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Drug Resistance in Human Helminths: Current Situation and Lessons from Livestock

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INTRODUCTION

In recent years, several reports of apparent failures in the treatment of human schistosomes and nematodes have been published (33, 81, 116, 132). Although the interpretation and the implications of these studies are still being debated, they have led to an increased awareness of the potential problem of anthelmintic resistance (AR) in the treatment and control of human helminths.

In view of the short but worrying history of AR in livestock, such concerns are not superfluous. At present, AR is the most important disease problem of the sheep-farming industry in Australia, South Africa, and possibly South America (140, 146, 147). Twenty years ago, however, many scientists considered drug resistance in livestock helminths an unimportant phenomenon. High prevalences of AR, often exceeding 50%, have now been reported in all parts of the world for gastrointestinal helminths of sheep, goats, and horses kept in industrial livestock systems. Surprisingly, up to now very few problems with AR have been noticed in cattle helminths (58). Table 1 summarizes the helminth species and the anthelmintic classes most frequently involved.

Even multiple drug resistance is not uncommon in helminths of veterinary importance. In parts of Paraguay (95) and South Africa (140), helminths are resistant to all available broad-spectrum anthelmintics and farmers have started to give up sheep farming because of insurmountable problems with AR (138).

For purposes of discussion, AR is defined as a heritable reduction in the sensitivity of a parasite population to the

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TABLE 1. Main helminth species of livestock for which drug resistance has been reported

Host	Parasite	Resistance to[a]:		
		BZ	LEV-MOR	AVM-MIL
Sheep and goats	*Haemonchus contortus*	+		+
	Ostertagia spp.	+	+	+
	Trichostrongylus spp.	+	+	+
Horses	*Cyathostomes*	+		

[a] BZ, benzimidazoles; LEV-MOR, levamisole-morantel; AVM-MIL, avermectins-milbemycins.

action of a drug. The reduction is expressed as the decrease of the frequency of individual parasites affected by exposure to the drug, compared to the frequency observed in the same population upon initial or prior to exposure (31). Although not unequivocal but generally considered the most adequate, this definition encompasses two biologically distinct but not always distinguishable processes: (i) existing drug-tolerant parasite lines may become more frequent, particularly under drug pressure, and (ii) previously susceptible parasites may undergo genetic mutations, possibly induced by drug exposure, and be selected under drug pressure.

The term "tolerance" refers to the innate unresponsiveness of a parasite to a drug, independent of prior exposure to that drug or to others belonging to the same class.

In advancing the cause for the widespread use of drugs to control human helminths, Cerami and Warren (20) believed that "helminths are less likely to develop resistance or would do so more slowly" compared to other infectious agents because they multiply at a lower rate. This assumption has certainly not appeared valid for livestock helminths, justifying caution in the treatment of human helminths as well. AR may not be a medical problem yet, but for all we know the few reports so far may represent only the tip of an iceberg. Veterinary experiences have shown that the problems becomes apparent only when it is too late and reversion to susceptibility is no longer possible (31). Individual treatment failures may often remain unnoticed, since most helminth infections lead only to subclinical disease. Epidemiologically, there have been few efforts so far to examine or monitor the problem. The development of drug resistance, and AR in particular, usually follows a sigmoidal pattern: a long period of incubation with only a few scattered cases is followed by a sudden explosion of the problem (145). Once AR becomes apparent, it may very quickly become a major problem in both clinical and preventive medicine.

For more than a decade, veterinary researchers have drawn the attention of the medical community to the risk of AR development in human helminths, such as schistosomes and hookworms (26, 28, 62, 128). Drawing from the lessons and errors in their own field, they urged medical workers to use anthelmintics more carefully in order to avoid or at least to delay the development of AR. Nevertheless, the widespread drug use for the control of schistosomiasis, onchocerciasis, and geohelminths has been increasingly advocated by scientists and international organizations, with drug companies willing to offer assistance (1, 17, 113, 151). In light of these issues, in this paper we critically review the available evidence for drug resistance of human helminths at present and discuss the prospects for the future, taking the veterinary experiences into account.

REPORTS ON DRUG RESISTANCE IN HUMAN HELMINTHS: A CRITICAL ANALYSIS

Early reports on possible resistance to santonin in *Ascaris lumbricoides* (86) and diethylcarbamazine (DEC) in *Onchocerca volvulus* (143) were not well documented and cannot be assessed for accuracy and relevance. In this section, we concentrate on the more recent and better documented reports on AR of human nematodes (hookworms) and trematodes (schistosomes). AR of human cestodes has not yet been reported. Also, livestock cestodes do not seem to develop drug resistance easily; only a single report of drug resistance in tapeworms of sheep (*Moniezia expansa*) has been published (144).

Drug Resistance in Nematodes

Use of anthelmintics. The main drugs used to treat human nematodes nowadays are mebendazole, albendazole, pyrantel pamoate, and levamisole for intestinal nematodes, ivermectin (IVM) for onchocerciasis, and DEC alone or DEC-albendazole and IVM-albendazole combination treatments for filariasis (1, 35, 154). Depending on local epidemiology, availability, and cost, these drugs have been widely available in most health care systems for the curative treatment of clinical cases for many years. In addition, the use of anthelmintics is now being strongly advocated in a preventive, population-based way as well (1, 17, 113, 151, 155). It is estimated that some 1.3 to 2.0 billion people in the world suffer from helminth infections. Although direct mortality is low, intestinal helminth infections are believed to contribute to "general morbidity." Both intestinal helminths and schistosomiasis have been associated with anemia, stunted growth, poor nutritional status, and reduced physical and intellectual abilities (17, 18, 151); onchocerciasis has been associated with severe itching, skin diseases, poor health, and even reduced chances for marriage. By providing single-dose anthelminthics on a regular basis to entire populations or high-risk groups (such as schoolchildren and pregnant women), it is hoped to reduce both morbidity and transmission. It has even been proposed to combine albendazole, IVM, and praziquantel (PZQ) at a low dose in a single tablet and to distribute it to virtually all school-age children in the developing world (148, 149). The proponents of these strategies recognize the risk of emergence of AR but usually judge it to be insignificant. As mentioned above, veterinary experiences dictate otherwise. The recently published reports on AR in human helminths must thus be taken seriously, yet examined critically.

Problems of defining drug resistance in hookworms. It should first be noted that complete cure of hookworm infection (and most other helminth infections for that matter) is usually not achieved with any drug. Depending on the dosage and the coprological method applied (with lack of standardization and control methods being a noteworthy problem), cure rates as low as 61% (400 mg) and 67% (800 mg) for albendazole, 0% (single dose) and 23% (repeated dose) for levamisole, 30% (single) and 37% (repeated) for pyrantel pamoate, 27% for thiabendazole, 19% (single) and 45% (repeated) for mebendazole have been reported (35, 88).

Thus, at least some hookworm populations show some degree of (innate) tolerance to at least one of the drugs currently in use. The different susceptibilities of the two species *Ancylostoma duodenale* and *Necator americanus* is well established. Most probably, the degree of tolerance varies regionally, even locally, within a species.

Second, the results of field trials depend critically on the coprological methods used. The number of hookworm eggs per

gram (EPG) measured by the Kato-Katz method, commonly used for schistosomes, is unreliable if not strictly standardized. This method consists of measuring 25 to 50 mg of sieved stools in a punched template, after which the sample is allowed to clear with glycerin. Since hookworm eggs tend to dissolve quickly and uncontrollably, the slides must be examined within 30 to 60 min of preparation (96, 110). In the field, however, Kato slides are often difficult to read, unless the thick fecal smear has been allowed to clear for at least several hours, particularly when the feces are hard or dark or when quantities over 25 mg are examined, such as in the commonly applied Kato-Katz technique (83, 106, 109, 131). To quantify hookworm eggs correctly and certainly to compare the number of EPG between individuals or groups or over time, the method must be strictly followed. Qualitative methods, such as $ZnSO_4$ flotation or Ridley's formol-ether concentration, allow only semiquantitative determinations at best. The most sensitive method, stool culture, is laborious and also only semiquantitative. It is noteworthy, however, that the few therapeutic trials in which this method was applied have resulted in considerably lower cure rates than were reported with other methods, and this holds for most of the drug regimens in use (88). Finally, even correctly measured egg counts or EPGs must be interpreted cautiously, since they are only an indirect measure of worm counts (the actual outcome indicator of transmission and treatment) and are subject to inter- and intraindividual variations (38, 74).

In contrast to veterinary helminthology, in which methods and cutoff values to define AR are well established and standardized (27), there are no such guidelines in human helminthology. In vitro methods for the biological confirmation of AR have not been developed or validated for human nematodes. Also, the local endemic situation and the timing of follow-up are of paramount importance in tests for the detection of AR, and this is true in different ways for different species and drugs. In endemic situations, people (particularly children) who were cured are reinfected quickly and may reach the pretreatment level of infection within a few months. Moreover, they may carry prepatent infections which are affected by some drugs but not by others such as mebendazole, which is hardly absorbed.

Therapeutic trials for treatment of human helminths demand rigorous statistical methods, since the worms are overdispersed (i.e., a large number of worms are present in a small proportion of the hosts) within a population due to physiological, immunological, ecological, and behavioral factors. The study and control populations must therefore be large enough and randomly selected, and upon analysis any cluster bias must be excluded. A few "wormy" people in one or another group may lead to fatal flaws in the analysis of the results (3, 18). Clearly, lack of validated methods and reference data, many confounding factors, and complex statistics complicate the interpretation of low drug efficacy.

Reports of drug resistance in hookworms. Two recent publications have invoked AR as the probable cause of failure of anthelmintic treatment of human hookworms. Both are community-based studies in field conditions, not clinical observations. De Clercq et al. (33) described a failure of mebendazole to treat *N. americanus* in Mali, whereas Reynoldson et al. (116) reported poor efficacy of pyrantel pamoate against *A. duodenale* in northwestern Australia. The salient features of both reports are summarized in Table 2. The authors mentioned other possible causes of reduced drug sensitivity of the hookworms such as a genetic change in the susceptibility of the local strain of hookworms (i.e., not through selection pressure by the drug) or host factors (such as local diets) which might have altered the pharmacodynamic properties of the drug. However, some features which were present in one or both localities are suggestive of possible drug resistance. Since regions in Mali and Australia are remote, relatively isolated areas with probably a rather limited influx of infected foreigners, local helminth populations may have been isolated with little dilution or replenishment by (susceptible) helminths from elsewhere. Under these circumstances, AR would develop more rapidly, because of the lack of influx of susceptible genotypes (2).

