Drug resistance in veterinary helminths

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At present, there is no effective alternative to chemical control of parasitic helminths where livestock are grazed intensively. Resistance to anthelmintics has become a major problem in veterinary medicine, and threatens both agricultural income and animal welfare. The molecular and biochemical basis of this resistance is not well understood. The lack of reliable biological and molecular tests means that we are not able to follow the emergence and spread of resistance alleles and clinical resistance as well as we need. This review summarizes some of the recent findings on resistance mechanisms, puts forward some recommendations for limiting its impact and suggests some priorities for research in this area.

The use of effective compounds to kill pest species frequently leads to the development of resistance. If the organisms in question cause disease, this can mean a loss of our ability to treat or control that disease. This is true of viruses, bacteria, arthropods, parasitic protozoa, cancer cells and parasitic helminths. The introduction of an effective therapy can be accompanied by a period of complacency before biological reality reasserts itself, and we are left contemplating the reappearance of a problem previously thought solved. In the case of veterinary helminths, widespread resistance to anthelmintics (Box 1) has left us searching for ways to maintain their effectiveness until novel forms of control are found. We need to devise effective strategies to minimize the impact of anthelmintic resistance. This requires a much better understanding of the basic biology of the parasites, how they become resistant and what are the alternative means of control.

The extent of the problem

In small ruminants, anthelmintic-resistant nematodes are already a serious problem [1]. In Australia, for example, the prevalence and severity of resistance now threatens the profitability of the entire sheep industry [2]. Resistance has arisen to all of the major families of broad-spectrum anthelmintics [3], the benzimidazoles (BZ), levamisole (LEV) and the other nicotinic agonists, in addition to the avermectins and milbemycins (AM)

(including ivermectin, doramectin and moxidectin). Nematodes that are resistant to other, narrow-spectrum anthelmintics, such as closantel, have also been reported [3]. The situation in cattle is currently less severe, but there are cattle nematodes resistant to multiple anthelmintic classes in New Zealand and South America [4,5], and this will probably become more widespread. In horses, BZ resistance is that which widespread among the cyathostomins: the AM are still effective for cyathostomins, but not for *Parascaris* in foals [6–8]. This could change as AM are used more frequently and selection pressure increases (Box 2).

Although resistance in flukes has not yet reached the levels present in nematodes, resistance exists for the salicylanilides, rafoxanide and closantel, with evidence of cross-resistance to the halogenated phenol, nitroxynil [9]. Of greater concern is the spread of resistance to triclabendazole, the main drug used to treat fluke infections because of its high activity against the migrating immature stages. Resistance was first reported in Australia in 1995 [10] and has since been described in The Netherlands, UK and Ireland. At the same time, there has been a dramatic resurgence of fasciolosis as a result of climate change and the advent of milder, wetter weather [11]. In the UK, for example, the 2002–2003 season in Scotland and northern England was described as the worst on record for the number of fasciolosis cases (see: http://www.endoparasite.net). Abattoir data indicate that ∼50% of cattle livers and >20% of sheep livers were condemned as a result of fluke infection [12].

Anthelmintic resistance is a threat to agricultural incomes, already under pressure in many parts of the world, and the increase in disease also poses a threat to animal welfare. The absence of viable alternative methods of worm control means that we must understand how resistance works to limit its impact as far as possible.

Mechanisms of resistance

Drug resistance can arise in a limited number of ways (Box 3): (i) a change in the molecular target, so that the drug no longer recognizes the target and is thus ineffective; (ii) a change in metabolism that inactivates or removes the drug, or that prevents its activation; (iii) a change in the distribution of the drug in the target

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Box 1. Anthelmintic resistance and how to measure it

A definition of what constitutes resistance and the ability to measure the level of resistance are crucial in any management strategy.

What is resistance?

