Is anthelmintic resistance a concern for heartworm control?
What can we learn from the human filariasis control programs?

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Abstract

Heartworm prophylaxis is currently largely dependent on the ability of avermectins and milbemycins to arrest the development of third and fourth stages of *Dirofilaria immitis* for prolonged periods, without producing adulticidal effects. Major control programs, dependent on the activity of ivermectin, are being implemented for human onchocerciasis and lymphatic filariasis. The avermectins and milbemycins act on glutamate-gated and γ-aminobutyrate-gated chloride channel subunit proteins in nematodes. Ivermectin resistance has been widely described in trichostrongylid nematodes of ruminants. There is evidence that when ivermectin resistance occurs in nematodes, there may be selection on some, but not all of the genes that code for ligand-gated chloride channel subunit proteins as well as on some ABC-transporter genes, whose products may be involved in regulating macrocyclic lactone drug concentrations at receptors, and on some structural protein genes of amphidial neurones. Although ivermectin resistance has not been reported in filarial nematodes, there have recently been reports of suboptimal responses to ivermectin in *Onchocerca volvulus*. Evidence has been found of ivermectin selection on at least ABC-transporter genes and some neuronal structural protein genes in *O. volvulus*. To date, there is no evidence of avermectin/milbemycin resistance in *D. immitis*, also a filarial nematode. Chemotherapy against trichostrongylids of animals, human filariae, and *D. immitis*, relies on avermectins or milbemycins. However, control involves targeting different stages or processes in the nematode life cycle, different control strategies, different proportions of the nematode population in refugia, and different药 dosage rates. Consideration of the proportion of the *D. immitis* population normally in refugia, the life cycle stage targeted, and the anthelmintic dosages used suggest that it is unlikely that significant avermectin/milbemycin resistance will be selected in *D. immitis* with current treatment strategies.

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1. Introduction

Chemotherapy with anthelmintics has played a major role in the control of nematode parasites of animals and humans. Avermectins, such as ivermectin (IVM), have played a key role in control of filarial nematodes, such as the heartworm *Dirofilaria immitis*; *Onchocerca volvulus*, which causes human onchocerciasis (river blindness); and *Wuchereria bancrofti*, which causes lymphatic filariasis or elephantiasis in humans. Other avermectins, such as selamectin and milbemycins, such as moxidectin and milbemycin oxime, are also important in the prevention of heart-
worm disease. Avermectins and milbemycins are also of major importance in the control of other nematode parasites in farm and companion animals. Avermectin, and to a lesser extent, milbemycin resistances have become widespread problems in the control of nematode parasites of ruminants (Kaplan, 2004).

Given that avermectin/milbemycin resistance has developed in many nematode parasites and that these anthelmintics are of key importance for the control of filarial nematodes in humans and animals, this review focuses on the situation in human filarial parasites where major regional and global control programs have been launched. These programs entirely depend on chemoprophylaxis with anthelmintics, how the avermectin/milbemycin anthelmintics work, our knowledge of the genetics and mechanisms of avermectin/milbemycin resistance, and selection factors that determine the rate of resistance development, in order to address the question of whether anthelmintic resistance is likely to develop from current heartworm control practices.

2. Anthelmintic resistance in nematode parasites

2.1. Geo-nematode parasites of animals

The most common anthelmintic resistance problems have occurred in geo-nematodes of ruminants and horses. Benzimidazole (BZ) resistance is widespread in trichostrongylid nematodes and small strongyles of small ruminants and horses, respectively. Resistance to the imidazothiazole/tetrahydropyrimidines is also common in these nematode parasites. Resistance to the avermectins is widespread in some trichostrongylid nematodes of ruminants, and there are increasing numbers of reports of developing resistance to the milbemycin anthelmintics in trichosstrongylid nematodes of ruminants and in Parascaris equorum in horses. The anthelmintic resistance status in nematodes of animals has recently been reviewed (Kaplan, 2004).