The possible development of resistance to mebendazole in human hookworms (Mali study) would not altogether be surprising, since benzimidazoles (BZ) are known to be relatively good selectors of AR (8, 118). In helminths of livestock, BZ resistance has appeared quickly and spread easily (31). On the other hand, the drug pressure in the Mali community was not especially noteworthy, as far as data are available (no history of previous mass treatments).

Pyrantel/morantel resistance in livestock helminths developed mainly as cross-resistance due to widespread use of levamisole (125). In the Australian study (116), there might be a plausible case for intense pyrantel pressure having led to specific resistance: it had been used for passive case detection as well as active community treatment for decades. Albendazole, which had not previously been used in this population before, worked perfectly, thereby also validating the hypothesis.

The hypothesis of drug resistance in the Australian situation was inspired by clinical suspicion of resistance in an area where pyrantel pamoate had been used for a considerable length of time in the community. The reported efficacy of pyrantel pamoate (cure rate [CR] 13%; egg reduction rate [ERR], 46%) at the given (relatively low and single) dose and for the particular species is below those documented elsewhere, although CR as low as 19% have been described (35). The reported ERR is based on Kato slides from a small number of subjects and may therefore be biased. The study did not include an untreated control group, a necessity for the correct interpretation in light of egg output variations or statistical bias due to aggregation. The follow-up period of 7 days was relatively short, and no in vitro confirmation was attempted. In conclusion, the situation and the data are suggestive but fall short from providing conclusive evidence.

In the Mali study, drug resistance was discovered within the context of a research project on schistosomiasis. Since there was no history of intense treatment or clinical suspicion of drug resistance, the local situation was not different from any other area of endemic infection in Africa. Single-dose mebendazole treatment is known to be of low efficacy, with a reported CR as low as 18% and an ERR as low as 46% (35). Few data are available from sub-Saharan Africa. Therefore, the low CR and ERR in the treated groups may be due to a general low susceptibility of African hookworms to that drug regimen, as well as to local resistance. Also, pyrantel, the control drug used, is known to have little activity against human hookworms (88). Furthermore, the Mali study relied on Kato-Katz slides from "overnight samples that were processed and examined on the same day" (33), which may have led to some overclearing of the slides and consequent underestimation of hookworm egg counts. The 4-week interval between treatment and examination was too long to distinguish treatment failure from rapid reinfection and/or maturing prepatent infections, particularly in a relatively high-transmission area and for a drug such as mebendazole, which does not affect immature infections.

Both a negative and a placebo group were included, showing ERRs of 37.5 and 32.5%, respectively. This may be considered suggestive of the poor efficacy of mebendazole but also of statistical and methodological bias. The in vitro confirmation

TABLE 2. Important features of reports on treatment failures of human hookworm infections[a]

Characteristic	Mali (*N. americanus*)	Australia (*A. duodenale*)
Helminth species		
Initial prevalence and transmission	High	Moderate
Previous drug exposure	In health centers	Community treatment
Anthelmintic drug	Mebendazole (Vermox)	Pyrantel (Combantrin)
Dose	500 mg/person	10 mg/kg
Treatment regimen	Single dose	Single dose
Study design		
No. of subjects	103	29
Random selection of subjects	Yes	Yes
Control group, other drug	Pyrantel	Albendazole
Control group, no treatment	Yes	No
Placebo	Yes	No
Coprological method	Kato-Katz	ZnSO4 flotation + Kato
EPG[b] after treatment (wk)	4	1
Cure rate (%)[c]		
Treated group	22.9	13.3
Control group, no treatment	25.0	ND[e]
Control group, other treatment	44.8	100
Placebo group	22.6	ND
Egg reduction rate (%)[d]		
Treated group	−6.5(increase)	−46.1(increase)
Control group, no treatment	39.5	ND
Control group, other treatment	75.0	100.0
Placebo group (vitamin C)	32.7	ND
In vitro assay (drug resistance)	Egg hatch test	ND

[a] Data from references 33 and 116.
[b] EPG, eggs per gram of feces.
[c] Percentage of treated (infected) persons becoming negative after treatment.
[d] Percent reduction of EPG after treatment compared to EPG before treatment.
[e] ND, not done.

of the Mali results was based on the egg-hatching technique, accepted in veterinary medicine but not yet standardized for human hookworms. A 50% reduction of egg hatchability was found compared to a laboratory strain; it is unclear if this difference is statistically or biologically significant. Strain differences, processing of the field samples, delays during transport, etc., may have affected the results. Again, this study is at best suggestive, but does not provide conclusive evidence for reduced mebendazole efficacy. This study has since been repeated using a more rigorous study design, in which the efficacies of three anthelmintics (mebendazole, albendazole, and pyrantel) against *N. americanus* were compared (121a). Participants were examined 10 days after treatment. After controlling for the drift in the fecal egg counts (opposite trends in male and female subjects) in the placebo-treated subset, age, sex, fasting, and intensity of infection, single-dose mebendazole (500 mg) treatment showed efficacies (ERR) ranging from 60.9 to 89.9%, depending on the method used for the evaluation of the results. The efficacies obtained using albendazole (single dose of 400 mg) and pyrantel (12.5 mg/kg) ranged from 92.1 to 99.7% and 4.8 to 89.7% respectively (121a). These results are more or less consistent with those reported elsewhere (35, 88). Thus, it remains a matter of conjecture whether pyrantel and mebendazole lack efficacy against *N. americanus* or whether resistance is beginning to develop.

In conclusion, AR in human hookworms might already be present, but the evidence to date is doubtful. Future studies should be carried out under well-controlled conditions and using standardized methods for trial design, calculation of summary data relating to drug efficacies, and statistical analysis to confirm the presence or absence of drug resistance in these or other human hookworms populations (121a). Ideally, clear hypotheses, standard protocols (in vivo as well as in vitro), and indisputable cutoff values should be established by a governing body and/or multidisciplinary groups of scientists, such as has been the case in veterinary medicine by the World Association for the Advancement of Veterinary Parasitology (WAAVP).

However, the doubts about the reported data should not lead to optimism or complacency. If anything, the critical review of these and earlier data shows that tolerance traits are indeed present in many hookworm populations. Even without taking into account the possibility of mutations, experience in veterinary practice suggests that these traits might quickly and irreversibly become dominant in helminths under drug pressure.

Drug Resistance in Schistosomes

Use of antischistosomal drugs. Praziquantel (PZQ) is the most common drug for the treatment of human schistosomiasis (32, 89, 155), since it is active against all the *Schistosoma* species (*Schistosoma mansoni*, *S. haematobium*, *S. japonicum*, *S. intercalatum*, and *S. mekongi*). In the field, particularly in community treatment, the usual dosage is 40 mg/kg of body weight in a single dose; higher dosages or split regimens result in lower compliance (89). In hospitalized patients, particularly for *S. japonicum* and *S. mekongi*, and for heavy infections with the other species, the recommended dose is 30 mg/kg, up to

three times daily, for two consecutive days (32, 35, 89). The drug is safe, with few or limited side effects; in heavy infections with *S. mansoni*, acute abdominal cramps and bloody diarrhea are frequent but always transient. CR with 40 mg/kg are usually between 70 and 90%; ERR are above 90% (32, 71, 89).

In endemic conditions, reinfection is the rule rather than the exception, particularly in children, who are heavily exposed and appear to be (innately or immunologically) more susceptible to infection than adults (72). Nevertheless, when the intensity and duration of infection decrease, treatment considerably reduces individual pathology and community morbidity (89, 155).

Several brands and generic formats of PZQ are now on the market. Although there is no indication so far that substandard products are a problem (103), some products are of unclear origin; it is advisable to select reputed production or wholesale companies complying with international quality control procedures. International competition has brought the initial high price back to about 40 cents per average dose. The World Health Organization has therefore recently called for a major effort to bring the drug within reach of all primary health care systems (101).

In several countries with major endemic infections, the drug is not only widely available for treatment but is also being actively distributed to prevent or control disease ("morbidity control"). Community-based treatment after active screening, through indiscriminate mass treatment, or in specific target groups is now the major control strategy in Egypt, China, Brazil, the Philippines, and several other countries (89, 155). For example, all school-age children and millions of adults are screened and, if necessary, treated every 6 to 12 months in Egypt. In high-prevalence areas, treatment is now given indiscriminately to the entire population (46). Out of concern for the appearance of drug resistance under such high drug pressure, an elaborate national monitoring system has been set up in which stool samples from apparent treatment failures are referred to regional research centers and subjected to in vivo and in vitro tests.

Oxamniquine, used at a dosage of 15 to 40 mg/kg, is active only against *S. mansoni*, with CR (>80%) and ERR (>95%) usually somewhat higher than with PZQ (71, 155). Although by and large a safe drug, oxamniquine may have troublesome side effects in some individuals, such as drowsiness, severe dizziness, and seizures. It is used mostly in Brazil and is not on the market any more in most of Africa because of the commercial dominance of PZQ.

Metrifonate is another, inexpensive drug, active only against *S. haematobium*, that was available until recently, but it is no longer available for the treatment of schistosomiasis.

Thus, there is presently only one general schistosomicide available, PZQ. The single available alternative, oxamniquine, is active only against *S. mansoni*. The emergence of resistance is therefore a frightening prospect, not only for disease control or prevention but also for curative use in clinical practice.

Reports on resistance to schistosomicides. As with nematodes, it should first be noted that CR and ERR in therapeutic trials with any drug for human schistosomes rarely reach 100%, even in situations where reinfection is excluded (32, 71). Moreover, reported cure rates considerably overestimate real CR. Many light infections (with EPGs below the detection limit of the coprological techniques) that persist after treatment are not detected by the usual diagnostic methods but require repeated or very sensitive examinations (37, 70). Thus, the recommended doses of schistosomicides should be considered subcurative (41). In light of these data, it is safe to assume that in schistosome populations, some individual parasites are tolerant to the drug to some degree, at least at the usual dosages.