The definition for resistance occurring is: 'when a greater frequency of individuals in a parasite population, usually affected by a dose or concentration of compound, are no longer affected (or a greater concentration of drug is required to reach a certain level of efficacy). Resistance is inherited' [54]. For each chemical class of anthelmintic, resistance to one member usually confers resistance to the other members. It is possible, and increasingly common, to have multiple resistances, where parasites develop resistance sequentially and independently to several anthelmintic classes. Once resistance is present in a population, reversion or loss of resistance has never been observed [30]. When an anthelmintic class is first introduced, the frequency of resistance alleles is low, indicating that, in the absence of treatment, resistance alleles confer a neutral or negative reproductive fitness. Resistance is not an inevitable consequence of drug use, and selection for resistance will depend on the relative reproductive fitness conferred by susceptibility and resistance alleles at the given level of drug usage.

For some parasite and drug use situations, resistance might never develop. When resistance to a drug does occur, there are three phases linked to the accumulation of resistance alleles:

(i) Establishment of resistance is largely a random event influenced by: population size and diversity; the mutation rate for the gene(s) in question; and the relative fitness of individuals with the mutation compared with the wild-type gene. The resistance allele frequency is usually low.

(ii) Development occurs in response to a selective agent that kills susceptible worms, but allows resistant ones to survive and reproduce. Drug treatment is a powerful selector of resistance alleles. With continued selection, the frequency of resistance alleles increases and disperses through the population.

(iii) Emergence occurs as selection continues and resistance becomes noticed. R alleles are at high frequency.

The presence of a resistance genotype always precedes observation of clinical resistance (field resistance or treatment failure in the emergence phase). As drugs are often used in the field at doses higher than the minimum required to kill most of the worms, selection could produce a high frequency of R alleles before clinical resistance is noticed.

Measuring resistance

Most surveys of clinical resistance involve treatment of infected hosts with a recommended dose of drug followed by a calculation of faecal egg count reduction compared with pre-treatment egg counts or those of untreated controls. Reductions of <95% (based on group arithmetic means, for sheep) score as clinical resistance [55]: in hosts other than sheep, a different figure can be accepted. Measures of resistance can be: (i) efficacy at a certain dose rate; (ii) the concentration required to kill 50% of worms *in vitro*; or (iii) the proportion of farms with clinical resistance. Measuring resistance during the development phase is difficult and more-sensitive tests are required. Some *in vitro* assays are available. Genetic tests detect the presence of specific resistance alleles in a population: to form the basis of useful tests, such alleles must be present in the majority of cases of resistance. The development of assays to measure gene frequency in populations, especially field populations, is a major challenge. Desirable tests should be cheap, reproducible, use a single sample collection and, preferably, use technology available in a field laboratory. They should detect resistance to the major chemical classes and important species (of one host) in a single set of steps. Describing tests as 'cow-side' (on the farm) and 'dipstick' are used to encapsulate these characteristics.

Box 2. Factors influencing selection pressure for resistance

There are several factors that influence selection pressure for resistance which are outside management control. The following factors represent those that can increase selection for resistance:

Parasite genetics

• Resistance alleles might be dominant, as suggested for resistance to the avermectins and/or milbemycins [56]. If heterozygotes are resistant, then clinical resistance will be apparent at much lower allele frequencies than if resistance is recessive.

• There might be few genes, or only one, involved in resistance. The bigger the effect of each individual change, the faster resistance will tend to develop.

• The high genetic diversity of parasitic helminths [57], coupled with their large populations, increases the likelihood that resistance alleles will already be present in a population, possibly at relatively high frequency.

• If resistant worms have enhanced fitness compared to susceptible individuals, or if resistance is linked to other fitness genes, then resistance will tend to spread in the population.

Parasite biology

• Parasites have a short generation time and high fecundity: therefore, production of many individuals of several generations in a short time increases the spread of resistance alleles through the population.

• Direct life cycles mean that the fitness associated with resistance alleles is not dissipated by passage through an intermediate host.

• Parasite populations tend to be mobile, especially if the hosts are moved.

• There might be low levels of untreated parasites in refugia (Box 3).

Host–parasite relationships

• Infection with pathogenic worms requires treatment to control disease, so selection pressure could be higher for these parasites.

• Reduced hypobiosis (larval inhibition or arrested larval development – a temporary halt in development at a specific point in the life cycle) could shorten life cycles and reduce the refugia of arrested larvae inaccessible to the drug.

Managing selection pressure

The following represent factors that are under management control and indicate where we can decrease the selection pressure:

• The inherent nature of the chemical and its propensity to select for resistance.

