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Anthelmintic resistance in human helminths: a review

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Summary We briefly review reports on drug resistance in human helminths and compare the factors which contribute to the development of anthelmintic resistance in livestock and man, i.e. high treatment frequency, single-drug regimens, targeting and timing of mass treatments and underdosing. Conclusions are drawn from the mistakes in the treatment and control of livestock helminths. The advantages and inconveniences of current methods for the detection of drug resistance in helminths of livestock are discussed and some suggestions are put forward to standardize the tests for the detection of resistance in human helminths. Finally, based on veterinary experience, some recommendations are made to reduce the risks of development of drug resistance in human helminths. The dramatic and rapid spread of resistance to all major classes of veterinary anthelmintics should be a warning against too strong a reliance on drugs in helminth control programmes.

keywords anthelmintic resistance, livestock, man, review

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Introduction

Over the past 20 years the perception of the problem of drug resistance in helminths of livestock has drastically changed. At the end of the 1970s, when the first reports of anthelmintic resistance appeared, the phenomenon was not always taken seriously by the veterinary scientific community. Waller (1985) still asked 'Is drug resistance a real problem or merely a paper tiger?'. Only 12 years later in 1997 the question put forward by van Wyk was 'How long before drug resistance makes it impossible to control some field strains of *Haemonchus contortus*?' Table 1 summarizes some figures on anthelmintic resistance in sheep nematodes in countries where the situation has become serious to such an extent that the future of sheep farming is threatened (Van Wyk *et al.* 1999). A similar situation might occur with drug resistance (DR) in human helminths. Currently the problem seems just to be emerging. Within the next decade medical doctors might be faced with similar problems as the veterinarians if the problem is not taken seriously.

In this paper, the reports on DR in human helminths are briefly reviewed. The factors which contribute to the development of DR in livestock and man are compared and conclusions drawn from the mistakes in the treatment and control of livestock helminths. Finally, some guidelines are formulated in order to delay the development of DR in human helminths.

Drug resistance in human helminths: current situation

Table 2 summarizes the species of helminths in man for which DR is suspected or proven. There is a lot of arguing on whether the reports on DR in hookworms are real cases of resistance. Geerts and Gryseels (2000) have made a detailed analysis of the data concerning *Necator americanus* (De Clercq *et al.* 1997) and *Ancylostoma duodenale* (Reynoldson *et al.* 1997) and conclude that these reports are suggestive for the development of DR, but fall short of providing conclusive evidence. Concerning the suspected resistance to praziquantel (PZQ) in Senegalese strains of *Schistosoma mansoni*, in spite of suggestive laboratory and field trials, so far there is no real evidence of DR (Geerts & Gryseels 2000; Gryseels *et al.*, this issue). In-depth studies and models indicate that the low cure rates in Senegal may be explained by the specific epidemiological situation in the northern region of the country (high initial worm burdens and high transmission intensities) but also that currently available methods would probably not detect a reduction of sensitivity of less than 5–10%. Resistance of *S. mansoni* to oxamniquine in Brazil has been well documented (Cioli *et al.* 1992, 1993) and there is some evidence that

Table 1 Prevalence of anthelmintic resistance in sheep nematodes

Country	No. farms examined	Resistance to (%)		
		BZ	IVM	LEV
South Africa	80	79	73	23
Brazil	182	68	7	19
Paraguay	37	70	67	47
Uruguay	242	61	1	29

Source: Van Wyk *et al.* (1999); Waller *et al.* (1996).

resistance to PZQ may be occurring in Egypt (Ismail *et al.* 1999). Development of resistance to ivermectin (IVM) in *Onchocerca volvulus* has not yet been proved, but reduced susceptibility of microfilariae has been observed in some regions of the West-African Onchocercosis Control Programme (R.K. Prichard, pers. comm.). Several authors have already warned of the development of resistance to IVM in large-scale programmes against onchocerciasis which use the same drug continuously over many years (Shoop 1993; Geerts *et al.* 1997). Recently, Grant (2000) has drawn the attention to the possible development of DR in adult macrofilariae, which would even be more disastrous than DR in microfilariae.