Unlike for nematodes, robust parasitological methods for the measurement of egg counts are available for schistosomes, such as the Kato-Katz method for fecal eggs and urine filtration for urinary schistosomiasis (83, 106, 155). Moreover, the detection and quantitation of circulating antigens in blood and urine have added another quantitative tool for the evaluation of drug efficacy (34). On the other hand, day-to-day variation of egg output and antigen levels is substantial; e.g. the coefficient of variation of EPGs in seven consecutive stool examinations varied between 28 and 245% (50), and the relation between worm numbers in the blood and egg counts in excreta is even more indirect and statistically complex than for nematodes (37, 70).

Resistance of schistosomes to oxamniquine is undisputably documented, both in vivo and in vitro (23, 25). Epidemiologically, the phenomenon has remained remarkably limited to scattered areas in Brazil. Possibly, the resistance trait is disadvantageous to parasite survival and/or reproduction of schistosomes; also, the mutation may actually be induced by exposure of individual schistosomes to oxamniquine (16). Combined, these factors would explain a self-limiting process even under drug pressure. Since the use of oxamniquine is by and large confined to Brazil and since it is being replaced by PZQ, oxamniquine resistance is not considered to be a major problem.

Recent reports on the possible development of resistance to PZQ have generated much more unrest, particularly since this drug is at the basis of current control strategies aimed at the reduction of morbidity through population-based treatment (152, 153, 155). The first field report came from a new, intense, and epidemic focus in northern Senegal (72, 132). In a community with extremely high prevalences and intensities of infection, a CR of only 18% was observed using PZQ, much lower than is usually reported from other (even comparably intense) foci (132). However, ERR were still over 80%. Heavy initial infections, intensive transmission, prepatent parasites, and immunological naivety were considered the most likely explanations for these low CR. The possibility of drug resistance or tolerance could not be ruled out, however. Another hypothesis was that in such an epidemic focus, a clonal parasite population may have sprung from a few tolerant worms.

The matter was further investigated in a systematic series of field studies, the results of which can be summarized as follows. (i) The low CR with PZQ at 40 mg/kg (18 to 36%) in the field were confirmed in four more study cohorts, consisting of various age and infection-intensity groups, in different seasons, with different timings of follow-up surveys, and with circulating antigen detection (72, 130, 137). (ii) CR remained abnormally low when the dose was increased to two consecutive doses of 30 mg/kg at a 16-h interval (73). CR for oxamniquine at 20 mg/kg in a single dose, however, were normal (84%) (132). (iii) CR with PZQ at 40 mg/kg rose to normal when the treatment was repeated after 2 to 4 months and were also normal in children originating from the area of endemic infection but living in an urban area with no transmission (108; A. Mbaye, D. Engels, L. Tchuente, and B. Gryseels, unpublished results). (iv) The efficacy of PZQ could be related to age and pretreatment intensity but not to other host factors, including behavioral and immunological parameters (137). (v) Application of a statistical model relating egg counts more accurately to worm numbers showed that the poor CR could be explained by the initial high intensity of infection, even if over 95% of the worms were killed (S. J. de Vlas, D. Engels, A. Mbaye, and B. Gryseels, Schistosomiasis Res. Project Conf. Proc., p. 211, 1998).

The overall conclusion that may be drawn from these observations is that there is no convincing field evidence of reduced susceptibility of *S. mansoni* to PZQ and that the observed low CR may be explained by the specific epidemiological situation. Unfortunately, there is no reliable in vitro test available to determine PZQ resistance. In fact, a major problem in developing such a test is precisely the lack of a reference schistosome strain that is resistant.

Several experimental in vivo studies have recently been conducted to unravel the problem in Senegal. In short, these studies have shown the following. (i) It was possible to select from a mixture of *S. mansoni* strains kept for years in the laboratory a parasite population that was almost insensitive to PZQ treatment (51). However, it is probable that this result can be explained by the experimental protocol, in which mice were treated after 35 days of infection. Parasite lines with a slower maturation time would not yet be susceptible to PZQ at that time and would be selected under drug pressure as a "resistant" strain (22). (ii) In the same protocol, a "wild" Senegalese strain appeared to be less susceptible to PZQ (53). Remarkably, this observation was not consistent with the high ERR observed in the field, indicating reduced susceptibility at most. Again, it is quite probable that the result was an artifact of the early treatment of the infected mice. Subsequent studies with experimental treatment after 60 days of infection showed a markedly improved efficacy, albeit lower than in other geographical strains (52). (iii) In another laboratory, schistosomes isolated from Senegalese patients who had undergone several treatments but still (or again) excreted eggs did not show any reduction in susceptibility to PZQ (21, 22).

The consistent field observation of low cure rates with PZQ can apparently be explained statistically by the high initial worm burdens and possibly heavy immature infections (against which PZQ is not very effective), in combination with the inherent limits of the diagnostic system. Biologically, this hypothesis is supported by the high levels of circulating antigen (indicating heavy infections) and the results of repeated treatments and treatment in areas with no endemic infection, which gave normal cure rates. The "normal" results with oxamniquine can statistically be explained by a somewhat stronger inherent schistosomicidal effect. The results of the mouse experiments are conflicting; the only methodologically indisputable observation on reduced susceptibility is the geographical strain difference (52). Although geographical differences in drug susceptibility have not been described for PZQ, they are well known for hycanthone and oxamniquine, even leading to region-specific dosage recommendations (4, 32).

If anything, these studies lead to the conclusion that only a very substantial reduction in susceptibility can be detected reliably by current field methods. Laboratory confirmation is still compromised by the lack of standardization and reference material. The international effort to establish at least some tentative protocols and to coordinate the collection of data and material is therefore most welcome (114, 157).

Other, well-documented clinical and experimental reports come from Egypt, an area of endemic infection which, due to extensive drug usage, would seem predestined for the appearance of PZQ resistance. A nationwide monitoring system was set up to detect and investigate cases in which PZQ did not lead to cure, even after repeated treatment (9, 46). From several dozen cases, largely clustered in one geographical area, parasites were isolated that showed a reduced susceptibility in mice and in vitro compared to Egyptian reference strains (10, 81, 81a). Again, the lack of standardized methods, particularly in vitro, do not yet allow definite conclusions. At the very least, however, the possibility that less susceptible strains are (and possibly always were) present and are emerging more prominently under drug pressure cannot be excluded (10).

Conclusions

The recent reports on possible emerging drug resistance in human nematodes and schistosomes do not provide conclusive evidence for the increase of innately tolerant strains or for the appearance of newly mutated resistant strains. However, they strongly suggest that such tolerant or resistant strains can and do exist and that these strains may emerge more prominently under drug pressure (hookworm in Australia, schistosomes in Egypt) or under specific circumstances (schistosomes in Senegal). Perhaps even more important, the published studies show that available tools, methods, and reference materials are so far insufficient to detect problems of AR in a timely fashion, if at all. Therefore, we will review in more detail the knowledge of the veterinary world, which has a longstanding experience with AR, and analyze how it can be used to clarify and possibly remediate the situation in humans.

DRUG RESISTANCE IN LIVESTOCK HELMINTHS AND ITS RELEVANCE FOR HUMAN HELMINTHS

As described above, AR in livestock is now a well established fact. Several contributing factors have been identified and studied.

Contributing Factors for the Development of Resistance

High treatment frequency. Barton (6) and Martin et al. (98, 99) have shown in well-controlled trials that a high treatment frequency selects for resistance more strongly than do less frequent dosing regimens. There is also strong evidence that resistance develops more rapidly in regions where animals are dewormed regularly. Serious problems with AR in *Haemonchus contortus* were reported in some humid tropical areas where 10 to 15 treatments per year were used to control this parasite in small ruminants (42).

Drug resistance, however, can also be selected at lower treatment frequencies, especially when the same drug is used over many years. Several authors (7, 19, 29, 59) have reported the development of drug resistance even when only two or three treatments were given annually. This observation is important, since similar treatment frequencies are advocated for the control of intestinal nematodes in humans (17, 115, 148, 151).

Single-drug regimens. Often a single drug, which is usually very effective in the first years, is continuously used until it no longer works. In a survey of sheep farmers in the United States, Reinemeyer et al. (112) found that one out of every two flocks were dosed with a single anthelmintic until it failed. Long-term use of levamisole in cattle also led to the development of resistance, although the annual treatment frequency was low and cattle helminths seem to develop resistance less easily than do worms in small ruminants (58, 61). Frequent use of IVM without alternation with other drugs has also been reported as the reason for the fast development of resistance in *H. contortus* in South Africa and New Zealand (127, 139). In the light of these data, the frequent and continuous use of single drugs such as albendazole for the control of intestinal helminths, IVM for onchocerciasis, or PZQ for schistosomiasis in humans may raise concern. The quickness with which AR to BZ in livestock nematodes has spread is described above; if similar strategies are to be applied in humans, there is no reason why the same problems would not arise as well.

Because resistance of *H. contortus* in sheep and goats to

IVM has been widely reported (31), Shoop (127) has warned of the risk of AR problems in the onchocerciasis control programs in western Africa, which are increasingly based on periodic community-based treatment with ivermectin (113). Although the initial objectives of drug-based control strategies in schistosomiasis and helminthiasis were restricted to the reduction and prevention of disease in humans, they are now also advocated for the control and even interruption of transmission (17, 113, 156). Two IVM treatments per year for a period of at least 10 years are recommended to interrupt transmission of *O. volvulus* among humans (156). In countries such as Egypt, active antischistosomal community treatment with PZQ has been going on for more than a decade already and will be continued, even intensified, for the foreseeable future (46). AR probably will not develop as easily in helminths with an indirect life cycle (having the multiplicative part of their cycle in arthropods or molluscs) as in directly transmissible intestinal helminths. However, given sufficient time, intensive treatment strategies such as in Egypt may provide opportunities for resistant strains to appear and/or become dominant.