• The pharmacokinetics of the drug – it is generally considered preferable to use short-acting drugs to prevent worms being exposed to the sub-therapeutic concentrations that result from an extended half-life of a drug.

• It is important to avoid under-dosing and ensure that treatments are fully efficacious.

• Drugs should be used in ways that maintain refugia (Box 3).

• Treatments should be planned, through timing and management, to reduce the survival of free-living stages in the environment. Where practical, the access of free-living stages to the next host should be reduced by measures such as removal of faeces and alternate grazing of different hosts.

• Use of other control methods to complement anthelmintics, or the use of alternative chemical classes.

BZ act by inhibiting the polymerization of tubulin to form microtubules and it is clear that resistance is associated with mutations in β-tubulin genes that prevent drug binding. However, several different polymorphisms of the β-tubulin genes have been correlated with BZ resistance [13]. The well-known Phe–Tyr polymorphism at codon 200 of β-tubulin isotype 1 was the first described [14] and has frequently been considered the most important mutation conferring resistance to these

organism that prevents the drug from accessing its site of action; or (iv) amplification of target genes to overcome drug action. Those mechanisms implicated in anthelmintic resistance are summarized in Table 1.

Box 3. Genetic mechanisms and the evolution of resistance

There are many possible genetic processes that can lead to resistance. The simplest examples are resistances caused by a change in a gene encoding a drug receptor that then weakens drug binding. However, a change in gene expression could lead to resistance by increasing the production of the drug target. Enhanced detoxication or drug removal are other possible mechanisms. Selection by some parasite–drug combinations could involve a single gene as a first-step in the development of resistance and other alleles of that gene or other, unrelated, genes could be selected on by further drug treatment. The first step, such as Tyr 200 of β-tubulin in benzimidazole (BZ) resistance, is the most important target if we want to develop a test for resistance, but subsequent steps are also important to comprehend the whole picture. The selection of additional alleles or genes that contribute to resistance could increase the level of resistance (decrease efficacy of drugs) further.

Refugia are subpopulations of parasites that are not selected by drug treatment. They are important because the higher the proportion of the population in refugia, the slower the selection for resistance. Examples of refugia are organisms in the environment (e.g. larvae on pasture) or inhibited larvae (especially encysted horse cyathostomins) that are not susceptible to the effects of some drugs. Another refugium is the worms in untreated members of a herd. Climatic conditions have fundamental effects on the numbers in refugia. Few free-living stages survive in arid climates, so the pasture refugium is small. The appearance of avermectin resistance in *Telodorsagia* spp. in Western Australia after only two treatments with the drug illustrates the power of selection in arid areas [3]. Cattle dung pats can also represent a reservoir of infective larvae for up to 12 months, ensuring a large refugium and slow selection for resistance in cattle parasites.

Resistant isolates

While resistance can arise in the field, it can also be selected in the laboratory. By using marginal (selective) dose rates and no refugia (infecting the next host only with larvae that survive treatment), resistant isolates can be achieved rapidly. However, different selection protocols can select for different resistance phenotypes and, presumably, genotypes [33]. It is important in describing isolates, that their selection history is known.

compounds. However, even in the early studies, highly resistant populations of *Haemonchus contortus* were also known to possess a deletion in β-tubulin isotype 2. More recently, a second Phe–Tyr polymorphism, at codon 167 of β-tubulin isotype 1, was detected in BZ-resistant populations of *H. contortus*. The same two polymorphisms also occur in the β-tubulin isotype 2 gene of *H. contortus*, and they too can confer BZ resistance [13]. The codon 167

