenotypic and genotypic susceptibility (Van Wyk, 2001; Soulsby, 2007).

The concept of refugia was first described by ecologists, who considered that a refugium is any local environment that has escaped regional ecological change and therefore provides a habitat for endangered species. The concept was also used by researchers working on insecticide resistance to describe areas where members of a population were not exposed to chemical control and were therefore unaffected by treatment (Georgioud and Taylor, 1977). However, this concept cannot be directly applied to parasitic communities where the 'endangered' (anthelmintic susceptible) population exists alongside the 'dangerous' (anthelmintic resistant) population in both the pre-parasitic and parasitic environments and moreover the two populations can interbreed freely. The simplest definition for a population in refugia that has become widely accepted amongst the parasitological community (Van Wyk et al., 2002), which is largely concerned about the maintenance of susceptibility to anthelmintic treatment, refers to those subpopulations from within either the infrapopulation (the parasitic stages within the host) or suprapopulation (free-living stages on pasture) that are not exposed to anthelmintic treatment. The obvious major pre-requisite for the effective maintenance of anthelmintic susceptibility through the exploitation of the population in refugia is that the parasites in refugia must complete their life cycle and pass on susceptible alleles to the next parasitic generation. Environmental and management factors that markedly affect the bionomics of the free-living stages are also important due to the influence they exert on the effective maintenance of refugia. Martin et al. (1981) were the first to apply the concept of refugia to a population of GI nematodes, however, it is only relatively recently that members of the parasitological research community have considered the idea (Van Wyk, 2001). If a proportion of a population is left unexposed to drugs then these worms should be able to help to maintain the alleles for susceptibility within the population. The pre-parasitic stages derived from these worms in refugia can dilute the resistant genotypes on pasture thus reducing the proportion of resistant parasitic adult worms that are likely to mate with other resistant adults (Van Wyk et al., 2006).

Although there are several parasitological and management factors which may influence the rate of development of resistance, such as treatment frequency and underdosing, Van Wyk et al. (2001) suggested that the proportion of the parasite population in refugia could be the most important factor determining the rate of development of resistance and should be considered, above all else, throughout the development and implementation of any control strategies (Van Wyk, 2001).

In recent years a considerable amount of research effort has been directed towards how refugia can be used in the management of anthelmintic resistance. In particular the participants in the EU-funded PARASite SOLutions project (PARASOL, [www.parasol-project.org](http://www.parasol-project.org)) have examined regionally and production system appropriate means of exploiting refugia. The purpose of this review is, using the findings from recent research, to identify refugia-based methods which can be used to reduce the rate of development of anthelmintic resistance. The review will describe the sources of refugia, factors that impact on it, ways in which the concept of refugia can be used in the management of anthelmintic resistance and some of the potential markers that can be used as indicators for treatment. In addition, factors affecting the implementation of refugia-based strategies on farms are discussed.

## 2. Sources of refugia

There are several sources of refugia for anthelmintic susceptible GI nematodes which have direct lifecycles: where sub populations within in the suprapopulation or infrapopulation can serve to maintain susceptible alleles. Although it is usually the infrapopulations in untreated hosts that provide refugia in fact any lifecycle stage in the host which is refractory (but not resistant, e.g. histotropic larvae) to a particular drug treatment can also do so (Fleming et al., 2006).

In temperate areas, the majority of the parasite population (up to 95% of the total worm population, Barnes et al., 1988) is usually found on pasture, and therefore, provides a relatively large reservoir of susceptibility. In tropical areas, however, this may not always be the case, since the extreme heat desiccates the free living worm stages on pasture within as short a period as 4 weeks (Barger, 1994). Drought conditions can also increase mortality amongst the free living stages thus reducing the proportion of the parasite population in refugia. Treatments given under these circumstances can thus select more strongly for resistance (Papadopoulos et al., 2001). Although it is relatively easy to determine the composition of the population that has contaminated the pasture, it is difficult to predict the composition of a derived suprapopulation accurately, as there are a number of factors that influence parasite development and survival. In particular, environmental/climatic factors which influence egg and larval development and survival have the potential to skew the genetic structure of surviving infective larval populations. Differences in the relative fitness of resistant and susceptible populations might be important factors that need to be taken into account when considering the management of refugia.