Targeting and timing of mass treatment. Prophylactic mass treatments of domestic animals have certainly contributed to the widespread development of AR in helminths. Although no data are available from experimental studies, computer models (5) indicate that the development of resistance is delayed when 20% of the flock is left untreated. This approach would ensure that the progeny of the worms surviving treatment will not consist only of resistant worms. Given the well-known overdispersed distribution of helminths, leaving part of the group untreated, especially the members carrying the lowest worm burdens, should not necessarily reduce the overall impact of the treatment.

In worm control in livestock, regular moving of the flocks to clean pastures after mass treatment and/or planning to administer treatment in the dry seasons is common practice to reduce rapid reinfection. However, these actions result in the next helminth generation that consists almost completely of worms that survived therapy and therefore might contribute to the development of AR (128, 134). For example, Coles et al. (29) reported problems with AR in the helminths of sheep and goats on some small Greek islands which suffered from extended drought; in contrast, no AR developed under similar management and deworming practices on the mainland.

In contrast to livestock, where nearly 100% of the animals of the herd or the flock are treated, population compliance is usually less than 80% in community-based mass treatment of humans: people are absent, not interested, ill, or pregnant. Often, compliance decreases further after the first few treatments, if only because of the reduction of morbidity. Moreover, populations are often not stable, and there may be an influx of neighboring or traveling communities (47, 48). Timing of treatment in dry, low-transmission periods has been proposed (155). In some areas of China, synchronized treatment of cattle and humans is applied in the hope of reducing transmission (121). However, such strategies are difficult to apply, if only because of organizational and logistical problems.

It may be hoped (but not guaranteed) that these typically human factors will delay (but not prevent) the occurrence and spread of AR in humans. However, if regular treatments are focused mainly on school-age children (intestinal worms) or in isolated communities (onchocerciasis), groups in which participation is well controlled and even reinforced and in which transmission may occur in a relatively closed ecological system, the situation and risks may be not that different from those in livestock.

Underdosing. Underdosing is generally considered an important factor in the development of drug resistance, because subtherapeutic doses might allow the survival of heterozygous resistant worms (128). Several laboratory experiments have shown that underdosing indeed contributes to the selection of resistant or tolerant strains (43, 78). Some indirect field evidence further supports this assumption. Recently, it was shown that the bioavailability of BZ and levamisole is much lower in goats than in sheep and that goats should be treated with dosages 1.5 to 2 times higher than those given to sheep (77). For many years, however, sheep and goats were given the same anthelmintic doses. The fact that AR is very frequent and widespread in goats may be a direct consequence. Recent modeling exercises suggest that the field situation of AR is not always as simple (129). Depending on the initial frequency of the resistance alleles, there might be a range of dose levels where underdosing promotes resistance and a range of dose levels where it actually impedes resistance.

Although further research on the impact of underdosing on resistance development is necessary, current knowledge advises against the use of subcurative dosages. To reduce the costs of anthelmintic treatment campaigns in developing countries, the use of lower dosages than the recommended therapeutic ones has been advocated (151). Such practices should clearly be avoided. As shown above, most of the currently applied anthelmintics are in fact subcurative in at least part of the population. This is considered acceptable for morbidity control, but in the long run such strategies may contribute to the development of AR as well.

Underdosing in humans occurs widely in many developing countries. Drugs are commonly shared or used at half (or less) the normal doses by poor families. Furthermore, generic products of substandard quality, repacked and/or reformulated products, and expired drugs are widespread in pharmacies and general markets. Also, the presence of poor-quality drugs has been documented in human as well as in veterinary medicine (104, 126, 141). Human drugs, especially antibiotics and anthelminthics, are produced by a large number of unlicensed companies all over the world. Quality control of these drugs is usually lacking.

Mechanisms of Drug Resistance

Benzimidazoles. The best known mechanism of resistance is the one to BZ. No information is available about the resistance mechanisms present in BZ-resistant human hookworms, but veterinary helminthologists have studied BZ resistance of *H. contortus* in detail. The BZ exert their anthelmintic activity by binding to β-tubulin, which interferes with the polymerisation of the microtubuli. Several authors (9, 120) showed that there is an extensive polymorphism of the β-tubulin gene in susceptible *H. contortus* populations. Roos et al. (120) proved that selection for resistance to BZ is accompanied by a loss of alleles at the locus of β-tubulin isotype 1. Kwa et al. (91) nicely demonstrated that resistance to BZ is correlated with a conserved mutation at amino acid 200 in β-tubulin isotype 1 (with Phe being replaced by Tyr).

The same mutation was shown to occur in BZ-resistant fungi such as *Aspergillus nidulans* and *Venturia inaequalis* (82, 85). The functional importance of this amino acid substitution was shown by heterologous expression of the β-tubulin isotype 1 (isolated from BZ-susceptible *H. contortus*) in BZ-resistant *Caenorhabditis elegans*. Expression of the *H. contortus* gene altered the phenotype of transgenic *C. elegans* from resistant to susceptible. Conversely, when Phe was replaced by Tyr at

amino acid position 200 of this gene by in vitro mutagenesis, the reverting activity was lost (92).

A second resistance mechanism was identified in some *H. contortus* populations showing higher levels of resistance and in which a deletion of the β-tubulin isotype 2 locus was shown (120). However, Beech et al. could not confirm this in other BZ-resistant *H. contortus* populations (9). These authors also showed that changes in allele frequencies rather than novel rearrangements induced by exposure to the drug explained changes associated with BZ resistance. A similar stepwise selection of BZ resistance also occurs in some *Trichostrongylus colubriformis* and *Ostertagia circumcincta* populations (45, 68). Furthermore, Kerboeuf et al. (84) recently provided indirect evidence that P-glycoproteins (P-gp) also play a role in BZ resistance in *H. contortus*. P-gp are involved in multidrug resistance in mammalian tumor cells, *Leishmania*, and *Plasmodium* and in resistance to toxic compounds in *C. elegans*. Rhodamine 123, a P-gp transport probe, associated with the reversal agent verapamil (an inhibitor of multidrug resistance-associated proteins), gave significantly higher levels of fluorescence in eggs from *H. contortus* resistant to BZ and IVM than in susceptible eggs. These results confirm those obtained with biological drug assays using both anthelmintics and verapamil and reinforce the probability of a P-gp-like dependent efflux in nematode eggs, which could be involved in resistance to xenobiotics. However, Kwa et al. (90), using a P-gp gene probe from *H. contortus*, were not able to correlate polymorphism to any of the (multi)drug resistances examined in different *H. contortus* populations. It should be noticed that the DNA used by Kwa et al. (90) was prepared from pooled L3 larvae and not from individual parasites, so that no estimates of allele frequencies could be made (2). Since at least 14 P-gp genes seem to be present in *C. elegans*, it is also possible that P-gp other than those characterized by Kwa et al. (90) or multidrug resistance-associated proteins might be involved in drug resistance. Blackhall (personal communication) recently found that the same gene, encoding a P-gp which is responsible for resistance to IVM and moxidectin, is also involved in BZ resistance.

Since specific BZ resistance seems to be due to similar point mutations in several fungi and nematodes of veterinary importance, it is not unlikely that it would be relevant for resistance in human nematodes as well. Since similar molecules are used in human and veterinary medicine, it would be worthwhile to look for the presence of these point mutations in human helminths as well.

Levamisole. Levamisole and the related anthelmintics pyrantel and morantel are cholinergic agonists with a selective action on nematode receptors. The mechanism of resistance to levamisole has not yet been elucidated. Sangster (122) thoroughly reviewed the pharmacology of levamisole resistance. It is thought to be caused either by a reduction of the number of nicotinic acetylcholinesterase receptors or by a decreased affinity of these receptors for the drug. Hoekstra et al. (79) were able to clone the gene *Hca 1*, encoding the nicotinic acetylcholinesterase receptor from *H. contortus*. Although polymorphism at the amino acid level could be demonstrated, these authors could not find evidence that alleles at this locus were involved in selection for resistance to levamisole. A similar gene, *tar-1*, was identified on the X chromosome in *T. colubriformis* (150). However, although statistical comparison of allele frequencies from individual male and female worms was consistent with sex linkage of *tar-1*, no correlation was found with levamisole resistance status.

Ivermectin. IVM and other macrocyclic lactones affect gastrointestinal nematodes by causing starvation and/or paralysis by opening chloride channels, which are thought to be associated with alfa-subunits of glutamate-gated ion channels located on muscles of the pharynx and possibly the somatic musculature (122). Rohrer et al. (117) compared IVM-resistant and -susceptible *H. contortus* populations and found that resistance is not due to an alteration in the binding of IVM to glutamate gated chloride channel receptors. Nevertheless, Blackhall et al. (13) did report that one allele of the putative alfa-subunit gene is associated with resistance to the drug. Recently, Blackhall et al. (12) reported considerable genetic variation of a P-gp locus in *H. contortus*. In several drug-selected strains of the parasite, selection for the same allele was observed. Using different approaches, Xu et al. (158) and Sangster et al. (124) came to the conclusion that P-gp might be involved in resistance to IVM in this helminth species. Other mechanisms of resistance may be present as well, as suggested by Gill et al. (64) and Gill and Lacey (65). The latter described five possible types of resistance to IVM in *H. contortus* based on different behavior in in vitro tests (larval development assay and L3 motility tests), different sensitivity to paraherquamide (an anthelmintic with a completely different structure and different binding sites from IVM), and different inheritance (in at least two of the five resistance types). Gill and Lacey (65) also suggested that the mechanism of resistance to IVM might be different from one species of helminth to another, because the critical events leading to expulsion have been shown to be different, e.g., when *O. ostertagi* is compared to *H. contortus* and *T. colubriformis*. Further research is needed to confirm these observations, to which the relevance to human *O. volvulus* is at present not clear.

Antischistosomal drugs (oxamniquine and praziquantel). The mechanism of action of oxamniquine is closely associated with its irreversible inhibition of nucleic acid synthesis in schistosomes (23). Based on cross-breeding experiments using susceptible and drug-selected schistosome strains exhibiting stable resistance, Cioli et al. (24) suggested that oxamniquine is not bioactivated in resistant worms, allowing them to survive the drug action. The activating enzyme, which is present in sensitive and absent in resistant schistosomes, seems to be a sulfotransferase. There is no clear understanding of the mode of action of PZQ, which also hampers the elucidation of possible mechanisms of resistance to PZQ. Redman et al. (111) have reviewed the existing knowledge and consider the PZQ-induced Ca^{2+} influx across the tegument as vital in the effect of this drug. However, the mechanisms leading to this alteration in Ca^{2+} homeostasis are not clear at all (22).