polymorphism was also present in BZ-resistant *Teladorsagia circumcincta*, but not in *Trichostrongylus colubriformis* [15]. Mutations at codon 167, but not 200, were found in several highly BZ-resistant cyathostomin species from horses [16]. Binding studies with recombinant *H. contortus* β-tubulins indicated that mutations at codon 167 of isotype 1 or 2 reduce affinity for BZ [13]. However, genotyping two *H. contortus* field populations showed that Tyr at codon 200 is required for BZ resistance. This was not true for *T. circumcincta*: worms homozygous for Phe at codon 200, but heterozygous or homozygous for Tyr at codon 167, survived BZ treatment [15]. Of these surviving *T. circumcincta*, a similar proportion was heterozygous at codon 167 as were homozygous for Tyr. This implies that the genetics of BZ resistance in *T. circumcincta* are different from those of *H. contortus*, and investigators using molecular techniques to evaluate field resistance levels should bear this in mind. In horse cyathostomins, the β-tubulin isotype 1 codon 200 polymorphism is not the only, and probably not even the most important, mutation with respect to resistance [17–19]. However, it remains to be seen to what extent codon 167 mutations contribute to resistance in these worms. The codon 200 polymorphism has been described in the cattle nematode, *Cooperia oncophora*, and found to occur in BZ-resistant populations [20,21]. Apart from such target gene changes, multiple data suggest that modulation of the activity of the cell-membrane efflux pump P-glycoprotein (Pgp) could also contribute to BZ resistance in trichostrongyles [22].

The fasciolicide, triclabendazole, is an atypical BZ with a very narrow spectrum of activity. Observations by microscopy indicate a microtubule-directed action typical of BZs. The active form of the drug, triclabendazole sulfoxide (TCBZ.SO), blocks the movement of tegumental secretions, leading to widespread sloughing of the tegument. This is reminiscent of early ultrastructural studies on mebendazole involving nematodes and cestodes. In addition, TCBZ.SO inhibits mitotic division of the vitelline and spermatogenic cells, and causes a reduction in the intensity of tubulin immunostaining, changes typical of microtubule disruption that were not seen in a triclabendazole-resistant isolate. However, resistance to triclabendazole does not appear to be associated with mutations in

Table 1. Possible mechanisms of resistance to the major anthelmintic families[a]

Anthelmintic family	Mechanism of resistance	Comments
Benzimidazoles	β-tubulin isotype 1 mutations: F200Y, F167Y	The best studied mutations and probably the most important. F200Y seems to be the most important mutation in *Haemonchus contortus*, but this might not be true for all species.
	β-tubulin isotype 2 mutations: F200Y, F167Y, deletion. Altered metabolism and/or uptake.	Also present in *H. contortus*, field importance unknown. Might be important in triclabendazole-resistant flukes: importance in nematodes unknown, but probably minor.
Avermectins and milbemycins	Mutations in GluCl and/or GABA-R genes	Molecular evidence from *Cooperia oncophora*: population genetic evidence from *H. contortus*.
	Overexpression of P-glycoproteins	Population genetic and some pharmacological evidence. The relative importance of these two mechanisms is yet to be determined.
Levamisole	Changes in nicotinic acetylcholine receptors	Physiological and pharmacological evidence: no molecular data to date.

[a]Data obtained from Anthelmintic Resistance in Veterinary Parasites – what is the best way forward? A workshop sponsored by the Biotechnology and Biological Sciences Research Council, held 1–3 February 2004, in Bath UK.

β-tubulin. Tyrosine is present at position 200 in β-tubulin from both susceptible and resistant isolates [23], and no amino acid polymorphisms are present in any *Fasciola* β-tubulin isolated to date. An alternative mechanism is required to explain resistance. One possibility is enhanced metabolism of triclabendazole, in that resistant flukes can metabolize TCBZ.SO to the relatively inert sulfone metabolite to a greater extent than can their susceptible counterparts [24]. Preliminary data also indicate that the uptake of triclabendazole and its metabolites is altered in resistant flukes (C. Lanusse *et al.*, unpublished).