Unfortunately, since large-scale comparative studies have not been undertaken, it is still unclear whether there are any consistent fitness differences between susceptible and resistant parasites. Some studies have suggested differences in characteristics such as pathogenicity, fecundity and development and survival rates that influence the epidemiology of *Haemonchus contortus* (Kelly et al., 1978), *Teladorsagia circumcincta* (Leignel and Cabaret, 2001) and *Cooperia oncophora* (Coles et al., 2001). By way of contrast, other studies have reported few, if any, differences between susceptible and resistant isolates of *H. contortus* (Scott and Armour, 1991) and *T. circumcincta* (Barrett et al., 1998; Elard et al., 1998).

In some cases, stage specific differences in susceptibility to an antiparasitic compound that are not attributable to resistance may provide some refugia even in treated animals. For example, it is known that the immature stages of some *T. circumcincta* populations are refractory to levamisole treatment (Grimshaw et al., 1996), which is the basis for conducting faecal egg count reduction tests (FECRT) on day 7 post-treatment (Coles et al., 2006). Under these circumstances levamisole treatments may inadvertently maintain refugia. In contrast treatments with the macrocyclic lactones, which have efficacies approaching 100% against all developmental stages, result in negligible levels of refugia.

### 3. Which factors impact on the maintenance of refugia?

There are several factors which affect the management of refugic parasite communities that can be classified as being parasite, host, environment or management associated (Jackson and Waller, 2008).

There are a number of parasite associated factors which may influence the proportion of parasites that are required to be untreated to maintain an effective refuge. These factors include the frequency of the resistant alleles in the parasite population, the biotic potential of the parasite and the longevity of both the supra and infrapopulations. The frequency of resistant genes influences the rate at which homozygous resistant parasites appear in the population. If the proportion of resistance genes is high, then there is an increased probability that heterozygotes will have the opportunity to mate and produce homozygous resistant offspring. The degree of biotic potential of a parasite species determines the numbers of individuals of that species required to provide a given level of effective refugia. For example, species with high biotic potential, e.g. *H. contortus*, can provide a high degree of pasture contamination from relatively few individuals and therefore, effective refugia can, assuming successful completion of the lifecycle, be maintained by leaving a relatively small proportion of the host population unexposed to treatment. In other species with lower biotic potential, e.g. *T. circumcincta*, a greater proportion of the host population would need to be left unexposed to treatment to provide the same level of refugia.

Host-associated factors with an effect on proportions of parasites in refugia include the level of resilience to parasitic infection and the extent of the expression of immunity. Resilient animals continue to perform whilst

under parasite challenge and therefore require less anthelmintic treatments than non-resilient animals in any given season (Bisset et al., 2001a). Furthermore, lambs in temperate regions can develop significant protective immunity to some helminth species by the end of their first grazing season (Waller and Thomas, 1978; Waller and Thomas, 1981), thereby effectively controlling their worm burdens. Under these circumstances, it is possible that the contribution of parasite eggs to pasture contamination is reduced as a competent immune response is developed, thus throughout the grazing season an increasing proportion of animals may need to be left untreated to provide a significant contribution to refugia.

In addition to drought and other environmental conditions that influence the development of eggs and larvae on pasture, management practices can also impact upon the maintenance of refugia. For example, drench and move regimes which consist of treating all animals in a flock and moving them onto clean pasture (where there are no or few parasites in refugia) will result in the contamination of the clean pasture with eggs of only those parasites that survived treatment (Abbott et al., 2004).

The growing problem of anthelmintic resistance in the UK led to the development of the 'Sustainable Control of Parasitic Diseases in Sheep (SCOPS)' initiative, which offers advice to farmers and vets on methods of managing the problem. Amongst the advice promoted by SCOPS, the practice of dosing and moving animals to clean pasture is now actively discouraged. Molento et al. (2004) suggested that animals should be moved prior to drenching and treatment delayed until the desired levels of refugia have built up on the new pasture to ensure that unselected parasites were transferred to clean pasture. Even better is, if practicable, to selectively treat only those animals which would 'most benefit' from treatment (see also Section 4.3). Furthermore, the sharing of common grazing by groups of animals that have different treatment regimes and different susceptibilities to infection are likely to provide a continual turnover of parasites in refugia, and may well provide an explanation for the lack of anthelmintic resistance observed in the Greek mainland (Papadopoulos et al., 2001) and in Ethiopia (Sissay et al., 2006).