Genetics of Drug Resistance

Nematodes. Nematode parasite populations are genetically heterogeneous and thus able to respond to selective pressures, i.e., anthelmintic drugs (67). Widespread drug pressure will favor and select parasite lines carrying tolerance or resistance alleles. The rate at which resistance spreads in the parasite population depends on many factors. One key factor is the proportional contribution that helminths surviving therapy will make to the next generation. This contribution is influenced by the drug pressure (frequency and timing of treatment), the drug efficacy, the gene flow (the introduction of susceptible genotypes from elsewhere), the generation time and fecundity of the worms, the frequency of resistance alleles prior to drug use, the number of genes involved, and the dominance or recessiveness of these genes. Since it is quite difficult to set up experiments to examine the influence of these different factors, several mathematical models have been developed to simulate the development of AR in gastrointestinal helminths (5, 63, 128, 129). Although these models have their limitations and

must certainly be interpreted with caution (39), models such as the one of Barnes et al. (5) concerning *Trichostrongylus colubriformis* in grazing sheep provide interesting insights. The model allowed up to three genes for drug resistance, each with two alleles, that were combined independently under random mating. Worms of all genotypes were assumed to be equally fit in the absence of the anthelmintic. The initial frequency of resistance alleles in the worm population was assumed to be very low and was set at 0.01%. To examine the effect of using either mixtures of two drugs or rotations of a single one, two independent genes for resistance to two drugs (with different mechanisms of action) were simulated, with resistance being codominant and each drug killing 99, 50, and 10% of worms of homozygous susceptible (SS), heterozygous (RS), and homozygous resistant (RR) genotypes, respectively. The simulations were run for a period of 20 years, with treatment once a year for the ewes and three times a year for the lambs. These resulted in little development of resistance when the two drugs were used together (mixture). Substantial resistance, however, developed for all rotation strategies, 1-, 5-, and 10 yearly, with slowest development of AR in the annual rotation strategy. Assuming equal initial drug efficacy and equal resistance allele frequency, resistance developed more rapidly if it was determined by a single gene than when two or more genes were involved. Furthermore, resistance evolved fastest when it was dominant, slower when it was codominant, and slowest when it was recessive. When 20% of the flock was never treated, resistance was delayed at the expense of worm control.

It should be noted, however, that this and most other models are deterministic, ignoring the overdispersed distribution of free-living and parasitic helminth stages. Smith et al. (129) used a stochastic model to examine the effect of aggregated parasite distributions on parasite mating probabilities and the spread and maintenance of rare (resistant) genotypes. They concluded that spatial heterogeneity in transmission might be a significant force in promoting the spread of resistant genotypes, at least when infection levels are low.

When modelling exercises are compared with current knowledge of genetics of AR in helminths of livestock, the most striking and alarming observation is the high frequencies of resistance alleles observed in untreated populations of livestock helminths of veterinary importance. Beech et al. (9) analyzed individual genotypes of susceptible *H. contortus* before any exposure to BZ and reported initial frequencies of resistance alleles of 46 and 12% at the isotype 1 and isotype 2 β-tubulin loci, respectively. Anderson et al. (2) suggested that similar high frequencies associated with IVM resistance might occur in unselected lines of the same helminth species. The numbers of Beech et al. (8) may be overestimations, but they indicate that resistance alleles in untreated helminth populations of livestock—and maybe also humans—might be much more common than is usually assumed in the theoretical models.

Contradictory reports have been published regarding the number of genes involved in AR and their dominance or recessiveness. The available information, mainly on *H. contortus*, has been summarized by Anderson et al. (2). BZ resistance in this parasite seems to be polygenic; at least two, possibly three, genes with recessive alleles are involved. Levamisole resistance in *H. contortus* and *T. colubriformis* is probably due to one single major gene or gene cluster, the alleles of which are autosomal recessive for the former and sex-linked recessive for the latter (2). Resistance to IVM in *H. contortus* appears to be mediated by a single gene or gene complex with primarily dominant effects. IVM resistance might thus develop quite fast, as appears to be confirmed by field observations in South Africa, where IVM resistance in *H. contortus* developed after only three treatments (139). Avermectin and mylbemycin resistance is now widespread in *H. contortus* and *O. circumcincta* of small ruminants all over the world but remarkably not in *T. colubriformis* (123).

Obviously, these veterinary experiences and findings are of considerable relevance to humans. The presence of tolerant strains to anthelmintics in any parasite population has been demonstrated; as far as biological observations and statistical extrapolations allow, the proportion of innately resistant helminths is on the order of percentages (10^{-2}), not of 10^{-3} or less, as previously thought. Virtually all strategies proposed and implemented to date for human intestinal helminth control are based on a single-drug approach, without combination or rotation, and at a minimal frequency of once a year for a considerable length of time. Although the situation with livestock is different from that of humans and the results or simulations cannot be automatically extrapolated, the biological, epidemiological, and pharmaceutical similarities are of concern. Research should focus on genetic and related phenotypic similarities with relevance to AR in livestock and human helminths. Modeling and simulation studies, which have been applied to advance the cause for large-scale treatment programs in humans (17, 113) should be used to project possible side effects and AR in particular.

Trematodes. The genetics of resistance of schistosomes to oxamniquine are quite well known, but this is not the case for PZQ. In contrast to the development of classical drug resistance in helminths, which spreads gradually through a population as a consequence of selection of resistant phenotypes present at low frequency, resistance to hycanthone-oxamniquine appeared universally in the first filial progeny of parasites exposed to the drug (16). This strongly suggests that resistance is induced rather than selected from preexisting forms (16). The crossbreeding experiments of Cioli et al. (23, 25) and Pica-Mattocia et al. (107) have clearly shown that oxamniquine resistance is controlled by a single autosomal recessive gene. Resistance to oxamniquine does not appear to spread easily within communities but, rather, tends to remain limited to individual cases. According to Cioli et al. (25), this could be due to a selective disadvantage of resistant schistosomes in the absence of drug pressure. The fact that resistance is induced rather than selected might also contribute to this phenomenon.

Little is known about the genetic or biochemical background of possible resistance to PZQ. Recently, genetic differences have been demonstrated between a laboratory strain of *S. mansoni* selected for resistance to PZQ and the parent susceptible strain (105). Although these authors did not detect any major genomic rearrangements in these strains, they showed that mRNA encoding a fragment of the subunit 1 of cytochrome *c* oxidase was overexpressed about 5- to 10-fold in the resistant strain compared to the susceptible one. Further research is necessary to examine whether a similar phenomenon is also present in field strains suspected of resistance to PZQ and whether other genes are also differentially expressed in resistant strains of *S. mansoni*.

Detection of Drug Resistance

Fecal egg count reduction test. The most commonly used test to detect problems of anthelmintic resistance is the fecal egg count reduction test (FECRT), which compares the egg count before and after treatment with an anthelmintic drug. A standardized protocol for the FECRT is available for the detection of anthelmintic resistance in nematodes of veterinary

importance (27). In small ruminants, fecal samples are taken from two groups of at least 15, preferably young animals, which have been bred on the farm and not treated in the previous 8 to 12 weeks. Animals are randomly distributed into a treatment and a control group. Fecal samples are collected 10 to 14 days after treatment. To reduce the workload, no pretreatment samples may be taken; it has been shown that comparing treatment and control groups posttreatment is as reliable as comparing pre- and posttreatment samples. Egg counts are performed using a standardized McMaster method (27). The EPG in the feces of the control group should be higher than 150 to allow valid comparison. The following formula is used to calculate the percent reduction of the EPG: ERR = $100(1 - X_t/X_c)$, where X is the arithmetic mean EPG and c and t indicate the control and treated groups respectively. According to the guidelines of the WAAVP, drug resistance in helminths of small ruminants is considered to be present when ERR <95% and the lower 95% confidence interval is below 90%. If only one of both criteria is met, resistance is suspected (27).

This protocol could help guide the development of a standard approach for AR in humans, but modifications must be made because of significant differences between animals and humans. To start with, the objective is different: in livestock, the test is used as a routine local confirmation of known AR. In humans, the challenge is still to demonstrate that AR exists at all. Furthermore, study populations of humans are much more heterogeneous than are those of animals: there is a loss of compliance in follow-up, sample collection is not evident, and individual behavior (concerning exposure as well as health-seeking behavior) can have an important impact on the test parameters. Finally, the infecting worm species are different and require other coprological methods.

Taking into account the methodological problems experienced in the past in defining drug resistance in human helminths (see "Problems of defining drug resistance in hookworms" above), a standard protocol to detect AR in humans under field conditions could include a number of standard elements, such as study groups and parasitological methods.

(i) Study groups. Studies to confirm suspected drug resistance, particularly for a compound for which this has not yet been convincingly reported, should include at least a treatment group (with the compound under study) and a nontreated group (possibly placebo). Preferably, a "positive" control group, treated with another, nonrelated and presumably efficacious drug should also be included. The drugs should be of undisputable origin and quality, and adequate dosages should be used, i.e., those recommended for clinical use, not the subcurative doses applied for community-based morbidity control, with individual dosages adapted to actual body weight. The tablets must be swallowed under direct observation; particularly in young children appropriate syrup or suspension formats should be used. People who vomit or have severe diarrhea shortly after treatment should be excluded from the cohort. Apart from toxicity reasons, pregnant women and people with systemic illnesses should also be excluded, since pharmacological and immunoparasitological dynamics may be disturbed. Pharmacodynamical studies are not essential from the start but should be conducted before conclusions about drug resistance are made.

Sample sizes should be determined using a statistical power analysis based on a quantified hypothesis; i.e., for each tested anthelmintic, a normal and an abnormal CR and ERR should be defined beforehand. As is made clear by the above discussion, there are currently no generally accepted normal rates. An international concerted action to determine reference data would be useful.