Levamisole is the most widely used cholinergic anthelmintic, acting as an agonist at nicotinic acetylcholine receptors (nAChR) at the nematode neuromuscular junction (NMJ) and causing a spastic paralysis. Nematodes resistant to levamisole are also resistant to other nicotinic agonists such as morantel and pyrantel. Membrane preparations from resistant nematodes have reduced binding affinity at a low affinity site for a levamisole analogue [25]. In *Caenorhabditis elegans*, levamisole resistance can result from the absence of levamisole receptors, which form one of two populations of nAChR present at the NMJ [26]: the second is preferentially activated by nicotine. The levamisole-insensitive population could allow resistant worms to survive without functional levamisole receptors. The presence of two pharmacologically distinct nAChR at the NMJ, one of which is activated by levamisole, has been confirmed in *Ascaris suum* [27]. The pattern of channel subtype expression could be altered in resistant *Oesophagostomum dentatum* [28]. The use of protein kinase inhibitors has indicated that receptor phosphorylation regulates levamisole sensitivity, suggesting that different channel subtypes could result from post-translational modifications of the receptors [29]. However, the molecular basis for the physiological and pharmacological differences between levamisole-sensitive and -resistant worms remains obscure. Nematodes possess a large family of nAChR and molecular cloning efforts have so far failed to reveal any polymorphisms associated with resistance [3]. There is genetic evidence that resistance is a result of a single, sex-linked, gene in *T. colubriformis* and *O. dentatum*, but in *H. contortus* multiple genes could be involved [30]. We need more information on parasite nAChR before we can clearly define the molecular basis of levamisole and/or pyrantel resistance.

With resistance to the AM anthelmintics, the picture is even more confused. These drugs act on ligand-gated channels, including glutamate- (GluCl) and GABA-gated (GABACl) chloride channels, a family of receptors widely distributed in nematodes that regulate locomotion, feeding and reproduction [31,32]. The AM have effects on all of these functions, but it is likely that their relative importance in the overall anthelmintic activity varies between species, and thus mechanisms of resistance could also vary. Indeed, even different AM-resistant isolates of one species, *H. contortus*, each have different phenotypes [33]. Parasites resistant to one AM, such as ivermectin, are generally resistant to the others. Genetic studies have found that ivermectin resistance is dominant in *H. contortus*, perhaps reflecting a gain-of-function mutation, although it could be that true resistance results

from polymorphisms in several closely linked genes. Multiple mutations are required for high-level AM resistance in *C. elegans* [34], although the relevance of these studies to parasites could be questioned. It is possible to select *H. contortus* that have not previously been exposed to ivermectin for resistance in only three generations under laboratory conditions (G.C. Coles and A.J. Wolstenholme, unpublished) indicating that a pool of resistance alleles is present in the unselected populations. Binding studies failed to find any consistent changes in radiolabelled ivermectin binding to membranes from resistant *H. contortus* or *T. circumcincta* [35], suggesting that target-site mutations do not cause resistance in these species, but did find an increase in a low-affinity L-glutamate-binding site in ivermectin-resistant isolates of *H. contortus* and *T. circumcincta* [35,36]. The nature of this site has not been investigated further. There are suggestions that P-glycoproteins (Pgp) are involved in AM resistance [22]. Population genetics studies in *H. contortus* found evidence for the association of Pgp genes with AM resistance [37], but failed to find consistent associations [38] and a segregation study indicated that a particular Pgp gene was not the major determinant of resistance in one isolate [39]. Other studies found selection at GluCl and GABACl genes in individual resistant isolates [40–41]. Ivermectin increased the GABA response in cells transfected with an unselected, wild-type allele of a *H. contortus* GABACl subunit gene, whereas in cells transfected with the AM selected allele, ivermectin attenuated the GABA response [32]. A polymorphism in a GluCl subunit from an ivermectin-resistant isolate of *C. oncophora* caused channels formed in *Xenopus* oocytes to be less sensitive to both glutamate and ivermectin [42], although this polymorphism was not found in resistant *H. contortus* [35]. An intriguing further observation is that the amphids (sensory structures in the nematode head) are altered in AM-resistant *H. contortus* [43] – there are similar findings from *C. elegans*, where virtually all of the mutations that confer low-level resistance (5–10 fold) are amphid mutations, and it is possible to select for alleles of genes known to be expressed in amphids that have no obvious amphidial defects, but are ivermectin resistant (W. Grant, pers. commun.). The amphids form a pathway from the environment into the interior of the worm, so defects could prevent drugs gaining access to their target sites.