### 4. How can the concept of refugia be used to reduce the development of resistance?

The proportion of the parasite population in refugia has been shown to be important in influencing the rate of development of resistance. Control programs that leave relatively low or no parasites in refugia, i.e. spring treatments against a parasite species that overwinters poorly or dose and move to clean pasture, select strongly for resistance (Michel, 1985). It has been known for some time that a higher proportion of larvae in refugia can slow the development of resistance (Martin et al., 1981). The proportion of resistant alleles present also influences how refugia affects the rate of development of resistance. When the proportion of resistance alleles is low then there is potential for a substantial refugium to exist. However, once resistant alleles become very common in the population

then the opportunity to maintain a population in refugia decreases (Gaba et al., 2006). The concept of refugia can be applied to slow the development of resistance through the use of approaches such as dilution of resistant with susceptible parasites when the proportion of resistance alleles is high, and targeted treatments and selectively targeted treatments when resistant alleles are less common.

#### 4.1. Dilution of resistant with susceptible parasites

Once anthelmintic resistance has been identified to a particular drug on a farm it may be possible to substitute the resistant parasite community for a susceptible one. To date, several studies have been conducted to examine the feasibility of replacing BZ resistant *H. contortus* (Van Wyk and Van Schalkwyk, 1990) or *T. circumcincta* (Moussavou-Boussougou et al., 2007), or multi-drug resistant nematodes (Bird et al., 2001; Van Wyk et al., 2001; Sissay et al., 2006) with susceptible nematodes on pasture. These studies have shown that it is possible to reintroduce susceptible nematodes either by reducing the resistant parasites to negligible levels on pasture and infecting either entire flocks or seeder lambs with susceptible parasites to re-contaminate pastures (Van Wyk and Van Schalkwyk, 1990; Bird et al., 2001; Van Wyk et al., 2001; Moussavou-Boussougou et al., 2007) or by reducing resistant nematodes to very low levels in the hosts and transferring the hosts to pastures with high levels of nematodes in natural refugia (Sissay et al., 2006). Whilst most of these studies were conducted in subtropical and tropical areas where high temperatures during the summer months (Van Wyk and Van Schalkwyk, 1990; Bird et al., 2001; Sissay et al., 2006) or cold, dry winters (Van Wyk et al., 2001) render the pasture safe or even clean of infective larvae in only a few months, the experiment by Moussavou-Boussougou et al. (2007) was performed in a temperate region where infective larvae can survive for many months on pasture. Although this approach has the potential to reduce the prevalence of resistant parasites on a given area, there are practical limitations which may prevent its widespread use, e.g. the availability of large numbers of pathogen-free susceptible larvae with which to infect seeder animals and the ability to reduce pasture contamination to negligible levels. In addition, the success of reseeding pastures with susceptible parasites, particularly when the anthelmintic to which resistance has been previously identified is to be used, is likely to be dependant on the incorporation of management strategies that are designed with refugia in mind.

#### 4.2. Targeted treatments

Targeted treatments can be defined as whole flock treatments given at the most appropriate times bearing in mind the need to maintain refugia. These treatments differ from strategic treatments which are generally given prophylactically on the basis of historical knowledge of parasite epidemiology in a given area and are used to protect animals and prevent disease over a substantial period. The targeted treatment approach serves to reduce

the numbers of anthelmintic treatments given to a flock and thus minimise pasture contamination with resistant genotypes. Effective targeting should also lead to an increased interval between treatments, allowing susceptible genotypes a better opportunity to establish on pasture and so reduce the risk of heterozygotes mating and producing homozygous resistant offspring. Recent examples of the use of targeted treatments include studies conducted in Western Australia (Besier and Love, 2003) and Italy (Cringoli et al., 2008) where treatments were stopped during summer months when few parasites were present on pasture and given instead at other times when more parasites were present in refugia.