Although the reports mentioned in Table 2 do not always provide conclusive evidence about emerging DR in human nematodes and schistosomes, the data indicate that tolerance traits are indeed present in some helminth populations of man. As suggested earlier (Geerts *et al.* 1997; Geerts & Gryseels 2000), experience in veterinary practice shows that such traits might quickly and irreversibly become dominant in helminths under continuous drug pressure.

Genetics of drug resistance

Our knowledge about the genetics of DR in helminths has a lot of gaps. Nevertheless some features derived from the study of worms of livestock might be very relevant for human helminths. It is well known that the rate at which DR develops in a given helminth population depends on many factors, among them the frequency of resistance alleles in the initial untreated population. Usually this frequency is estimated at a very low level (10^{-6}–10^{-10}). However, in untreated *H. contortus* populations the initial frequencies of alleles responsible for resistance to benzimidazoles (BZ) at the isotype 1 and 2 β-tubulin loci were 46 and 12%, respectively, which is surprisingly high (Beech *et al.* 1994). Similarly high (10–20%) frequencies of alleles for IVM resistance have been reported in unexposed *H. contortus* (Anderson *et al.* 1998) and in feral horses, which had never been treated with anthelmintics, the subpopulation of IVM resistant cyathostomes (small strongyle worms) was estimated at 4% (Young *et al.* 1999). The fast development of resistance to IVM and BZ in helminths of livestock can certainly partially be explained by this phenomenon. It would be worthwhile to examine whether similar high frequencies of DR alleles occur in human helminth populations, which were not exposed to drugs previously.

The number of genes involved in resistance and their dominance or recessiveness are other factors with an important impact on the rate at which DR spreads. Although contradictory reports have been published on *H. contortus* (the best studied helminth of veterinary importance), there is consensus that resistance to BZ is polygenic with at least two, possibly three, genes with recessive alleles involved. Levamisole resistance seems to be caused by one gene or gene cluster, the alleles of which are autosomal recessive, and IVM resistance appears to be mediated by a single gene or gene complex with primarily dominant effects (Anderson *et al.* 1998). Although some argument is still ongoing about the number of genes involved in resistance to these different anthelmintics, there is a general agreement that reversion to susceptibility is rare once DR has developed in livestock helminths, even when other drugs with completely different working mechanisms are used for prolonged periods. This is supported by many field and experimental data, reviewed by Conder and Campbell (1995). Although the findings in veterinary helminths cannot be extrapolated directly to humans, the chances that similar phenomena might occur in the medical sector are real.

Table 2 Human helminths with suspected or proven drug resistance

Species	Drug	Country	Reference
Necator americanus	Mebendazole	Mali	De Clercq *et al.* (1997)
Ancylostoma duodenale	Pyrantel	Australia	Reynoldson *et al.* (1997)
Schistosoma mansoni	Oxamniquine	Brazil	Cioli *et al.* (1992, 1993)
	Praziquantel	Senegal	Stelma *et al.* (1995)
		Egypt	Ismail *et al.* (1999)
Onchocerca volvulus	Ivermectin	West Africa	R.K. Prichard, pers. comm.