At first sight, these data seem contradictory and confusing, so what hypotheses can be formulated to explain them? Two main possibilities stand out. Resistance to the AM anthelmintics could be caused by a gain-of-function mutation in a Pgp gene, leading to more rapid removal of the drug from the worm. Such mutations could either: (i) cause increased expression of a pump capable of carrying the AM, which might lead to changes in gene expression that could be detected by microarray or proteomics experiments; or (ii) increase the affinity for these substrates, detection of which might require careful studies on reconstituted worm Pgp preparations. Alternatively, parasites could become resistant by the accumulation of one or more mutations in the GluCl and other genes. Such multigenic resistance would be slower to

appear, requiring tens of generations rather than four or five, and the genes involved would vary between species and even isolates, depending on their relative importance in drug action and the genetic constitution of the populations. Because GluCls are expressed in amphid and extrapharyngeal neurones [31,34,44], defects in these neurones could also cause the observed changes in the response to AM. A more detailed comparison of the GluCl, GABACl and Pgp genes between many sensitive and resistant species and isolates is needed: the first step is to determine the number, structure and sequence of such genes in several parasite species.

How to deal with anthelmintic resistance

Anthelmintic resistance is a major problem that we do not really understand, and it is here to stay – there is no evidence for reversion to anthelmintic susceptibility, even where the drug has been withdrawn [30]. In the short to medium term, there are no realistic alternatives to the continued use of current chemicals for parasite control. Pasture management can reduce the number of anthelmintic treatments required, but cannot replace them entirely [45]. Effective vaccines, new cost-effective compounds and non-chemical means of control are all some distance in the future – so it is vital that we maintain the efficacy of current treatments for as long as possible. This will require good communication with the users of these products to reduce practices that encourage the emergence and spread of resistance (Box 2). Selection pressure for resistance is largely affected by the degree of refugia [46] (Box 3). Experimental and field studies have suggested that treating selected animals based on infection do not need to have negative effects on production yields [47] and such strategies could reduce the selection pressure for resistance by increasing refugia.

Closantel, oxyclozanide, nitroxynil, clorsulon and albendazole are active against adult triclabendazole-resistant flukes [48–50], but are not very effective against juvenile flukes. Clinically, this is important because the migratory stages represent the most damaging phase of the disease. Another possibility is the use of synergistic drug combinations, with the aim of at least slowing down the spread of resistance. A combination of triclabendazole with either clorsulon or luxabendazole has been shown to be effective against six-week-old triclabendazole-resistant flukes. Other combinations of drugs are active against salicylanilide-resistant *F. hepatica* [9].

Management in endemic regions

The aim of management is to make parasite control sustainable. This implies the use of a range of control measures and a stabilization of resistance. At the same time, the economic cost of parasitism (due to production losses, and the costs of prevention and treatment) must be kept down. While they continue to work, anthelmintics remain the most cost-effective method of parasite control and so will remain in use. Although there might be some benefits in using mixtures of chemicals, existing resistance and regulatory issues will limit the future of chemical use alone, so approaches focus on refining chemical usage and augmenting this with grazing, nutritional and immunological control methods. The sustainability of any approach is difficult to ascertain, but knowledge of resistance mechanisms, selection factors and mathematical modeling will provide clues on how to design sustainable systems. To some extent, the better worms are controlled by drugs, the faster resistance develops. What to advise is a complex matter. Where pathogenic species such as *H. contortus* are prevalent, parasite control is essential and there is less latitude for using non-chemical control.

Assuming drug use is required, there are several ways to use them to reduce selection for resistance. Wherever possible, treatment should be confined to animals suffering from parasitism, and animals that can tolerate existing infections should be left untreated leaving an unselected refugium (Box 3). Such approaches mean the host suffers some parasitism, and farmers could experience some loss of productivity. Testing for infection and only treating when infections reach a threshold is 'curative' treatment that is likely to reduce resistance selection compared with 'strategic' control programs. Curative treatments might be on a herd basis (e.g. by egg counts on a subsample of a flock), on a random basis (leaving 2–5% of animals untreated (R. Dobson, pers. commun.) or an individual basis. Treatment on an individual basis requires the identification of those animals to be treated, and this has an economic cost. In the FAMACHA system employed in South Africa, an operator assesses the severity of infection by visual scoring of anaemia (a sign of haemonchosis, but also of fluke infection, so it is important to check local causes of anaemia before using this system more widely) and treating affected animals only. Selection of the most appropriate drug class for use on an individual farm can be made by testing for anthelmintic efficacy. Combinations (given together as mixtures or sequentially) of anthelmintics from different classes are the best way of reducing the rate of development of resistance. This is certainly true before resistance develops; it is less helpful once resistance to one component of a combination is present. Anthelmintics from novel classes will also help control, but ensuring their longevity through appropriate use is essential.

