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Drug resistance in nematodes of veterinary importance: a status report

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Reports of drug resistance have been made in every livestock host and to every anthelmintic class. In some regions of world, the extremely high prevalence of multi-drug resistance (MDR) in nematodes of sheep and goats threatens the viability of small-ruminant industries. Resistance in nematodes of horses and cattle has not yet reached the levels seen in small ruminants, but evidence suggests that the problems of resistance, including MDR worms, are also increasing in these hosts. There is an urgent need to develop both novel non-chemical approaches for parasite control and molecular assays capable of detecting resistant worms.

Many parasitic nematodes of veterinary importance have genetic features that favor the development of anthelmintic resistance. Among the most important of these are rapid rates of nucleotide sequence evolution and extremely large effective population sizes that give these worms an exceptionally high level of genetic diversity [1,2]. In addition, most nematode species that have been studied demonstrate a population structure consistent with high levels of gene flow, suggesting that host movement is an important determinant of nematode population genetic structure [2]. Thus, these worms possess not only the genetic potential to respond successfully to chemical attack, but also the means to assure dissemination of their resistant genes through host movement.

Brief history of anthelmintic resistance

The initial reports of anthelmintic resistance were to the drug phenothiazine in the late 1950s and early 1960s, first in *Haemonchus contortus* (barber pole worm) of sheep [3] and then in cyathostomins (small strongyles) of horses [4–6]. In 1961, thiabendazole was introduced as the first anthelmintic that combined efficacious broad-spectrum nematocide activity with low toxicity. The rapid acceptance and widespread use of thiabendazole and then other benzimidazole anthelmintics marked the beginning of the modern chemical assault on helminth parasites. However, within a few years, resistance to thiabendazole was reported, again first in the sheep nematode *H. contortus* [7,8] and then in the equine cyathostomins*. Reports then appeared of benzimidazole resistance in the other major gastrointestinal trichostrongylid nematodes of sheep – *Teladorsagia* (*Ostertagia*) *circumcincta* (brown stomach worm) and *Trichostrongylus colubriformis* (black scour worm). These reports led to studies investigating the prevalence of resistance, which found that, by the mid-1970s, multiple-species nematode resistance to benzimidazole anthelmintics was common and widespread in both sheep and horses throughout the world. This same pattern repeated itself in the 1970s and 1980s following the introduction of the newer imidazothiazole–tetrahydropyrimidine and avermectin–milbemycin classes of anthelmintics and, by the early 1980s, reports of multiple-drug resistant (MDR) worms appeared for the first time (reviewed by Refs [9–16])

By the 1990s, anthelmintic resistance was no longer a potential problem of the future. Widespread reports of MDR worms, including resistance to avermectin–milbemycin drugs, had elevated the issue of anthelmintic resistance from being one of academic interest to being a major threat to small-ruminant production in many areas of the world [17]. Presently, MDR (to all three major anthelmintic classes) *H. contortus*, *T. circumcincta* and *T. colubriformis* have been documented throughout the world, and MDR *H. contortus* now threaten the viability of small-ruminant industries in much of South America [18–21], South Africa [22], Malaysia [23,24] and southeast USA [25]. Recent reviews of the situation in Australia [26] and New Zealand [27] indicate that the problem of anthelmintic resistance, although severe, has not yet reached the crisis levels seen in some of the more tropical areas of the world. However, recent reports of moxidectin resistance in both Australia [28] and New Zealand [29] suggest that the problem may be more severe than past surveys have indicated. In other areas of the world, such as Europe and Canada, MDR worms have been only infrequently reported, and resistance is less of a concern. Nevertheless, in the UK, where drug resistance in nematodes of sheep is not nearly as severe a problem as it is in many other areas of the world, the problem is important enough that a national workshop was recently convened to develop a set of national strategies and recommendations to slow the development of resistance†; see Tables 1 and 2 for summaries of the resistance situation.

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* Drudge, J.H. and Lyons, E.T. (1965) Newer developments in helminth control and *Strongylus vulgaris* research. In *11th Annual Meeting of the American Association of Equine Practitioners*, held 6–8 December 1965, pp. 381–389, American Association of Equine Practitioners, Denver, CO, USA.

† L. Stubbings (2003) Internal Parasite Control in Sheep. In *Proceeding of a Workshop to decide: short term strategies to slow the development of anthelmintic resistance in internal parasites of sheep in the United Kingdom*, held 11–12 March, 2003, in London, UK.