#### 4.3. Targeted selective treatments (TST)

Targeted treatments can be further enhanced by selective treatment of only those animals that will most benefit from treatment, leaving the rest of the flock untreated. GI parasite populations in small ruminants are highly aggregated and over-dispersed within the host, with approximately 80% of the worms found in only 20–30% of the hosts, whilst the vast majority of hosts possess low worm burdens (Sreter et al., 1994). Selective treatments should be directed towards those animals that are disease susceptible (non-resistant and/or non-resilient) or those that most contaminate pasture, however this requires the ability to identify these individuals within a flock. Targeted selective treatments (TST) exploit both epidemiologically appropriate treatment times and treat only those animals that would benefit from treatment. In this way the TST approach provides a continual source of worms in refugia even at times when treatments are being administered.

#### 5. Potential markers as indicators for targeted or targeted selective treatment (TST)

There are several methods that have been suggested to target or selectively target anthelmintic treatments, which range from visually identifying the animals to treat, to using parasite-based or pathophysiological markers to indicate that treatment is required (Besier, 2008). Although it is clear that the extent of the parasite induced penalty is influenced by many factors including host nutrition, immunological responsiveness, genotype and the species of nematode, the exhibition of clinical signs can generally be considered to be density dependant, with greater parasite challenge associated with more severe disease. Challenge density dependant affects are one of the key factors that determine which are the most appropriate markers to use under the prevailing circumstances.

An ideal indicator for use in targeted or selectively targeted treatments would be cost-effective, simple to use, require minimal operator training and allow treatment decisions to be made 'sheep-side'. Pathophysiological markers such as anaemia and dag score, parasite-based markers such as FEC and production indices such as milk production and live weight gain (Bisset et al., 2001b; Van Wyk and Bath, 2002; Riley and Van Wyk, 2009) have been examined as potential markers for TST regimes and in

theory, wool production and body condition score may also be suitable (Van Wyk and Bath, 2002; Riley and Van Wyk, 2009). The potential indicators and their suitability for use as targeted treatment or TST markers are discussed below.

### 5.1. Pathophysiological indicators

#### 5.1.1. Anaemia caused by haematophagous parasites

The main proof-of-concept studies for the use of TST were conducted in South Africa, where the FAMACHA® method was devised and evaluated (Bath et al., 1996; Van Wyk et al., 1997; Malan et al., 2001; Vatta et al., 2001). FAMACHA® assesses the colour of the conjunctival mucosal membrane, on a five-point scale, to estimate the degree of anaemia present as caused by the blood-feeding nematode, *H. contortus*. Anaemic animals, scoring 4 or 5 on the FAMACHA® scale, are at risk of disease and are treated (Bath et al., 2001; Vatta et al., 2001, reviewed by Besier, 2008). However, the physiological status of the sheep needs to be taken into consideration. For instance, lactating ewes are considerably more susceptible than non-lactating or heavily pregnant ewes, with the result that these animals require more liberal treatments. The advice for the use of FAMACHA has been updated and Van Wyk and Bath (2002) now recommend that all sheep in FAMACHA® category 3 should be drenched routinely and animals with a FAMACHA® score of 2, or even all animals, should be drenched when there are signs of unabated worsening of the average level of anaemia in a flock despite treatment of FAMACHA® categories 3–5. This system can effectively target those animals requiring treatment and it has been shown to reduce the number of anthelmintic treatments required in any one season. Preliminary trials indicate that animal performance may be unaffected or only marginally affected (Vatta et al., 2001) by treating animals on the basis of FAMACHA score. Although the system is labour intensive and is suitable for the identification of infection by haematophagous parasites only, the benefits include the ability to make the decision to treat ‘sheep-side’, it is low cost, and requires low levels of expertise and training to carry out the assessment (Van Wyk and Bath, 2002).