Efforts to reduce selection pressure on a property will be wasted if worms carrying resistance alleles are introduced from elsewhere. Quarantine treatments, usually mixtures of several anthelmintic classes, are the best way to exclude parasites [51]. They could theoretically select for high levels of resistance, but the alleles will be present anyway (in worms in untreated hosts). If the number of introduced animals is small, the probability will be low that any one animal will contain worms with the gene combination that will allow survival of both female and male worms that are resistant to each anthelmintic class used in the mixture.

A range of non-chemical methods might contribute to control. Parasites compete with the host for nutrients and, as a result, hosts lose weight and their immune responses become downregulated. Energy and protein supplements are useful in stimulating immunity. Where additional pastures or other stock (such as older sheep, cattle
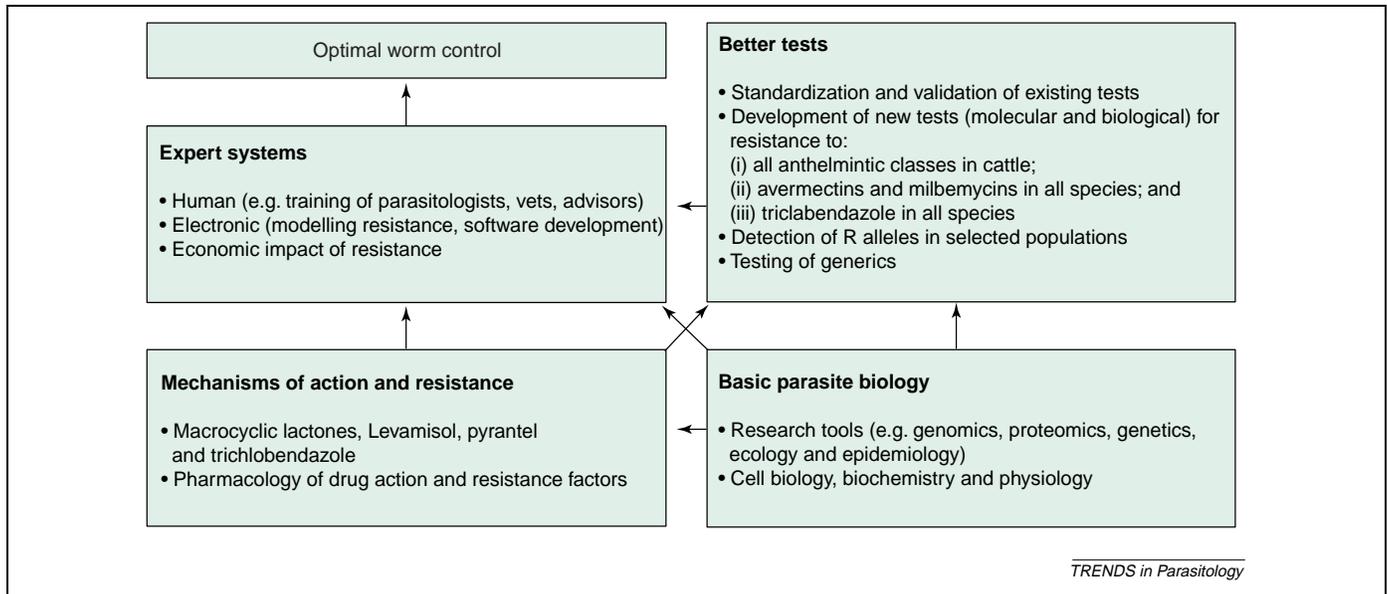
Figure 1. Priorities in anthelmintic research. Optimal worm control, defined as the best level of control, consistent with maintaining efficacy in the medium term, requires: (i) more basic knowledge on the biology of parasites; (ii) a better understanding of drug action and how resistance works; and (iii) the development of better molecular and biological tests for resistance. These will allow the training of human and electronic expert systems to advise on use of anthelmintics to achieve the best outcome for farmers over the short and medium terms. The arrows indicate how these various areas of research interact with each other.

or horses) are available, worms can be controlled by alternate grazing [45]. In hot moist climates, where rapid development occurs all year, rotation systems are useful [45]. For horse parasites, breaking the life cycle of the worm is a possibility. Removal of faeces from pastures removes most sources of re-infection including resistant worms. Finally, host selection, through testing for worm immunity or genetic correlates, can improve herd immunity to parasites.