The study group composition must be statistically similar for age, sex ratio, and pretreatment mean egg count, and this includes averages as well as distribution. Children and adults should be considered different populations. Other possible confounding factors which may lead to differential exposure patterns, such as socioeconomic class, occupation, school attendance, and religion, must be avoided as well. The groups should ideally be selected from one more or less homogeneous population (e.g., one village) and should be studied simultaneously to avoid spatial and temporal variations of transmission. None of the study subjects should have received treatment with the drug or a related compound in the previous 3 (nematodes) to 12 (schistosomes) months, since such subjects may be in the process of "rebuilding" their parasite load.

Given these requirements and the unavoidable dropout rate of study subjects, initial sample sizes should probably be not less than 50 children or adults in each study group, if only to validly test the distribution pattern of the egg counts. The pretreatment egg counts should be sufficiently high to allow meaningful statistical interpretation, taking into account the detection level of the coprological method.

All ethical conditions must be met: fully informed consent of subjects and/or their parents; treatment of negative controls immediately after follow-up or earlier if clinically necessary; monitoring and management of side effects; and permission of local and national health authorities.

(ii) Parasitological methods. A standardized egg-counting technique should be used to determine individual egg counts. For schistosomes, *Ascaris* and *Trichuris*, the Kato technique can be used in a standard way, as described by Katz et al. (83), Peters et al. (106), or Polderman et al. (109). Slides should preferably be stored for later reference and quality control.

For hookworms, utmost care must be taken to validate and standardize the Kato technique. Martin and Beaver (96) recommended reading the slides after 30 min and not later than after 60 min. This was based on only a few clinical samples, however. In the field, stool consistency and transparency can vary widely between individuals and communities. In any case, Kato slides based on stool samples of more than 25 mg, such as the standard Kato-Katz, can hardly be read after only 1 h (106) and are thus not suitable for standardized quantitative hookworm research. Reading all slides within a narrow window of time after preparation requires a rigidly organized and supervised field setup. Ideally, an adapted Kato technique in which hookworm eggs are preserved or alternative methods comparable to the veterinary FECRT should be developed. There is a great need for the development, optimization, and validation of a standard protocol, without which further field studies on AR in hookworms will remain severely handicapped.

Fecal helminth egg counts show strong day-to-day, interindividual, and intraindividual variations, both for nematodes and for schistosomes (50, 69, 75). To obtain more accurate schistosome egg counts at the individual level, a minimum of three stool samples must be examined (49, 50).

If the focus is on CR (e.g., to establish fully curative doses), the most sensitive coprological (qualitative) methods should be used in conjunction with the quantitative ones, such as glycerine sedimentation for schistosomes and cultures for hookworms (8). The qualitative methods are essential in at least a subsample to determine the exact species involved.

The statistical interpretation of mean egg counts is complicated. Scientific accuracy demands the use of models which relate the egg count to worm burdens, the underlying outcome parameter of treatment. Direct use of EPG assumes a proportional relationship, which is far from the biological and statistical truth. Practical statistical tools to that end are not readily

available and have so far only been developed for schistosomes (38). For simplicity, mean EPGs can be used for a first crude analysis and may be sufficient to reject the hypothesis of resistance. As shown by the Senegalese experiences with PZQ, however, more sophisticated analysis is essential before definite and far-reaching conclusions can be drawn. In veterinary science, arithmetic mean egg counts are preferred over geometric mean counts because they are more sensitive and allow an earlier detection of resistance (102). This may be justified in situations where AR is known to exist and needs only to be confirmed in a particular situation. Statistically, however, arithmetic means are by no means valid, due to the strongly aggregated helminth egg counts, which usually follow a negative binomial distribution (3). Geometric means are more appropriate although not yet ideal, since the distribution patterns change after intervention.

The interval between treatment and sampling should be adapted to each parasite species and to the drugs used. For example, for the evaluation of the efficacy of BZ in treating hookworms, a period of about 2 weeks is appropriate. A longer period would allow immature or even new infections to become patent, while a shorter one may overestimate efficacy, since some drugs temporarily suppress egg production without killing the worms.

For schistosomes, the problem of distinguishing active from immature or even past infections is somewhat more complicated. Since the worms live in the blood vessels, eggs follow a long and difficult path from this intravascular location to the outside world and may be excreted up to 6 to 8 weeks or even longer after their production. The Kato method does not distinguish dead from live eggs. On the other hand, immature infections, which are not affected by PZQ, can become patent days after successful cure of adult worms. Newly contracted infections may result in egg excretion within 4 to 6 weeks. The ideal solution for this dilemma would be to consider only patients outside the area of endemic infection and to evaluate cure after 8 to 12 weeks or even longer. In practice, this can be done only for tourists, who usually have uncharacteristically light infections. A pragmatic and generally accepted compromise is to evaluate cure in an area of endemic infection after 5 to 6 weeks of treatment (71, 72, 114). However, the results will always have to be interpreted in the light of possible reinfection (including maturation of prepatent infections) in high-transmission areas. If possible, treatment trials should take place in a non- or low-transmission season.

The quantification of circulating antigens, particularly in serum, can be a useful complementary tool (34). Cure can be assessed within a few days to a week after treatment, and so is much less sensitive to rapid reinfection. However, antigen detection cannot fully replace egg counts, since 5 to 30% of the infections are still missed (34); the assay is not commercially available and requires much more laboratory infrastructure than does the egg count method.

It may be clear from the above that valid data to confirm AR in the field requires considerable expertise in parasitology and epidemiology, well-trained field teams, careful organization, and strict quality control and that it is vital for further studies to improve and establish appropriate methods and standard protocols (157).

Laboratory tests for detection of resistance in livestock helminths. A variety of different laboratory tests have been described for the detection of AR in livestock helminths (31). Those which are most commonly used and which might be applied to detect AR in human helminths are briefly described here.

(i) Egg hatch test. The egg hatch test is an in vitro test, which is used only for the detection of BZ resistance in livestock helminths; it is based on the ovicidal activity of this group of molecules. The original test was described by Le Jambre (94); a standardized protocol was adopted by the WAAVP (27). Freshly collected fecal samples (within 3 h of being shed) are needed to obtain reliable data. If this is not possible, samples must be stored anaerobically; this storage does not influence the outcome of the test, at least for the major gastrointestinal helminths of small ruminants (80). Helminth eggs are purified and incubated with a series of concentrations of thiabendazole (TBZ). This compound was selected because it dissolves readily in dimethyl sulfoxide and because side resistance is usually present with other members of the BZ group. After 24 h, the number of hatched larvae is counted. When resistance develops, the ovicidal activity decreases, which results in a higher percentage of eggs that hatch. Based on vast experience with the test, WAAVP considers resistance to be present when the 50% effective dose is $\geq$0.1 μg/ml (27). This in vitro test has the advantage of requiring only one fecal sample. However, several authors have reported poor correlations between the results of the FECRT and the egg hatch test for helminths of livestock (14, 42).

Unfortunately, the FECRT and the egg hatch test detect resistance only when at least 25% of the worm population carries resistance genes, as shown by artificial infection of animals with mixtures of helminth populations with a known level of AR (97). Since reversion to susceptibility is considered to be possible only as long as resistance genes are present in less than 5% of the helminth population (119), FECRT and egg hatch assays allow the detection of AR only when it is too late to interfere. Field and experimental data for helminths of livestock indeed indicate that reversion to susceptibility to anthelmintic drugs in livestock helminths rarely occurs once resistance has been confirmed (31).

(ii) Larval development assay. The larval development assay is more laborious and time-consuming than the egg hatch test but allows the detection of resistance to the major broad-spectrum anthelmintic classes, including the avermectins-mylbemycins. It was originally described by Coles et al. (30) and further improved by several others (66, 93) and is now commercially available (DrenchRite; Horizon Technology). In the larval development assay, nematode eggs or L1 larvae are exposed to different concentrations of anthelmintics incorporated into agar wells in a microtiter plate. The effect of the drugs on the subsequent development into L3 larvae is measured. The results correlate well with those of in vivo tests. It is claimed that this test is more sensitive than FECRT and egg hatch test and detects AR when about 10% of the worm population carries resistance genes (40), but this remains to be proven.

(iii) Larval motility or paralysis test. Several in vitro assays to detect resistance to BZ, macrocyclic lactones or levamisole-morantel have been described which are based on the motility of larvae (31). For the latter group of anthelmintics, a clear-cut distinction between susceptible and resistant strains is not always possible (60, 142). A similar motility test has been used to evaluate the sensitivity of *O. volvulus* microfilariae to ivermectin (135). To render the interpretation more objective, a micromotility meter has been developed (11). Folz et al. (55, 56) used this apparatus to detect drug resistance in *H. contortus* and *T. colubriformis*, but other authors have found it less reliable (142; S. Geerts, unpublished results).

(iv) PCR. The first specific primers to detect drug-resistant parasitic nematodes were developed by Kwa et al. (91). These primers discriminated between heterozygous and homozygous

BZ-resistant *H. contortus* for the alleles in question (β-tubulin isotype 1), even when these genotypes are phenotypically indistinguishable, and could also identify BZ-resistant *T. colubriformis*. According to Roos et al. (120), PCR detected 1% of resistant individuals within a susceptible worm population, a tremendous improvement over other in vivo and in vitro tests.

Recently, Elard et al. (44) developed a more simplified method for the diagnosis of BZ-resistant *O.* (*Telodorsagia*) *circumcincta*. Using four primers (two allele-specific and two nonallele-specific ones) in the same PCR, adult worms were characterized for the mutation of residue 200 of isotype 1 β-tubulin. The technique has now been refined for use on a single worm, egg, or larva (M. H. Roos, personal communication). Since the frequencies of alleles associated with anthelmintic drug resistance might be quite high even in susceptible populations, it is indeed important to examine DNA from individual parasites. If DNA is prepared from pooled parasites, the association between particular alleles is likely to be obscured (2).

Since the same mutation is responsible for BZ resistance in many parasitic nematodes, this method may provide a means of investigating the frequencies of alleles bearing it in a wide range of animal and human intestinal nematodes.

Another interesting development is the availability of a P-gp gene probe for *Onchocerca volvulus* (90). Since it has been shown that P-gp plays a role in resistance to BZ and IVM in *H. contortus* (12, 84, 158), it can be expected that the same resistance mechanism might develop in many other helminths, including *O. volvulus*.