The FAMACHA® system has been used with success in many countries in addition to South Africa such as Guadeloupe (Mahieu et al., 2007), United States (Burke et al., 2007) and Central Kenya (Ejlertsen et al., 2006) and is particularly beneficial to resource poor farmers who, generally, have fewer animals and abundant access to labour sources. Recently there has been a large uptake of the system by farmers in the United States (Kaplan et al., 2004). In addition to its use to identify those animals that require anthelmintic treatment, FAMACHA® has also been evaluated as a method to aid in the identification of GI resistance/resilience traits in stud lambs in South Africa (Bisset et al., 2001b; Burke and Miller, 2008) and the USA (Riley and Van Wyk, 2009).

#### 5.1.2. Dag scores

The subjective measurement of the level of breech faecal soiling (dag scores) present in lambs could offer a

means to identify those animals requiring treatment. Although studies have shown that increased dag scores are associated with decreased FEC (Morris et al., 2000, 2005), one study in the UK suggests that at mid to late summer the situation is reversed, with a positive correlation between increased dag score and high FEC in lambs (Broughan and Wall, 2007). A recent study conducted in Western Australia used scouring as the primary and weight gain as secondary indicators to target anthelmintic treatments in groups of 12-month old Merino sheep. The majority of treatments were given due to the presence of scouring. Although weight gains were reduced by only 5% in the targeted group compared to a group where the whole flock was treated according to a strategic regime (Besier, 2008), the authors suggest that scouring is not a suitable indicator in 12-month old animals as by the time scouring was evident a loss in performance had already occurred (R.B. Besier, personal communication, 2008).

### 5.2. Parasitological indicators

#### 5.2.1. Faecal egg count (FEC)

FEC could potentially be used to target treatments effectively. Leathwick et al. (2006a) compared the effect of a suppressive drenching regime to that of a targeted regime where animals were treated on the basis of mean FEC (group mean exceeded 500 eggs per gram) on lamb performance and the parasite populations present. There was little difference between the numbers of anthelmintic treatments given to the two groups. The targeted treatment group received one less treatment than the suppressive treatment group (5 treatments compared to 6 in the suppressive treatment regime) during the experimental study. Results showed that there was no significant difference in live weight gain or fleece weight between treatment groups, although there were higher numbers of *H. contortus* and *Trichostrongylus colubriformis* present on the pasture of the targeted treated group. Thus, FEC is potentially a suitable indicator to target treatments more effectively, although the effects on anthelmintic resistance still require exploration.

Whilst FEC has potential for use as an indicator to target whole flock treatments more effectively, there are still some obvious limitations which will affect its use in practice. Generally, FEC samples have to be sent to a laboratory for analysis and the treatment decision is not, therefore instant or ‘sheep-side’. This approach can require animals to be gathered twice for treatment which may pose some practical difficulties. There are systems available that allow farmers to analyse FEC themselves on site which may overcome some of these shortcomings by minimising the interval between sampling and treatment. However, not only are there no widely accepted defined thresholds for treatment, it is also clear that these thresholds will vary between the different nematode species. In animals infected with *H. contortus* (Roberts and Swan, 1981; Coadwell and Ward, 1982) or *T. colubriformis* (Berajaya and Copeman, 2006) FEC is strongly correlated with worm burden. However, this relationship does not hold true for infection with other nematode species, especially *Nematodirus* spp. (Coles et al., 1986) and *T.*

*circumcincta* (Jackson and Christie, 1979). In addition, in areas where coinfection with many nematode species occurs, the high relatively high egg production of *H. contortus* may tend to mask the much lower egg production of species such as *T. colubriformis* and *T. circumcincta*.

### 5.3. Performance based indicators

#### 5.3.1. Milk production

Epidemiological studies in dairy goat flocks in France have shown that animals in their first lactation and high milk producing multiparous animals have higher faecal egg counts (Hoste et al., 2002a). Targeting anthelmintic treatments towards these two groups in a 2-year study reduced anthelmintic treatments by 48% and 66% in years 1 and 2 respectively, with no negative effects on milk production or worm egg excretion (Hoste et al., 2002b). The TST approach was then tested in 11 dairy farms in France in a 2-year survey resulting in the reduction of anthelmintic usage by 40% with no significant associated changes in egg output or milk production observed (Hoste et al., 2002c), compared to conventionally treated animals. These results show that milk production could be an appropriate marker to identify those animals requiring treatment, whilst maintaining production.