Contributing factors to the development of drug resistance

Table 3 compares the factors influencing the development of DR in helminths of livestock and man. *Treatment frequency* is certainly one of the most important factors in the selection of DR: the higher the drug pressure, the faster the selection of resistant helminth strains. Although treatment frequencies of 10–15 times a year have been reported in livestock (Dorny *et al*. 1994), most often the number of treatments is limited to 1–3 per year. Even at these lower treatment frequencies, many cases of DR have been reported in sheep and goat helminths (Geerts *et al*. 1990; Bauer & Failung 1992; Burger & Bauer 1994; Coles *et al*. 1995; Boudsocq *et al*. 1999). The current number of annual treatments in helminth control programmes in man also falls within this range: 1–3 for *Trichuris/Ascaris* (Warren *et al*. 1993; Renganathan *et al*. 1995), 1–2 for *O. volvulus* (WHO 1995) and 1–2 times a year for schistosomes (El Khoby *et al*. 1998). Especially when the same drug is used over prolonged periods, as is the case with IVM in the West-African Onchocercosis Control Programme and PZQ in schistosomiasis control programmes in Egypt, these lower treatment frequencies might be able to select for resistance. This has been clearly shown in livestock helminths, where farmers tend to use a single drug until it fails (Geerts *et al*. 1987; Reinemeyer *et al*. 1992). Computer models have shown that the development of DR in livestock helminths is delayed if drugs with a different working mechanism are used in combination and on condition that the initial frequency of resistance alleles is low. Annual rotation of two drugs is the second best choice, because it postpones resistance for a longer period than in case of rotation of drugs at each treatment or rotations at 5- or 10-year intervals (Barnes *et al*. 1995).

Table 3 Factors influencing the development of drug resistance in helminths of livestock and man

	Livestock	Man
Treatment frequency (No./year)		
Gastro-intestinal nematodes	1–3	1–3
Onchocerca volvulus	NA	1–2
Schistosoma spp.	NA	Up to 1–2
Single drug regimens		
Gastro-intestinal nematodes	BZ or IVM	ALB
O. volvulus	NA	IVM
Schistosoma spp.	NA	PZQ
Targeting of treatments		
Kind of treatment	Mass	Target
Coverage (%)	± 100	< 80
Underdosing		
Weight underestimation	+++	+
Economic reasons	+++	++
Substandard drugs	+++	++

NA: not applicable; ALB: albendazole; BZ: benzimidazoles; IVM: Ivermectin; PZQ: praziquantel.

The *target group* of the anthelmintic treatment is another important parameter which influences the development of DR. In the past, strategic or prophylactic mass treatments of livestock have been the rule and this practice is certainly responsible for many of the DR problems veterinarians are facing now. Especially the drench-and-move system, in which all animals in a flock are treated before they are moved to clean pastures containing few or no worms in refugia, is a strong selector of DR. The phenomenon of refugia, i.e. the proportion of the helminth population that is not exposed to drugs and thus escapes selection for resistance, is a very important factor, whose impact on the development of DR is too often overlooked (Van Wyk 2001). Only recently veterinary parasitologists have realized that a balance has to be found between treatment efficacy and delaying the development of DR. Models have shown that it is possible to delay the development of DR by not treating part (e.g. 20%) of the herd or flock, although this might have some consequences on productivity (Barnes *et al*. 1995). It is well known that there is an overdispersion of the helminth population, with a few individuals carrying a heavy worm burden, whereas the majority is only lightly infected. In South Africa, this principle has been applied in the development of the Famacha®, a colour card, which allows the farmer to identify anaemic sheep needing to be dewormed, on the basis of coloured drawings of the eye mucosa. In areas of South Africa where anaemia is mainly caused by *H. contortus*, this system has allowed to decrease significantly the number of animals treated and the treatment frequency without reducing productivity too much (Van Wyk *et al*. 1997a).

In control programmes of human helminths, indiscriminate mass treatments are no longer advocated by most of the medical parasitologists. Treatment is usually limited to target groups such as school children. This seriously reduces the selection pressure, which is even further reduced because the compliance is often less than 80% contrary to livestock where the coverage usually approaches 100% (Chitsulo *et al*. 2000).

Besides the target group, the *timing of the treatment* might also influence the development of DR. On some dry Greek islands, resistance in sheep helminths developed much more rapidly than in other regions with similar worm control practices but with a wetter climate. This can be explained by the fact that the helminth generation which develops after treatment in dry environments will almost

completely consist of resistant worms, whereas in wetter parts pre-parasitic stages of susceptible worms might survive on pasture and dilute the resistant genes in the next worm generation. This aspect should also be taken into consideration when treating helminths of man. Treatment in dry, low-transmission periods has been proposed by WHO (1992) for the control of schistosomiasis. The advantages and inconveniences of such treatments should be carefully evaluated.