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Table 1. Major anthelmintic classes used in the control of parasitic nematodes of small ruminants and horses

Drug	Host	Year of initial drug approval[a]	First published report of resistance[b]	Refs
Benzimidazoles				
Thiabendazole	Sheep	1961	1964	[7,8]
	Horse	1962	1965	[c]
Imidothiazoles–tetrahydropyrimidines				
Levamisole	Sheep	1970	1979	[57]
Pyrantel	Horse	1974	1996	[58]
Avermectin–milbemycins				
Ivermectin	Sheep	1981	1988	[59]
	Horse	1983	2002[c]	[37]
Moxidectin	Sheep	1991	1995	[60,61]
	Horse	1995	2003[c]	[d]

[a]The exact approval date will vary between countries.
[b]Dates given are for publication of the first documented resistance. In many instances, there are earlier published reports of suspected resistance and/or unpublished reports of resistance.
[c]Suspected resistance in *Parascaris equorum*, but not yet confirmed in a controlled efficacy study.
[d]Slocombe, J.O.D. (2003) *Parascaris* resistance to macrocyclic lactones. In *Proceedings of the World Association for the Advancement of Veterinary Parasitology 19th International Conference*, p. 180, held 10–14 August 2003, in New Orleans, LA, USA.

Such concern is predicated on the fact that levels of resistance can increase rapidly and there are few anthelmintics currently being developed. Two new classes of anthelmintics have emerged in the post-avermectin–milbemycin years: the cyclooctadepsipeptides [30,31] and the oxindole alkaloid, paraherquamide [32,33]. Various analogs of these drugs have demonstrated good-to-excellent efficacy against many species of nematodes in a variety of animal hosts. However, at the present time, no public information is available on the plans for the development of these drugs, and it is unknown whether a new product will be marketed in the foreseeable future.

Another consideration is the fact that reversion to susceptibility does not seem to occur, meaning that resistance is essentially everlasting. In theory, reversion to susceptibility might occur if use of a drug is discontinued and worms resistant to that drug suffer from a decrease in fitness. Likewise, reversion to susceptibility might also occur if counter selection is applied by treatment with a different drug. In theory, this should cause a decrease in the frequency of resistant alleles to the first drug. However, there is little evidence that true reversion occurs in the field, and where reversion to susceptibility has been demonstrated, it has proven to be short lived (reviewed by Ref. [27]). This occurs because worms carrying resistance alleles, although reduced in numbers, have a great selective advantage once the drug is reintroduced.

Anthelmintic resistance in cattle, horses and humans

Less attention has been given to the problem of anthelmintic resistance in cyathostomin nematodes of horses (now considered the principal parasitic pathogen of adult horses), although several studies have reported a prevalence of resistance to benzimidazole drugs greater than 75% [14]. Resistance to pyrantel (tetrahydropyrimidine class) appears to be much less common, but a recent study in southern USA found that over 40% of farms demonstrated resistance to this drug [34]. Interestingly, there are still no reports of cyathostomin resistance to ivermectin, despite over 20 years of use as the most commonly administered anthelmintic drug. One theory that is frequently proposed to explain the lack of resistance to ivermectin is the inability of this drug to kill mucosal larval stages of cyathostomins [14]. These mucosal larval stages tend to be much more numerous than the adult worms in the lumen, and therefore provide a large refugia‡. By contrast, there have been two recent reports of suspected ivermectin resistance in *Parascaris equorum*, which is the most important parasitic pathogen of foals [36,37]. These reports have not yet been confirmed with controlled efficacy studies, but *P. equorum* is the dose-limiting parasite for ivermectin in horses, therefore resistance might be expected to develop more quickly to this worm. The apparent excellent efficacy that avermectin–milbemycin drugs continue to have against the major strongyle nematode parasites of horses seems to have lulled this industry into a false sense of security. Considering the growing reliance upon this class of drugs, and the fact that avermectin–milbemycin resistance is becoming increasingly common in gastrointestinal nematode parasites of small ruminants and cattle, most equine parasitologists suspect that resistance in cyathostomins is inevitable [38–40].