#### 5.3.2. Liveweight gain

Liveweight gain could be a sensitive marker for the identification of animals requiring treatment, especially in areas where haematophagous parasites are not present, as it is relevant to both farm economics and the range of parasites present. Studies have shown that liveweight gain is reduced in animals infected with GI parasites (Coop et al., 1977, 1988; Hubert et al., 1979) and the reduction in liveweight gain in lambs infected with *T. circumcincta* occurred early in the infection prior to the appearance of overt clinical signs (Coop et al., 1977). To date, two approaches have been investigated to use liveweight gain as an indicator.

One approach is to leave a proportion of the heaviest animals untreated at each drenching occasion. Leathwick et al. (2006b) examined the effect of leaving the heaviest 15% of lambs untreated at each of 6 suppressive treatments throughout the grazing season in New Zealand and found that there was a slower development of resistance in *Teladorsagia* spp. in the targeted group. Another study where the heaviest 10% of the flock remained untreated found no significant differences in liveweight gain, compared to a flock where all animals were suppressively treated (Leathwick et al., 2006a). Increased numbers of *H. contortus* and *T. colubriformis* were found on pasture, although the long term implications of this with regard to increase pasture contamination leading to higher parasite challenge in subsequent years was not examined. Other studies with *H. contortus* have shown that, as judged by the effects of parasite challenge on grazing sheep, there was no further increase in pasture contamination beyond the first year in the paddocks of TST treated animals (Van Wyk and Bath, 2002; Van Wyk, 2006; Riley and Van Wyk, 2009).

The use of a threshold of liveweight gain as an indicator for treatment can be further refined by taking into account some of the external influences which may affect lamb growth. To this end, the efficiency of energy utilisation can be calculated using estimates of both energy intake and energy deposition in the carcass (Greer et al., 2009). This approach takes into account environmental influences such as herbage availability and quality and provides a treatment threshold efficiency value that can be applied across flocks and at different times both within and between grazing seasons. The results of the efficiency calculation were used to predict the weight gain of each animal and treatment was then given depending on whether or not each animal had gained the stated weight. Replicated field trials conducted over 2 years showed that lambs in the TST group grew either as well as, or had a small reduction in performance, compared to a suppressively treated group, but approximately 50% less drench was required in the TST group. Efficacy of ivermectin treatment was maintained in the TST group but dropped significantly in the suppressively treated group (Kenyon et al., unpublished data, 2008). The use of this index, coupled with electronic identification of animals and automatic weighing facilities may provide a method to accurately identify animals in need of treatment before major production losses occur, as was deemed necessary by Van Wyk et al. (2006). This is important in the meat-production focussed markets since there is evidence that lambs that suffer a check in growth will finish later than those that do not suffer a growth check (Coop et al., 1985). It should be noted that systems of suppressive treatment, such as the monthly treatments used by Kenyon et al. (unpublished, 2008) and Van Wyk (2008), are not sustainable, so TST systems should also be evaluated against conventional drenching practice instead of with suppressive treatment, as has been done to date.

## 6. Implementation of targeted and TST strategies

On farm implementation of refugia-based strategies is clearly dependant on researchers being able to define methods which can sensitively and specifically identify those animals that will gain either the greatest parasitological or production benefit from treatment, whilst allowing for effective monitoring of the animals to limit any production losses (Van Wyk et al., 2006). The most easily implemented TST indicators would have similar qualities to the ideal indicators suggested earlier, e.g. quick and simple to use, cost-effective, easily learned and allow treatment decisions to be made 'sheep-side'.