2.2. Geo-nematode parasites of humans

There are currently only a few reports that suggest possible occurrence of anthelmintic resistance in human geo-nematode parasites (De Clercq et al., 1997; Reynoldson et al., 1997; Albonico et al., 2003). In the past, control of human geo-nematode parasites has focused on reducing morbidity in school-age children with periodic and often sporadic mass treatment of this cohort without substantial concurrent efforts to reduce transmission from other cohorts of human hosts (e.g. adults) or effective concurrent environmental prevention of transmission. Under these circumstances, selection pressure for the development of anthelmintic resistance is likely to be low in human geo-nematode parasites. However, the World Health Organization (WHO, 2001) is setting the following minimal targets aimed at reducing morbidity due to soil-transmitted nematode infections by 80%, which can be achieved by all endemic countries, as an integral part of the health system: (1) regular chemotherapy for at least 75% of all school-age children at risk of morbidity from geo-nematodes by 2010; (2) access to essential anthelminthic drugs by health services in endemic areas, down to the most peripheral level, for the treatment of symptomatic cases, as well as children, women, and other groups at risk of morbidity.

Furthermore, entire communities, involving large numbers of people (350 million or more) are to be treated annually, with combination albendazole (ABZ) plus diethylcarbamazine (DEC) or ABZ plus IVM as part of the programs of the Global Alliance for the Elimination of Lymphatic Filariasis (GAELF). ABZ and IVM treatment to prevent transmission of lymphatic filariae will also remove geo-nematodes. These various control measures will increase selection pressure for anthelmintic resistance to develop in human geohelminth parasites. However, the free-living stages of these parasites often survive for long periods in soil, providing a very large refugium. Unless concurrent efforts are made to reduce environmental contamination and treatment frequencies increase from once per year, the large proportion of the nematode population in the unpressured free-living stages is likely to dilute out resistant worms that survive treatment, resulting in only a low level of selection for resistance in human geo-nematode parasites.

2.3. Filarial nematode parasites of humans

In 1987, WHO commenced using IVM to treat human onchocerciasis, following a generous donation from Merck & Co. (Molyneux, 1995). IVM, usually
given for onchocerciasis as an annual oral treatment at 150 μg/kg, is microfilaricidal, and therefore, reduces transmission and morbidity associated with the microfilariae. Single-dose IVM is not significantly macrofilaricidal, although repeated treatment may kill some *O. volvulus* macrofilariae (Gardon et al., 2002).

Of critical importance to the effectiveness of IVM against onchocerciasis, as a single annual treatment, is its ability to markedly impair reproduction in adult *O. volvulus* and to maintain inhibition of microfilarial production for several months (Awadzi et al., 1999). Annual IVM treatment has dramatically improved the social and economical well being of people in areas affected by this parasite. Unequivocal IVM resistance in *O. volvulus* has not been documented, despite more than 16 years of annual treatment in some parts of West Africa. However, recently the finding of patients with surprisingly high microfilarial counts, despite many rounds of IVM treatment (Ali et al., 2002; Awadzi et al., 2004) suggests the possibility of some suboptimal responses to IVM treatment. Among a number of possible causes of suboptimal response to treatment could be a developing drug resistance. Because IVM does not kill most of the adult worms but causes a temporary reduction in microfilarial production, and because there are no animal hosts for *O. volvulus* that would provide a means for experimental investigation of resistance, parasitological definition of resistance in onchocerciasis is very difficult. At the present time, it is not clear how IVM resistance would first manifest itself parasitologically in *O. volvulus*. However, drug resistance is a genetic process. Therefore, selection for anthelmintic resistance would be seen as a selection for particular alleles of genes that would improve the reproductive fitness of *O. volvulus* in the presence of an IVM-based control program. Information on the genetics of selection for IVM resistance will be considered below.

More recently, the GAELF has been launched. In parts of the world where onchocerciasis can be concurrent with lymphatic filariasis (mainly in sub-Saharan Africa), which precludes the safe use of DEC, IVM is being co-administered annually with ABZ. In the rest of the world, interruption of transmission of lymphatic filariae is usually being attempted with combination ABZ and DEC annual treatment, and in some areas, by DEC alone. There have been a few reports of suboptimal responses in *W. bancrofti* to DEC (e.g. Eberhard et al., 1991) and an increasing number of anecdotal reports of sub-optimal responses to DEC or ABZ plus DEC. Again, there is no animal host available for studies of possible anthelmintic resistance in *W. bancrofti*, so it is difficult to confirm anthelmintic resistance in lymphatic filariasis. Because the anthelmintics used to prevent transmission of lymphatic filariasis are not highly macrofilaricidal and have complex and long-term effects on reproduction as well as short-term microfilaricidal effects, it is not presently clear how anthelmintic resistance would be first manifested parasitologically in lymphatic filariae. Information on the genetics of selection for possible anthelmintic resistance in *W. bancrofti* will be considered below.