Finally, *underdosing* might also constitute an important risk factor for the development of DR. As is shown by the models developed by Smith *et al.* (1999), here again the impact depends on the initial (before exposure to a given anthelmintic) and the resultant (after treatment) frequency of resistance alleles in the helminth population. Depending on their ability to kill all or part of the susceptible homozygote, heterozygote and/or homozygote resistant helminths, there are dose levels where underdosing promotes resistance and others where resistance is impeded. Assuming that resistance is determined by a single major gene comprising two alleles at a single autosomal locus and low initial frequency of the allele for resistance, the most dangerous dose is the one that kills all susceptible homozygotes but none of the other genotypes. On the contrary, when the initial frequency of the allele for resistance is high, the dose which promotes resistance most strongly is that which kills all susceptible homozygotes and all heterozygotes, but none of the resistant homozygotes (Smith *et al.* 1999). Table 3 summarizes the most important factors resulting in underdosing: underestimation of weight, dilution of the drug for economic reasons and the use of substandard drugs (Shakoor *et al.* 1997; van Wyk *et al.* 1997c; Monteiro *et al.* 1998). All of them may be more important in veterinary than in human medicine. However, lower drug doses are sometimes advocated for the treatment of human helminths in order to reduce side-effects or when the objective is only morbidity control (Warren *et al.* 1993). Taking into account these restrictions, this approach might select for resistance under certain conditions.

Detection of drug resistance

The techniques which are most frequently used for the detection of DR in helminths of livestock are summarized in Table 4. Their sensitivity is rather poor, except for the PCR. Martin *et al.* (1989) did prove that the faecal egg count reduction test (FECRT) and the egg hatch assay are able to detect DR only when at least 25% of the helminth population carries resistance genes. For the larval development assay it is assumed although not proved that its sensitivity is slightly better, resistance being detected when 10% of the worm population is resistant (Dobson *et al.* 1996). As modelling exercises have shown that reversion to susceptibility might only be possible when less than 5% of the helminths carry resistance genes (Roos & Kwa 1994), these tests will obviously detect resistance when it is too late. Nevertheless a standardized FECRT is of crucial importance in order to allow comparison of data on a spatial and temporal basis. The World Association for the Advancement of Veterinary Parasitology (WAAVP) has developed standardized guidelines for the detection of DR in helminths of livestock (Coles *et al.* 1992). The important parameters of the WAAVP test are summarized in Table 5.

Table 4 Techniques for the detection of drug resistance in helminths of livestock

Test	Anthelmintic	Sensitivity
Faecal egg count reduction	All	Low
Egg hatch	BZ	Low
Larval development	All	Low
PCR	BZ, IVM	High

BZ: benzimidazoles; IVM: Ivermectin; PCR: polymerase chain reaction.

Table 5 Faecal egg-count reduction test for the detection of drug resistance (DR) in helminths of man and animals

Parameter	Animals*	Humans†
No. treated	≥ 15	≥ 50
No. controls	≥ 15	≥ 50
Technique	McMaster	Kato
Interval treatment–egg count	10–14 days (*N*)	2 weeks (hookw.) 5–6 weeks (schist.)
Criteria for DR: ERR (%)	95 (lower CI: < 90)	50 (*Trichuris*)‡ 70 (*Ascaris, Schistosoma*)‡

ERR: egg reduction rate; CI: 95% confidence interval; N: nematodes.
* Coles *et al.* (1992).
† Geerts and Gryseels (2000).
‡ WHO (1999).