Laboratory tests for detection of resistance in human helminths. Apart from the use of the egg hatch test for hookworms in the Mali study (33), in vitro tests for AR in human nematodes have so far not been developed, adapted, or validated. A major problem is obviously the lack of reference resistant strains. If these were available, the egg hatch test and the larval development assay, as well as the promising new PCRs, could probably easily be validated for human hookworms.

Laboratory tests for schistosomicide resistance, in particular to PZQ, consist mainly of measuring worm count reduction after treatment in experimentally infected mice. First, it must be stressed that white mice are highly unnatural hosts for schistosomes; these large blood-dwelling worms are giant foreign bodies in the tiny murine blood vessels. Proportionally, a single schistosome in a mouse (blood volume, 5 ml) corresponds to 10,000 worms in an adult (blood volume, 5 liters). Few mice survive high worm counts long enough to allow therapeutic trials, and so the statistical power is inherently limited. Mouse-based experiments are laborious and subject to considerable methodological pitfalls, including those involving different strain maturation times (9, 22, 52). Laboratory strains are usually maintained using eggs derived from livers of mice that have been infected for 5 to 6 weeks, resulting in the selection of parasites which mature much more rapidly, and become susceptible to PZQ much earlier, than natural strains. As mentioned above, such bias probably explains the first reports on induced and "natural" PZQ resistance in the laboratory (51, 53). Also, it is not easy to isolate homogeneous parasites, resistant or not. Usually, mice are infected with a mixture of cercariae from at least five snails to obtain bisexual, productive infections. These snails have in turn usually been exposed to three to five miracidia, often resulting in mixed infections. These miracidia, even if isolated from stools of one person not responding well to treatment, stem from an unknown number and variety of adult worm couples, of which only one or a few may be (partly) tolerant to the drug. Confirming and assessing drug resistance in such a model is thus a most tedious and tricky task. The standard protocol proposed by Fallon et al. (54), based on procedures and recommendations by Cioli in a series of European Community-supported consensus meetings in Leiden, The Netherlands, is a valuable basis for better standardization, but this mouse model remains difficult to handle and interpret.

There is thus a great need for in vitro tests. Adult schistosomes can be cultured in artificial media, providing an excellent opportunity for straightforward in vitro exposure tests for individual worms. Such tests are much more accurate, reproducible, and feasible than mouse experiments, and they allow the screening of a great number of individual worms and well defined isolates. It has allowed the in-depth research of resistance to hycanthone and oxamniquine (16, 23). However, for PZQ, the test cannot be established as long as there is no convincing resistant reference strain (D. Cioli, personal communication).

Therefore, the main priority in research on AR in human schistosomes and nematodes is to conduct field studies in communities where clinical and/or epidemiological suspicion warrants the investments needed, to isolate as many individual parasites as possible from noncured patients, and to confirm the results in animal models. Once such strains are established and consolidated, in vitro tests can be validated. These will then in turn allow much wider and faster testing of field isolates and in-depth research of the biology and genetics of AR in human helminths.

CONCLUSIONS AND RECOMMENDATIONS

There is as yet no unequivocal evidence that resistance to commonly used anthelmintics in humans is an emerging problem, either through new mutations or by the selection of innately tolerant strains. However, experiences with other infectious agents, particularly those with the quick and dramatic spread of AR in livestock, should warn the medical world against the widespread use of anthelmintics for the control of helminths.

The projected conditions in drug-based human helminth control may be different from those in livestock: the transmission dynamics are more complex (particularly for schistosomes and filariae); treatments may be less frequent, and coverage may be lower; different strategies can be proposed to reduce the appearance or selection of resistant helminth strains. However, these are all hypothetical and optimistic assumptions, which may delay but probably will not avoid the appearance of AR. The biological, epidemiological, and pharmaceutical similarities between human and livestock helminths are so great that optimism may amount to complacent neglect. In livestock, the problem is mainly economic, which is bad enough. In humans, widespread AR would be a serious public health problem. At present, our only certainty is the striking lack of adequate tools to detect AR in human helminths and the inability to remedy the problem once it is detected. The perspective is indeed extremely worrying. For major helminths affecting humans, there are a few drugs available which are both safe and efficacious; since the commercial benefits are low, there is little or no investment in research on new molecules.

If drug-based strategies are implemented, the following guidelines may delay the development of resistance. (i) The intervention should be targeted and justified. Indiscriminate mass treatment (without any previous screening of the population) should be applied only in areas and groups where the impact of helminths and the benefits expected outweigh the

costs and burden on the health system and where it can be integrated in a sustainable package of health care. Such a cost-benefit calculation must be made by local and national health authorities, taking into account a whole range of qualitative and quantitative parameters, for which no clear-cut model is available.

(ii) Other control measures should be incorporated. Although health education programs, construction of latrines, improved water supply, etc., are much more difficult to implement are than treatment programmes, they have a much wider impact on public health, improve the sustainability of the helminth control, and allow the number of treatments to be reduced in the long run. Mass treatment is easy and popular but can reduce the commitment to more fundamental advances in the improvement of the living conditions of the local population.

(iii) The number of treatments should be reduced. The most efficient way to delay the development of drug resistance remains the reduction of the selection pressure by the drugs, in particular the number of treatments, preferably to one per year at most. It is obvious that a reduced treatment frequency should be combined with other control measures (see above) to maximize its effect. Two or three treatments a year, as advised by Albonico et al. (1), were already sufficient to induce the development of AR in some livestock helminths.

(iv) Exposure of the whole parasite population to the drug should be avoided. As suggested by simulation models, limiting the exposure of the whole helminth population should delay the development of AR. Targeted treatment, e.g., aimed at schoolchildren, is preferable to indiscriminate mass treatment, although even in such programs over 50% of the parasite population may be exposed to anthelmintics (2). Timing of treatment to occur during low-transmission seasons may seem efficient in terms of reinfection but may contribute to the development of AR.

(v) The correct dosage should be used. The use of lower dosages of anthelmintics for morbidity control programmes has been advocated to reduce costs but should be avoided to prevent or delay AR. In fact, the costs of drugs make up only a minor part of treatment programs (87). Some of the currently recommended drug dosages, including PZQ at 40 mg/kg, IVM at 150 μg/kg, mebendazole at 500 mg, and albendazole at 200 mg and even 400 mg, are actually subcurative. Although the administration of higher doses might increase costs, the useful life of the drugs may be extended, a worthwhile investment.

Incorrect dosages due to substandard or counterfeit anthelmintics must and can be avoided by imposing adequate quality standards on wholesale suppliers for national health care systems and special control programs. Obviously, there is also an urgent need for drug quality control systems in the private and public curative sector.

(vi) Simultaneous or rotational use of different drugs should be implemented. The simultaneous use of two or more drugs with different mechanisms of action is able to postpone the development of resistance to each of the drugs used (15, 76, 133). The cost increase is a serious obstacle, however. A less effective alternative is the rotation of drugs belonging to different classes. In any case, strategies which depend exclusively on administration of one single drug during many consecutive years, as in current onchocerciasis and schistosomiasis control programs, seem bound to result in resistance problems.

(vii) The development of drug resistance should be monitored. Monitoring the development of AR should be an obligatory part of large-scale worm control programs. As made clear in this review, standardized reliable tests to detect AR are not yet available.

The most appropriate strategy would therefore seem not to embark on control strategies based on the widespread and frequent use of anthelmintics and to restrict their use to curative medicine and possibly targeted interventions in very-high-risk groups or areas, which can be identified through rapid appraisal methods or through the regular health information system. To that end (and many others), reinforcement of the general primary health care systems should be the first priority in the control of human helminths. Meanwhile, the most important scientific challenge is to develop the appropriate tools, methods, and protocols to reliably and quickly detect the appearance of drug resistance in human helminths.