### 3. Mechanisms of action of anti-nematode anthelmintics

The mechanism of action of the broad-spectrum anthelmintics has been studied primarily in trichostongylid and ascarid nematodes, such as *Haemonchus contortus*, *Ascaris suum*, and the free-living nematode *Caenorhabditis elegans*. In nematodes that have been studied, BZs act by binding to b-tubulin, inhibiting the polymerization of tubulin and the formation of microtubules. The lack of microtubules inhibits many cellular functions such as transport, cell division, neuronal transmission, and cell differentiation, ultimately leading to cell death. Imidazothiazole/tetrahydropyrimidines, such as levamisole and pyrantel act as agonists at nicotinic receptors at nematode neuromuscular junctions, causing a spastic paralysis in nematodes. The avermectin/milbemycins bind to glutamate- and γ-aminobutyrate (GABA)-gated chloride channels subunit proteins, irreversibly opening the chloride channels and causing a hyperpolarization of nerve or muscle cells, leading to a flaccid paralysis (Prichard, 2001). In nematodes that ingest nutrients via the mouth, the pharyngeal muscle seems to be most sensitive to avermectins (Geary et al., 1993).

Little work has been done on the mechanisms of action of anthelmintics in filarial nematodes. As microtubules are ubiquitous in nematodes and essential, it seems reasonable to expect that BZ anthelmintics would also bind β-tubulin and disrupt polymerization to microtubules in filariae. BZs are not...
very lethal to filarial worms, but do impair microfilarial production. In the case of *W. bancrofti*, impairment of reproduction may be sustained for a considerable period of time (Ottesen et al., 1999).

Adult filarial worms, such as *D. immitis*, are large and if killed rapidly, can release a large amount of worm antigen, which can lead to anaphylaxis and other pathologies. Levamisole causes rapid paralysis in some filarial worms and is contraindicated when adult *D. immitis* are present. In human filariasis, levamisole is not effective and will not be further considered.

DEC is used extensively for lymphatic filariasis, except in sub-Saharan Africa. It has larvicidal, microfilaricidal, and some macrofilaricidal effects. In the past, it was used for heartworm chemoprophylaxis. However, since the advent of the highly effective avermectins and milbemycins, it is now used less frequently for heartworm prevention. Although a competent host immune system is required for DEC effectiveness, the specifics of its mode of action in filarial nematodes are not at all understood. As a result, it will not be possible to discuss possible mechanisms of resistance to DEC. Nevertheless, the increasing amount of DEC being distributed to humans as part of the GAELP and increasing evidence of inadequate parasitological responses, as discussed above, warrant investigation of its actions, possible mechanisms of resistance, and the development of molecular markers for resistance in lymphatic filariae.

The mechanisms of action of the avermectin/milbemycins in filariae are not well understood, although it is assumed that these anthelmintics still act on glutamate- and GABA-gated chloride channels. Certainly, they cause paralysis to microfilariae in vitro (Tagboto and Townson, 1996), and filarial worms have these ligand-gated chloride channels (Cully et al., 1996). Nevertheless, there are some differences between filarial nematodes and other nematodes. The avermectin/milbemycins are not very lethal to macrofilariae, although, repeated IVM treatments do cause the loss of some adult *O. volvulus* (Gardon et al., 2002). However, some larval stages of filarial worms are very sensitive to avermectin/milbemycins. For *O. volvulus*, *W. bancrofti*, and *Brugia malayi*, dosage rates of 150 μg/kg are lethal to most of the microfilaria present at the time of treatment, whereas for *D. immitis*, dosages as low as 1–10 μg/kg of some avermectin/milbemycins are lethal for weeks and even months against developing L₃/L₄ stages. This exquisite sensitivity of the developing larval stages of *D. immitis* to avermectin/milbemycins is the basis of the chemoprophylaxis of heartworm. Although, IVM may also affect the developing larval stages of *Onchocerca ochengi* (Tchakoute et al., 1999), the chemoprophylaxis of heartworm is unique in specifically targeting these stages of the nematode life cycle. Another difference between filarial and other nematodes in the action of avermectin/milbemycins is in the mechanism of nutrient uptake. Most nematodes ingest nutrients through the mouth, and the pharyngeal muscle, which is highly sensitive to avermectin/milbemycins, plays a key role in pumping nutrients into the intestine where they are absorbed. In contrast, the pharynx is vestigial in filarial nematodes and nutrients are absorbed via the tegument. Thus pharyngeal paralysis is unlikely to adversely affect filariae, at least in the adult stages. However, the reproductive system of filarial nematodes seems to be very sensitive to long-term effects from avermectin/milbemycin treatment (Duke et al., 1991). While reproduction is affected by avermectin/milbemycins in non-filarial nematodes, the effects are more transient than in filarial nematodes. The involvement of ligand-gated chloride channels in nematode reproduction is not well understood, although normal muscle tone is necessary for reproduction. In humans, IVM has a half-life of about 18 h (Guzzo et al., 2002), yet some of the effects of IVM, on filarial worm reproduction, last in excess of one year. At present, we do not have a clear understanding of how this prolonged effect, which is the key effect for the impact of IVM in the human filariasis control programs, is achieved. Because of these marked differences between filarial nematodes and other nematodes, it is not possible simply to extrapolate from mode of action and resistance mechanisms of avermectin/milbemycins in non-filarial nematodes to filariae. Nevertheless, an understanding of resistance mechanisms and the genetics of resistance in non-filarial nematodes can provide useful information about possible mechanisms in filarial nematodes.