Similar slightly adapted parameters could be used for the detection of DR in human helminths. Given the fact that groups of children or adults are usually less homogeneous than herds or flocks of animals and taking into account the unavoidable drop-out rate, the number of people in the study groups should be higher (preferably 50 or more) than the number of animals (15) advised by the WAAVP. For schistosomes, *Ascaris* and *Trichuris*, the Kato technique can be used in a standard way as described by Katz *et al.* (1972) and Polderman *et al.* (1985). For hookworms the Kato slides should be read within 30 min after preparation in order to get reliable results (Martin & Beaver 1968). Given the strong day-to-day variations of schistosome egg output, at least three stool samples collected on different days should be examined (Engels *et al.* 1996). The interval between treatment and egg count has to be adapted to the helminth species: for hookworms or other nematodes and for schistosomes 2 and 5–6 weeks should be appropriate, respectively (Geerts & Gryseels 2000). DR is considered to be present in livestock helminths if the egg reduction rate (ERR) is less than 95% and if the lower 95% confidence interval is below 90% (Coles *et al.* 1992). WHO (1999) suggested cut-off values of 50 and 70% for *Trichuris* and *Ascaris*/schistosomes, respectively, below which the possibility of DR should be considered. However, drug efficacies can vary enormously depending on the manner in which summary statistics of drug trials are calculated (Sacko *et al.* 1999). Therefore, it is important to standardize the methods used for assessing and interpreting drug efficacy.

Given the low sensitivity of the FECRT and *in vitro* tests, more sensitive assays such as PCR are urgently needed. In the veterinary field PCR assays are available for the detection of resistance to BZ (Kwa *et al.* 1994; Elard *et al.* 1999) and IVM (Blackhall *et al.* 1998; Xu *et al.* 1998). As the mutation, which is responsible for resistance to BZ (point mutation of residue 200 of isotype 1 β-tubulin), seems to be present in many parasitic nematodes and even in fungi (Jung *et al.* 1992; Koenraadt *et al.* 1992), it might be anticipated that it will occur also in human nematodes, which develop resistance to BZ. However, other mechanisms of BZ resistance (deletion of isotype 2 β-tubulin, P-glycoproteins?) have been identified and should also be looked for (Roos *et al.* 1995; Kerboeuf *et al.* 1999).

Based on the research for the development of a PCR for the detection of resistance to IVM in livestock helminths, two putative P-glycoprotein coding genes have been identified in *O. volvulus*. These are expressed at a low level in IVM-sensitive microfilariae and at a high level in IVM-tolerant adults (Huang & Prichard 1999). This opens perspectives for the development of a PCR for IVM resistance in this important helminth species. Molecular tools for the detection of resistance to PZQ in *S. mansoni* are already available. Pereira *et al.* (1998) described an RT–PCR which allows to identify an overexpression of a fragment of subunit 1 of cytochrome-c oxidase in resistant strains. Recently, Tsai *et al.* (2000) discovered a primer for RAPD–PCR which was able to distinguish resistant from sensitive Egyptian strains of *S. mansoni*.

As the frequency of alleles associated with drug resistance may be quite high even in susceptible worm populations, as mentioned earlier, it is important not to pool DNA from different worms, but to examine DNA from individual parasites and to compare the results from suspected strains with those from susceptible strains. Therefore, well-characterized reference resistant and susceptible strains are urgently needed for use in either the classical or the molecular assays.

Conclusions

Although the present importance of DR in human helminths is not at all comparable with that in livestock, the dramatic and rapid spread of resistance to all major classes of veterinary anthelmintics should warn the medical world against the widespread use of anthelmintics for the control of helminths. Relying exclusively on drugs in helminth control programmes – as was too often the case in veterinary medicine – must be avoided if we want to maintain the efficacy of the currently available drugs. Based on the experiences with livestock helminths, we propose the following guidelines to delay the development of DR:

- Give priority to accessible diagnosis and treatment of symptomatic individual cases;
- Only use community-based treatment if really necessary, i.e. if morbidity is high;
- Avoid indiscriminate mass treatment;
- Reduce treatment frequency by combining the use of drugs with other measures such as health education programmes, construction of latrines, etc.
- Avoid exposure of the whole helminth population to the drug;
- Use the correct dose;
- Implement combined drug use or – if not possible – annual rotation of drugs and
- Monitor the development of drug resistance.

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