The proportion of animals that need to be left untreated to ensure that an effective parasite population remains in refugia is the subject of much debate within the scientific community. This proportion will depend not only on host susceptibility (e.g. lactating ewes) and the factors which influence the maintenance of refugia (e.g. the biotic potential of the parasites, infra and suprapopulation longevity and the extent of expression of immunity) but also the prevalence of resistant genes present within the population (Gaba et al., 2006) and the mechanism of resistance, i.e. whether resistance is conferred by multiple

or single genes. Optimal application of TST could also be expected to be farm specific, influenced by both the composition of parasite population and farm management. Studies conducted within a range of production systems in different regions are required to define the proportions of treated and untreated animals required to maintain effective refugia. It is also essential that farm advisors or vets fully appreciate the complex nature of these factors and their interactions prior to the recommendation of the most appropriate refugia-based approaches to farmers (Van Wyk and Bath, 2002; Van Wyk et al., 2006).

The acceptance by producers of TST strategies and their implementation may require a high level of input and education to the farming community. For many years, when parasite control mainly focussed on maximising animal production, farmers were encouraged to treat regularly and strategies such as dose and move were advocated to minimise pasture contamination. The need to consider the impact of refugia on the development of anthelmintic resistance has resulted in a change in the advice given, with farmers now being encouraged to leave some animals untreated to keep susceptible parasites present on their farms. In fact, Van Wyk (2006) goes so far as to suggest that since insufficient numbers of advisors with the necessary experience and the required funding remain, alternative methods such as on-farm automated decision support need to be developed. In order for refugia-based regimes to gain acceptance in the farming community, it is important that the suggested strategies minimise any loss in animal performance. For this reason it is imperative that extensive education is made available to both farmers and their advisors to ensure that the complex message of the new strategies designed to reduce the rate of development of anthelmintic resistance are fully understood. In addition, stakeholders must be made aware of the benefits of setting realistic performance targets and of using regular monitoring of animal performance and anthelmintic efficacy to acquire the necessary understanding required to achieve these targets, i.e. the trade off between anthelmintic usage for maximal animal production and selection for anthelmintic resistance. It is also crucial that practitioners/farmers and advisors are made aware not only of the cost benefits, and potential disadvantages, accruing from a TST approach but also those associated with extreme multi-drug resistance. The primary challenge for researchers is the need to develop strategies that can slow the development of resistance whilst still maintaining optimal performance and thus form the basis of sustainable integrated parasite management schemes (Van Wyk et al., 2006).

When using refugia-based control strategies it will be imperative to be able to effectively monitor any changes in anthelmintic resistance status occurring in the parasite population. Whilst the use of FECRTs to effectively monitor changes in the anthelmintic susceptibility of parasite populations is practicable, it also has its limitations due to the relative insensitivity of this test. The need for sensitivity in resistance tests has focussed attention on molecular markers for drug resistance. These are currently only available for mutations in the  $\beta$ -tubulin gene that arise in BZ resistance as there are currently no identified

markers for resistance to the macrocyclic lactone or levamisole families, although research is on-going in these areas.

In the future it may be possible to define immunological or genetic markers to identify resilient animals or those at most risk of parasitic disease. Knowledge of which breeding animals in the flock are most likely to require treatment would allow those animals to be monitored more closely for signs of infection and information on the resistance and resilience of individual animals would allow selective breeding to increase the proportion of resilient animals in a flock. Indeed, highly significant levels of correlation have been found between FEC and the FAMACHA score (Bisset et al., 2001b; Riley and Van Wyk, 2009) and heritability of FAMACHA values appears to be similar to that of FECs (Bisset et al., 2001a,b; Riley and Van Wyk, 2009) indicating that selective breeding using the FAMACHA system may increase both resilience and resistance within a given flock.

## 7. Conclusions

Maintaining an effective refugium for a proportion of the worm population is now considered one of the most important factors determining the rate of the development of resistance and should be included in any potential prophylactic control regime suggested for nematode parasites. The evidence to date suggests that targeted treatments or TST strategies make it possible to reduce anthelmintic usage in ruminants in such a way as to slow the development of anthelmintic resistance whilst still maintaining animal performance. However, there is a clear need for the development and validation of sustainable, objective, user-friendly and regionally specific indicators for treatment. In addition, trials should be conducted to develop a better understanding of the proportion of untreated animals necessary to maintain effective refugia under conditions of differing parasite species, environments and animal management regimes.

## Conflict of interest statement

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