4. The genetics and mechanisms of resistance to anti-nematode anthelmintics

Resistance to BZs is associated with mutations in β-tubulin genes that prevent the drugs binding to their
target (Prichard, 2001). A Phe-Tyr polymorphism at codon 200 of β-tubulin has been well characterized in trichostrongylid nematodes. More recently, a second Phe-Tyr polymorphism, at codon 167 of beta-tubulin, was detected in BZ-resistant populations of H. contortus. Investigations on BZ-resistant trichostrongyle field populations revealed that a codon Phe167-Tyr polymorphism may contribute to BZ resistance in Teladorsagia circumcincta in sheep (Silvestre and Cabaret, 2002) and small strongyle species from horses (Drogemuller et al., 2004). Recently, the presence of the BZ-resistance associated Phe200Tyr polymorphism has been found in populations of W. bancrofti (Schwab and Prichard, in press).

The genetics and mechanisms of resistance to the avermectin/milbemycin anthelmintics are still being unraveled. These drugs act on ligand-gated chloride channels, including glutamate-gated chloride channels (GluCl) and γ-aminobutyrate-gated chloride channels (GABACl), a family of receptors widely distributed in nematodes that regulate locomotion, feeding (in non-filarial nematodes) and reproduction (Brownlee et al., 1997; Feng et al., 2002; Yates et al., 2003). The avermectin/milbemycins have effects on all of these functions, but it is likely that their relative importance in the overall anthelmintic activity varies among species and life-cycle stages, and thus mechanisms of resistance could also vary. Indeed, even different avermectin-resistant strains of the same species, H. contortus, have varying phenotypes (Gill and Lacey, 1998). Gene knock-out studies in C. elegans have shown that deletion of 3 GluCl genes in the pharynx or extrapharangeal neurons causes loss of sensitivity to IVM in this free-living nematode (Dent et al., 2000). Binding studies have failed to find any consistent changes in radiolabelled IVM binding to membranes from resistant H. contortus or T. circumcincta (Païement et al., 1999; Hejmadi et al., 2000), suggesting that target-site mutations are not the major mechanism of resistance in these species. However, population studies found evidence for selection at GluCl genes and a GABA receptor-related gene in H. contortus and C. oncophora (Blackhall et al., 1998a; Blackhall et al., 2003; Njue and Prichard, 2004). IVM increased the response to GABA in cells transfected with an unselected wild-type allele of the H. contortus GABACl receptor subunit gene, whereas in the cells transfected with the avermectin/milbemycin-selected GABACl allele, IVM attenuated the response to GABA (Feng et al., 2002). Recently, Njue et al. (2004) found a polymorphism in a GluCl subunit from an IVM-resistant field isolate of Cooperia oncophora that caused the resultant channels to be less sensitive to both glutamate and ivermectin when expressed in vitro. So far, no evidence for IVM selection on GluCl or GABACl genes has been found in filarial nematodes (Eng and Prichard, in press).