The provision of information to farmers and their advisors is crucial in agricultural extension. This is especially true for worm control where the issues are complex. In Australia, for example, newsletters and internet resources play an important role in disseminating information (http://www.affa.gov.au and http://www.agric.nsw.gov.au/reader/2566 provide examples of communication and the points raised above).

Research priorities

A recent Biotechnology and Biological Sciences Research Council-sponsored workshop* produced a list of research priorities in anthelmintic resistance for the short to medium term (Figure 1). Generally, research is needed in four main areas: (i) the development of better tests; (ii) the mechanisms of drug action and of resistance; (iii) the generation of suitable expert systems; and (iv) more basic understanding of parasite biology. These four areas are interconnected and advances in all of them are necessary if we are to achieve our aim of optimal worm control – defined as minimizing costs while allowing for continued efficacy of current treatments.

There are few completely satisfactory tests for the detection of anthelmintic resistance. Even for the BZs, where several tests exist, we await a thorough evaluation of their reproducibility and the significance of the data

obtained. As a result, the true extent of resistance to these drugs in the field is not known. Results often correlate poorly between different tests [15,52,53] and different laboratories often obtain different results using the same test (G. von Samson-Himmelstjerna, unpublished). For this reason, the development of improved tests for anthelmintic resistance, including the detection of resistance alleles in a mixed population of worms, is seen as a major priority. Detection of resistance alleles requires a PCR-based test and this in turn requires that we understand the mechanism of resistance, or at least the polymorphisms responsible for it. Measuring the frequency of resistance alleles within a population requires either exhaustive PCR tests on multiple individual worms or the development of a quantitative PCR method that can be applied to populations. Even where reasonable tests for resistance exist, as for BZ resistance in sheep parasites, these need to be better standardized, so that laboratories can agree on what is meant by resistant worm populations (e.g. at what resistance allele frequency do we cease recommending the use of a particular drug?). If we are going to combat the spread of resistance effectively, or even reverse it, then we need to know far more about many aspects of basic parasite biology, including parasite ecology and epidemiology, and how these influence the spread and impact of resistance in different environments. Understanding the pharmacology of drug action and resistance might allow us to inhibit or evade resistance mechanisms and thus extend the usefulness of existing drugs. It is also important that the drugs that are administered are fully effective because under-dosing obviously risks promoting resistance (Box 2): some concern was expressed that generic formulations, especially of the AM, might not be correctly formulated to ensure that worms are exposed to a full dose. The development of novel methods of worm control will require better knowledge of the cell biology, biochemistry and

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* The workshop Anthelmintic Resistance in Veterinary Parasites – what is the best way forward? was held 1–3 February 2004, in Bath, UK.

physiology of the target species and this, in turn, will require better research tools. Post-genomic biology has yet to make an impact on parasitic helminth research, but proteomic and functional genomics methods should allow us to make rapid progress in these basic studies. Finally, none of this will have any impact on the situation unless the knowledge and expertise gained can be passed on to the end users, i.e. farmers and their advisors. Therefore, we need to develop expert systems: networks of human advisors trained in field parasitology, and electronic resources containing the latest findings, techniques and thinking, so that the practical decisions on which treatment to use and when and how to use it, are based on the best possible advice.

Concluding remarks

Anthelmintic resistance in veterinary parasites is a major problem worldwide. Little is known about how this resistance has arisen and how to reverse it. Until novel methods of worm control are developed, we need to implement strategies to maximize the effective lifetime of our current compounds. Such strategies will be based on a sound understanding of the biology, in its broadest sense, of resistance and more research in this area is urgently needed.

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