Reports of anthelmintic resistance in nematodes of cattle have been less common, and the general belief is that resistance is not yet an important issue in this host. However, no studies have been performed to investigate the prevalence of resistance in nematodes of cattle, so we are left only with clinical case reports describing the failures of treatment to control clinical disease as our measure. This is a very insensitive means to monitor the development of resistance because cases only become apparent once resistance reaches very high levels in a population. In recent years, avermectin–milbemycin resistance in *Cooperia* spp. of cattle has become increasingly common [41–47]; in addition, in Brazil, Argentina [41] and New Zealand [42], reports suggest that avermectin–milbemycin resistance in *Cooperia* spp. is starting to reach very high levels. Furthermore, in some of these reports, MDR worms were detected. In light of these recent findings, anthelmintic resistance in nematodes of cattle might be considerably more common than is currently recognized in places such as Europe and the USA, where anthelmintic resistance has not been reported and/or is not currently considered an important problem in cattle. Of additional concern is the observation that an ivermectin-resistant isolate of *Cooperia oncophora*

‡ Refugia is a term used to describe the proportion of a parasite population that is not exposed to a particular drug, thereby escaping selection for resistance. In practical terms, refugia are supplied by: (i) stages of parasites in the host that are not affected by the drug treatment; (ii) parasites residing in animals that are left untreated with a particular drug; and (iii) free-living stages in the environment at the time of treatment. Many parasitologists now consider levels of refugia as the single most important factor involved in the selection of anthelmintic-resistant parasites (see Ref. [35] for a more detailed discussion on refugia).

Table 2. General worldwide situation in levels of anthelmintic resistance among livestock hosts

Drug class	Hosts with high resistance[a,b]	Hosts with emerging resistance[c]	Major livestock-producing areas where drug is still highly effective in sheep, goats and horses
Benzimidazoles	Sheep, goats, horses	Cattle	None
Imidothiazoles–tetrahydropyrimidines			
Levamisole (ruminants)	Sheep, goats	Cattle	None
Pyrantel (horses)	Horses (USA only)	Horses	Unknown – few recent studies outside USA
Avermectin–milbemycins			
Ivermectin	Sheep, goats, cattle	Cattle, horses[d]	Horses – worldwide Sheep, goats – Europe, Canada
Moxidectin	Goats	Sheep, goats, cattle, horses[d]	Horses – worldwide Sheep – most regions

[a]In all cases, references to resistance relate to cyathostomin nematodes of horses and/or trichostrongylid nematodes of ruminants unless otherwise specified.
[b]High resistance is defined as a level and prevalence of resistance that is sufficient to warrant general concern of using that drug on a particular property without prior testing for efficacy. It should be understood that many species of gastrointestinal nematodes infect ruminants and high resistance in any one species is sufficient for inclusion in this list. If high resistance is known to occur in only a single country and/or region, then it is specifically mentioned. If high resistance is known to occur in more than one region, then no reference is made, but this does not necessarily mean that there is high resistance everywhere.
[c]Emerging resistance is defined as a situation where anthelmintic resistance is reported to occur, but prevalence is not known and the level and distribution of resistance is not recognized as a severe problem.
[d]Only in *Parascaris equorum*; presently, there is no evidence of resistance in cyathostomin or *Strongylus* spp. nematodes.

originating from the UK demonstrates a much higher level of pathogenicity than ivermectin-susceptible isolates [47,48]. Explanations for why resistance develops more slowly in nematodes of cattle has been reviewed previously [49], but the fact that resistance is much slower to develop in nematodes of cattle gives strong evidence that many factors other than the genetics of the worms are involved in the dynamic process of resistance selection. Relevant factors that affect the rate with which resistance develops include: the biology and epidemiology of the parasite, the dynamics of the host–parasite relationship, the treatment frequency and the treatment strategies that result in various levels of refugia. An additional factor that has not been fully investigated is differences in anthelmintic pharmacokinetics between host species. Anthelmintic drugs demonstrate considerably lower bioavailability in goats than in other livestock species, and it is frequently suggested that the extremely high prevalence of anthelmintic resistance in nematodes of goats is associated with this unique pharmacokinetic profile.