P-glycoproteins (Pgp) and other ABC-transporter genes are involved in avermectin/milbemycin resistance in trichostrongylid nematodes (Xu et al., 1998; Blackhall et al., 1998b; Kerboeuf et al., 2003) and are under selection by IVM in O. volvulus (Ardelli and Prichard, 2004, in press; Eng and Prichard, in press). IVM is an excellent substrate for these pumps (Pouliot et al., 1997) and Pgp inhibitors such as verapamil, enhance the activity of avermectin/milbemycins (Molento and Prichard, 1999). Higher levels of some ABC-transporter activities may increase rates of efflux of IVM and other avermectin/milbemycins, lowering anthelmintic concentrations at the drug site of action. Some avermectin/milbemycins may be more susceptible to ABC-transporter efflux mechanisms than other avermectin/milbemycins (Molento et al., 2004; A. Roulet, et al., personal communication).

An intriguing further observation is that the amphids (sensory structures in the head of nematodes) are altered in avermectin-resistant H. contortus (Freeman et al., 2003). The amphids form a pathway from the environment into the interior of the worm, so defects could preclude drugs gaining access to their target sites. In nematodes, bundles of amphidial neurons extend anteriorly from the nerve ring, which encircles the pharynx and terminate near the amphidial openings. These neurons have extensive microtubule bundles, which appear to be shortened and deranged in IVM resistant H. contortus (Freeman et al., 2003). It is interesting that Eng and Prichard (in press) have recently found evidence that there is selection for a particular allele of beta-tubulin in IVM selected O. volvulus. At first sight, these data seem complicated, so what hypotheses can be formulated to explain them? There are a number of possibilities. Resistance to the avermectin/milbemycin anthelmintics could be caused by a gain-of-function mutation in Pgp or ABC transporter genes, leading to more rapid removal of the drug from receptor sites in the worms.
Such mutations could either cause increased expression of a pump capable of carrying the avermectin/milbemycin, or change the specificity to increase the affinity for these substrates. These genes are very polymorphic in nematodes that have been examined, including *O. volvulus*, and mutations that alter pump expression or activity, if present at a low frequency, could be selected rapidly. Alternatively, parasites could become resistant by the accumulation of one or more mutations in GluCl or GABACl genes that may affect the primary action of the avermectin/milbemycin. The role of β-tubulin in avermectin/milbemycin resistance warrants further investigation, but mutations in this gene may affect the structure and uptake of avermectin/milbemycin via the amphids or affect ligand gated chloride channel function or transmission of neuromuscular signals, since microtubules are known to play a role in anchoring neurotransmitter channels at synapses. The exact genes involved may vary among species, depending on their relative importance in drug action. Since GluCls are expressed in amphid and extrapharyngeal neurones (Portillo et al., 2003; Liu et al., 2004) defects in these neurones could also cause the observed changes in morphology and response to avermectin/milbemycin. Further studies comparing GluCl, GABACl, ABC transport and β-tubulin genes in filarial nematodes that have been repeatedly exposed to IVM or are IVM-naive are needed: the first steps are to determine the number, sequence, and polymorphism of such genes and variations in genetic polymorphism in different treatment populations of filarial parasites.

5. Anthelmintic resistance selection in treatment of heartworm

Given current dependence on avermectin/milbemycin anthelmintics for heartworm prophylaxis, it is relevant to consider the experience with this group of anthelmintics in other nematode parasites, especially the human filarial nematodes, to ascertain possible selection pressure for resistance in *D. immitis*. Factors that will affect the rate of selection for anthelmintic resistance include the following:

- the extent of genetic polymorphism in the population;
- the initial frequency of resistance-contributing alleles;
- the number of genes involved and complexity of the resistance mechanism(s);
- the biology of the nematode;
- whether the resistance gene(s) are dominant or recessive in expressing the resistance phenotype;
- the extent of refugia;
- treatment coverage;
- the relative reproductive fitness of the wild-type (susceptible) and resistant genotypes in the absence and presence of the anthelmintic (possibility for reversion);
- treatment frequency;
- other management practices which may impact on parasite transmission;
- drug dosage in relation to the ED$_{99}$.

5.1. Genetic polymorphism

Some parasitic nematodes, such as *H. contortus*, exhibit extreme genetic diversity (Prichard, 2001). Little is known about genetic polymorphism in genes that may be required for the development of resistance in *D. immitis*. However, we do know that human filarial worms such as *O. volvulus* and *W. bancrofti* show moderate levels of genetic diversity (Ardelli and Prichard, 2004, in press; Eng and Prichard, in press; Schwab and Prichard, in press). The recent availability of the *B. malayi* genome (Ghedin et al., 2004) will improve the ability to analyze for genetic polymorphism in filarial nematodes. There is no reason to believe that *D. immitis* is not reasonably genetically diverse.