What about resistance in parasites of humans? To date, there have been no documented cases of anthelmintic resistance in nematodes of humans, although there have been several reports where treatment with mebendazole or pyrantel demonstrated efficacies at much lower levels than expected against hookworms [50–52]. Differentiating reduced efficacy from true resistance is more complicated in nematodes of humans than it is in nematodes of animals owing to several factors that might confound interpretation of fecal egg count data (reviewed by Ref. [53]). Additionally, in the case of human parasites, it can be quite difficult to prove whether reduced efficacies are due to resistance or to some other factor because the confirmatory controlled efficacy experiments carried out with animals cannot be performed on human subjects. Furthermore, we currently lack the molecular knowledge required to develop diagnostic assays that can reliably identify resistance for all drugs except the benzimidazoles. Even with benzimidazole drugs where specific mutations have been correlated with a resistant phenotype in several nematode species [54], we do not have validated tests for use in human parasites. Though the issue of anthelmintic resistance in parasites of humans has received scant attention, the potential is real and this reality should be taken into consideration when implementing drug-based control strategies [53]. Current mass treatment programs for onchocerciasis and lymphatic filariasis may be placing strong selective pressures for resistance on these filarial worm populations, as well as on the important gastrointestinal nematode species. It is of crucial importance that studies be performed to monitor the development of resistance in these nematode species so that these large-scale programs for control can be adjusted if necessary to prevent program failure on the eve of what appears to be their success.

Implications of anthelmintic resistance

The serious problem of anthelmintic resistance is easily appreciated. But what can be done about it? Beginning with phenothiazine in the 1950s, followed by the benzimidazoles in the 1960s, the imidazothiazole–tetrahydropyrimidines in the 1970s and the avermectin–milbemycins in the 1980s, a new class of anthelmintics was introduced into the marketplace each decade. This arsenal of highly effective and relatively inexpensive drugs led to recommendations for parasite control that were based almost solely on the frequent use of anthelmintics, the goals of which were to maximize livestock health, productivity and profitability. Though this approach was highly successful, history clearly suggests that this approach was short sighted and unsustainable. The prospect of a continuous flow of new classes of anthelmintics has not been realized; there has not been a new class of anthelmintics introduced into the marketplace in almost 25 years. During the post-ivermectin period, the investment in discovery and development of new anthelmintics has been greatly reduced and there are few new candidate drugs on the horizon. Development of the cyclooctadepsipeptides and/or paraherquamide would be a valuable addition to nematode parasite control, but it is unlikely that sufficient numbers of new drugs will be

developed to maintain a control paradigm based solely on frequent anthelmintic treatment.

The problem of resistance is by far the most severe in small ruminants and it is in these animals where the most dramatic changes in approaches to nematode control must be made. But what about horses and cattle? Effective cyathostomin control in horses, especially in the USA, is resting almost solely on the efficacy of a single class of anthelmintics. Despite this, it is unlikely that the message of change in parasite control methods being advocated by equine parasitologists will be heard in this industry until ivermectin resistance becomes common. In regard to cattle, do the current levels of resistance in cattle nematodes warrant a major change in how control is practiced? The seriousness of avermectin–milbemycin resistance in *Cooperia* spp. is unmistakable in some areas of the world, but should it be a major concern everywhere? Is this the beginning of high resistance in cattle with dramatic changes necessary to stall a situation reminiscent of small ruminants? These questions remain to be answered, but the potential implications of MDR nematodes in cattle demand that prevalence surveys be conducted to investigate this issue. In addition, we must develop molecular assays that can detect resistance while allele frequencies are still low. Detecting resistance before it becomes clinically apparent will permit implementation of changes in control strategies to preserve the effectiveness of that drug. Before such assays can be developed, we must develop a better understanding of the mechanisms of resistance. Research addressing mechanisms of anthelmintic resistance must therefore be a priority in this field.

Perspective

It is unlikely that development of new anthelmintics will rescue livestock producers from the inevitable losses in productivity and problems of animal welfare that result from a failure to control MDR worms adequately. Therefore, sincere efforts must be made to preserve the efficacy of the few drugs that remain effective. Now and in the future, anthelmintics must be thought of as highly valuable and limited resources to be preserved. The only realistic strategy for sustainable nematode parasite control is to develop novel non-chemical approaches that decrease the need for treatment and to use the anthelmintics that remain effective in a more intelligent manner [55,56]. Many such novel approaches are currently being investigated, but none of these is as effective as anthelmintics, and none of these will treat life-threatening disease. Therefore, as novel non-chemical control modalities become available and widely applied, anthelmintics will still be required for life-saving therapy when other control measures fail. Unless approaches for using anthelmintics in small ruminants dramatically and rapidly change, in many areas of the world there may be no effective anthelmintics remaining when that time comes. Horses and cattle might fare better, but the days of totally relying on anthelmintics for nematode control might also be nearing an end in these hosts.

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