5.2. Initial frequency of resistance-contributing alleles

Most of the information available deals with the Phe200Tyr mutation in β-tubulin that confers BZ resistance in nematodes. This can be quite high in unselected populations of *H. contortus* (Prichard, 2001). In *W. bancrofti*, initial frequencies of the Phe200Tyr polymorphism in β-tubulin appears to be moderately high (Schwab and Prichard, in press) as do polymorphisms in ABC-transporter and β-tubulin genes that are selected for with IVM treatment in *O.
volvulus (Ardelli and Prichard, 2004, in press; Eng and Prichard, in press). These moderately high initial frequencies of alleles that may contribute to resistance will facilitate resistance selection.

### 5.3. The number of genes involved and complexity of the resistance mechanism(s)

The evidence so far suggests that several genes can contribute to avermectin/milbemycin resistance and the mechanisms of avermectin/milbemycin resistance are complex (Prichard, 2001). Initial work on human filarial nematodes suggests that IVM is also selecting on several genes (Ardelli and Prichard, 2004, in press; Eng and Prichard, in press). A low level resistance may be expressed when a small number of resistance-contributing genes are present in the same individual. However, the level of resistance will increase with further drug selection as more individual parasites accumulate several resistance-contributing genes. The observation of a resistance phenotype during this process of augmentation of resistance alleles will depend on the anthelmintic dose rate and its relationship to the ED_{99} dose rate in the initial susceptible population.

### 5.4. The biology of the nematode

The biology of a particular nematode can greatly affect the rate of selection for resistance. A parasite that has a high reproduction rate per individual female and a high rate of natural parasite turnover, such as H. contortus, can rapidly transmit resistance. Another parasite with a long lived free-living stage, such as Ascaris lumbricoides could be slow to develop resistance, because susceptible free-living stages, not affected by the drug treatment in the host, will continue to be transmitted for many years, diluting out any parasitic stages that survived drug treatment. Filarial worms have relatively high reproductive rates, favoring resistance transmission, provided that a resistant individual is still able to reproduce despite anthelmintic treatment. There are no free-living stages, but stages in the insect vector are short lived, favoring resistance selection. Conversely, the parasitic stages in the definitive host are long lived which could mitigate against resistance development. However, if anthelmintic-susceptible filarial worms fail to establish, survive, or reproduce due to anthelmintic treatment, while resistant individuals successfully complete all of these steps, the longevity of filarial worms will have little effect on slowing the rate of resistance selection.

### 5.5. Is resistance dominant or recessive?

BZ resistance is usually recessive (Prichard, 2001), and this generally slows the rate of selection for BZ resistance, despite initial high frequencies of resistance alleles. A genetic study suggested that IVM resistance is dominant in H. contortus (Le Jambre et al., 2000). When resistance is dominant, the resistance phenotype may rapidly become apparent in a population under selection. However, the situation in filarial nematodes is unknown.

### 5.6. Refugia

The extent of refugia at the time of anthelmintic treatment can be extremely important for the selection of anthelmintic resistance. In trichostrongylid nematodes of small ruminants, a low level of refugia at the time of treatment, such as when treatment occurs during drought seasons or the use of treat-and-move to clean pasture management practices, has been found to be critically important for rapid development of anthelmintic resistance (Swan et al., 1994; Van Wyk, 2001). Almost all of the nematode population of filarial worms is in the definitive host, with only a very small fraction of the population occurring in the insect vector. This is because the life span in the vector is short compared with that in the definitive host and also because the number of parasites in each vector is very small compared with relatively huge numbers in the definitive host (including adults, microfilariae and developing third and fourth stages). If treatment eliminates virtually all of the larval stages in the definitive host and shuts down reproduction in the surviving adults, as occurs in the human filarial nematodes, selection pressure for resistance will be very high. However, the same situation does not prevail with heartworm. With heartworm prophylaxis, animals that already have a patent infection should not be treated with avermectin/milbemycins, resulting in no selection pressure on these important sources of transmission. Only animals with infections limited to developing third- and fourth-stage larvae and perhaps
immature adults or single-sex infections, i.e. animals with no occurrence of parasite reproduction, should be treated. Thus, most of the parasite population will be in refugia, and selection for resistance will be minimal.

5.7. Treatment coverage

In small ruminants, where anthelmintic resistance has developed rapidly, the practice has been to treat the whole herd. With onchocerciasis and lymphatic filariasis control, the programs aim at a high level of coverage, typically 65–85% of the eligible population in a community. This again is conducive to selection for resistance. Recently, the Famacha system of only treating individuals showing pathology from *H. contortus* infection has been recommended for small ruminants (Van Wyk and Bath, 2002). In the case of heartworm, treatment varies among pet owners, and stray animals, which are perhaps the most important source of transmission in some communities, are rarely treated. Thus, treatment coverage of animals for heartworm is likely to be low to moderate and this will reduce selection for anthelmintic resistance.

5.8. Relative reproductive fitness of susceptibility and resistance genotypes

Resistance alleles probably confer some small disadvantage in terms of overall reproductive fitness compared with susceptibility alleles, in the absence of anthelmintic treatment. Otherwise the resistance alleles would be very common and the drugs would not be developed as anthelmintics. However, the work that has been done so far on the frequency of resistance-associated alleles in parasitic nematodes suggests that resistance alleles are not initially rare (see discussion above). This implies that their disadvantage to overall reproductive fitness is minimal, and in fact, when resistance develops and use of the anthelmintic class is curtailed, reversion to susceptibility is often not apparent (Waller et al., 1988). However, when an anthelmintic is used and it either kills the susceptible worms or suppresses their reproduction for a prolonged period, as in the case of the human filarial control programs, then any resistant parasites will have an enormous reproductive advantage over susceptible individuals, and resistance is likely to be selected. Because selection for resistance is likely to be weak in heartworm as a result of other factors discussed here, the relative overall reproductive fitness of any resistant *D. immitis* is not likely to be currently important.

5.9. Treatment frequency

Frequent treatment has often been portrayed as being very important for the selection of anthelmintic resistance. Selection for resistance needs to be considered in the context of refugia at the time of treatment, relative fitness of resistant parasites, the parasite’s biology, and other factors that may play a more important part in selection pressure. The aim of heartworm control is for suppressive prophylaxis in animals under treatment. However, other animals in a community may not be under any treatment and be the main source of transmission. Suppressive treatment in individual animals will not have much effect on the rate of selection for drug resistance unless treatment coverage is high in the whole community.

5.10. Other management practices which may impact on parasite transmission

Parasite control measures, such as widespread vector control, in the case of filarial nematodes, may reduce the overall filarial nematode population, and relatively rare alleles may be lost. This may have an effect on initial rates of anthelmintic resistance in the absence of re introduction of those rare alleles by vector or host immigration. This could be relevant to areas that were under heavy vector larviciding in early years of the Onchocerciasis Control Program in West Africa. However, it is not likely to be important for resistance selection in *D. immitis*.

5.11. Drug dose rate in relation to the ED$_{99}$

The ED$_{99}$ of an anthelmintic varies for different nematode parasites and different stages of the parasite life-cycle. The dosage rate for some heartworm anthelmintics such as IVM and moxidectin is in the low $\mu$g/kg range. This is very low compared with that used against non-filarial nematodes or human filarial nematodes. In general, higher dosages are more
effective than lower dosages. Very high efficacy, unless it is 100%, imposes higher selection pressure for resistance than does low efficacy if resistance is dominant at that dose rate. However, the effect of dosage has to be examined in the context of percent efficacy. An ideal dosage rate is one that reliably produces 100% efficacy. This may be the case in heartworm prophylaxis, but the issue needs further evaluation.

6. Conclusions

Anthelmintic resistance is already a severe problem in some trichostrongylid nematodes of ruminants and small strongyles of horses. The global and regional control programs for human filarial nematode parasites are likely to impose significant selection pressure for anthelmintic resistance to develop, and already there are a few reports of sub-optimal responses and genetic changes consistent with resistance being selected. However, in non-filarial nematode parasites in humans, selection for anthelmintic resistance is likely to be weak unless chemotherapeutic control is intensified. Because of certain factors (i.e., the extent of refugia, treatment coverage, the low drug dosage rate used, the number of genes involved, and the complexity of the resistance mechanism(s) to avermectin/milbemycin anthelmintics), which are important for resistance selection, the selection for resistance in *D. immitis* is likely to be very low, and a resistance problem is not likely to occur with current heartworm control practices.

References