REFERENCES

1. **Albonico, M., D. W. T. Crompton, and L. Savioli.** 1999. Control strategies for human intestinal nematode infections. Adv. Parasitol. **42:**277–341.
2. **Anderson, T. J. C., M. S. Blouin, and R. N. Beech.** 1998. Population biology of parasitic nematodes: applications of genetic markers. Adv. Parasitol. **41:**220–281.
3. **Anderson, R. M., and R. M. May.** 1991. Infectious diseases of humans: dynamics and control. Oxford University Press, Oxford, United Kingdom.
4. **Andrew, P., H. Thomas, R. Pohlke, and J. Seubert.** 1983. Praziquantel. Med. Res. Rev. **3:**147–200.
5. **Barnes, E. H., R. J. Dobson, and I. A. Barger.** 1995. Worm control and anthelmintic resistance: adventures with a model. Parasitol. Today **11:**56–63.
6. **Barton, N. J.** 1983. Development of anthelmintic resistance in nematodes from sheep in Australia subjected to different treatment frequencies. Int. J. Parasitol. **13:**125–132.
7. **Bauer, V. C., and K. Failung.** 1992. Einsatz von Anthelminthika zur Nematodenbekämpfung bei Schafen in Westdeutschland: Ergebnisse einer Umfrage. Deutsch. Tieräertzl. Wochenschr. **99:**353–392.
8. **Beaver, P. C., R. C. Jung, and E. W. Cupp.** 1984. Clinical parasitology, 9th ed. Lea & Febiger, Philadelphia, Pa.
9. **Beech, R. N., R. K. Prichard, and M. E. Scott.** 1994. Genetic Variability of the β-tubulin genes in benzimidazole-susceptible and resistant strains of *Haemonchus contortus*. Genetics **138:**103–110.
10. **Bennett, J. L., T. Day, L. Feng Tao, M. Ismail, and A. Farghaly.** 1997. The development of resistance to anthelmintics: a perspective with an emphasis on the antischistosomicidal drug Praziquantel. Exp. Parasitol. **87:**260–267.
11. **Bennett, J. L., and R. A. Pax.** 1986. Micromotility meter: an instrument designed to evaluate the action of drugs on motility of larval and adult nematodes. Parasitology **93:**341–346.
12. **Blackhall, W. J., H. Y. Liu, M. Xu, R. K. Prichard, and R. N. Beech.** 1998. Selection at a P-glycoprotein gene in ivermectin- and moxidectin-selected strains of *Haemonchus contortus*. Mol. Biochem. Parasitol. **95:**193–201.
13. **Blackhall, W. J., J. F. Pouliot, R. K. Prichard, and R. N. Beech.** 1998. *Haemonchus contortus*: selection at a glutamate-gated chloride channel gene in ivermectin- and moxidectin-selected strains. Exp. Parasitol. **90:**42–48.
14. **Boersema, J. H., F. H. M. Borgsteede, M. Eysker, W. M. L. Hendrikx, and J. Jansen.** 1987. Prevalence of benzimidazole resistance of nematodes in sheep in The Netherlands. Res. Vet. Sci. **43:**18–21.
15. **Bonhoeffer, S., M. Lipsitch, and B. R. Levin.** 1997. Evaluating treatment protocols to prevent antibiotic resistance. Proc. Natl. Acad. Sci. USA **94:**12106–12111.
16. **Brindley, P. J.** 1994. Drug resistance to schistosomicides and other anthelmintics of medical significance. Acta Trop. **56:**213–231.
17. **Bundy, D. A., and N. R. De Silva.** 1998. Can we deworm this wormy world? Br. Med. Bull. **54:**421–432.
18. **Bundy, D. A., A. Hall, G. F. Medley, and L. Savioli.** 1992. Evaluating measures to control intestinal parasitic infections. World Health Stat. Q. **45:**168–179.
19. **Burger, H. J., and C. Bauer.** 1994. Antihelminthic resistant nematodes in farm animals in Germany, p. 63–68. *In* G. C. Coles, F. H. M. Borgsteede, and S. Geerts (ed.), Anthelmintic resistance in nematodes of farm animals. European Commission, Brussels, Belgium.
20. **Cerami, A., and K. S. Warren.** 1994. Drugs. Parasitol. Today **10:**404–406.
21. **Reference deleted.**
22. **Cioli, D.** 1998. Chemotherapy of schistosomiasis: an update. Parasitol. Today **14:**418–422.
23. **Cioli, D., L. Pica-Mattoccia, and S. Archer.** 1993. Drug resistance in schistosomes. Parasitol. Today **9:**162–166.
24. **Cioli, D., L. Pica-Mattoccia, and S. Archer.** 1995. Antischistosomal drugs: past, present. . .and future? Pharm. Ther. **68:**35–85.
25. **Cioli, D., L. Pica-Mattoccia, and R. Moroni.** 1992. *Schistosoma mansoni*: hycanthoone/oxamniquine resistance is controlled by a single autosomal recessive gene. Exp. Parasitol. **75:**425–432.
26. **Coles, G. C.** 1995. Chemotherapy of human nematodes: learning from the

problems in sheep. J. R. Soc. Med. **88:**649P–651P.
27. **Coles, G. C., C. Bauer, F. H. M. Borgsteede, S. Geerts, T. R. Klei, M. A. Taylor, and P. J. Waller.** 1992. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. **44:**35–44.
28. **Coles, G. C., J. I. Bruce, J. K. Kinoti, W. T. Mutahi, E. P. Dias, and N. Katz.** 1986. Drug resistance in schistosomiasis. Trans. R. Soc. Trop. Med. Hyg. **80:**347.
29. **Coles, G. C., E. Papadopoulos, and C. A. Himonas.** 1995. Tubulin, resistance and worms. Parasitol. Today **11:**183–185.
30. **Coles, C. G., J. P. Tritschler II, D. J. Giordano, N. J. Laste, and A. L. Schmidt.** 1988. Larval Development test for detection of anthelmintic resistant nematodes. Res. Vet. Sci. **45:**50–53.
31. **Conder, G. A., and W. C. Campbell.** 1995. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. Adv. Parasitol. **35:**1–84.
32. **Davis, A.** 1993. Antischistosomal drugs and clinical practice, p. 367–404. *In* P. Jordan, G. Webbe, and R. F. Sturrock (ed.), Human schistosomiasis. CAB International, Wallingford, United Kingdom.
33. **De Clercq, D., M. Sacko, J. Behnke, F. Gilbert, P. Dorny, and J. Vercruysse.** 1997. Failure of mebendazole in treatment of human hookworm infections in the southern region of Mali. Am. J. Trop. Med. Hyg. **57:**25–30.
34. **Deelder, A. M., Z. L. Qian, P. G. Kremsner, L. Acosta, A. L. T. Rabello, P. Enyong, P. P. Simarro, E. C. M. van Etten, F. W. Krijger, J. P. Rotmans, Y. E. Fillié, N. de Jonge, A. M. Agnew, and L. van Lieshout.** 1994. Quantitative diagnosis of *Schistosoma* infections by measurement of circulating antigens in serum and urine. Trop. Geogr. Med. **46:**233–238.
35. **De Silva, N., H. Guyatt, and D. Bundy.** 1997. Anthelminthics. A comparative review of their clinical pharmacology. Drugs **53:**769–788.
36. **Reference deleted.**
37. **de Vlas, S. J., and B. Gryseels.** 1992. Underestimation of *Schistosoma mansoni* prevalences. Parasitol. Today **8:**274–277.
38. **de Vlas, S. J., B. Gryseels, G. J. Van Oortmarssen, A. M. Polderman, and J. D. F. Habbema.** 1992. A model for variations in single and repeated egg counts in *Schistosoma mansoni* infections. Parasitology **104:**451–460.
39. **Dobson, R. J.** 1999. Modelling and forecasting. Int. J. Parasitol. **29:**93–94.
40. **Dobson, R. J., L. Lejambre, and J. H. Gill.** 1996. Management of anthelmintic resistance: inheritance of resistance and selection with persistent drugs. Int. J. Parasitol. **26:**993–1000.
41. **Doenhoff, M. J.** 1998. Is schistosomicidal chemotherapy sub-curative? Implications for drug resistance. Parasitol. Today **14:**434–435.
42. **Dorny, P., E. Claerebout, J. Vercruysse, R. Sani, and A. Jalila.** 1994. Anthelmintic resistance in goats in peninsular Malaysia. Vet. Parasitol. **55:**327–342.
43. **Egerton, J. R., D. Suhayda, and C. H. Eary.** 1988. Laboratory selection of *Haemonchus contortus* for resistance to ivermectin. J. Parasitol. **74:**614–617.
44. **Elard, L., J. Cabaret, and J. F. Humbert.** 1999. PCR diagnosis of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, *Teladorsagia circumcincta*. Vet. Parasitol. **80:**231–238.
45. **Elard, L., A. M. Comes, and J. F. Humbert.** 1996. Sequences of β-tubulin cDNA from benzimidazole-susceptible and -resistant strains of *Teladorsagia circumcincta*, a nematode parasite of small ruminants. Mol. Biochem. Parasitol. **79:**249–253.
46. **El Khoby, Y., N. Galal, and A. Fenwick.** 1998. The USAID/Government of Egypt's Schistosomiasis Research project (SRP). Parasitol. Today **14:**92–96.
47. **Engels, D., P. Ndoricimpa, and B. Gryseels.** 1993. Schistosomiasis mansoni in Burundi: progress in control since 1985. Bull. W. H. O. **71:**207–214.
48. **Engels, D., J. Ndoricimpa, S. Nahimana, and B. Gryseels.** 1994. Control of *Schistosoma mansoni* and intestinal helminths: 8-year follow-up of an urban school programme in Bujumbura, Burundi. Acta Trop. **58:**127–140.
49. **Engels, D., E. Sinzinkayo, S. J. de Vlas, and B. Gryseels.** 1997. Intraspecimen fecal egg count variation in *Schistosoma mansoni* infection. Am. J. Trop. Med. Hyg. **57:**571–577.
50. **Engels, D., E. Sinzinkayo, and B. Gryseels.** 1996. Day-to-day egg count fluctuation in *Schistosomiasis mansoni* and its operational implications. Am. J. Trop. Med. Hyg. **54:**319–324.
51. **Fallon, P. G., and M. J. Doenhoff.** 1994. Drug-resistant schistosomiasis: resistance to praziquantel and oxamniquine induced in *Schistosoma mansoni* in mice is drug specific. Am. J. Trop. Med. Hyg. **51:**83–88.
52. **Fallon, P. G., J. S. Mubarak, R. E. Fookes, M. Niang, A. E. Butterworth, R. F. Sturrock, and M. J. Doenhoff.** 1997. *Schistosoma mansoni*: maturation rate and drug susceptibility of different geographical isolates. Exp. Parasitol. **86:**29–36.
53. **Fallon, P. G., R. F. Sturrock, A. Capron, M. Niang, and M. J. Doenhoff.** 1995. Diminished susceptibility to praziquantel in a Senegal isolate of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. **53:**61–62.
54. **Fallon, P. G., L.-F. Tao, M. M. Ismail, and J. L. Bennett.** 1996. Schistosome resistance to praziquantel: fact or artifact. Parasitol. Today **12:**316–320.
55. **Folz, S. D., R. A. Pax, E. M. Thomas, J. L. Bennett, B. L. Lee, and G. A. Conder.** 1987. Development and validation of an in vitro *Trichostrongylus colubriformis* motility assay. Int. J. Parasitol. **17:**1441–1444.
56. **Folz, S. D., R. A. Pax, E. M. Thomas, J. L. Bennett, B. L. Lee, and G. A. Conder.** 1987. Motility response of benzimidazole-resistant *Haemonchus contortus* larvae to several anthelmintics. Proc. Helminthol. Soc. Wash. **54:**249–253.
57. **Reference deleted.**
58. **Geerts, S.** 1994. Anthelmintic resistance in nematodes of cattle, p. 25–30. *In* G. C. Coles F. H. M. Borgsteede, and S. Geerts (ed.), Anthelmintic resistance in nematodes of farm animals—1994. European Commission, Brussels, Belgium.
59. **Geerts, S., G. Bertels, B. Balis, J. Brandt, and V. Kumar.** 1990. Benzimidazole resistance in nematodes on a dairy goat farm in Belgium. Vlaams Diergeneeskd. Tijdschr. **59:**90–92.
60. **Geerts, S., J. Brandt, F. H. M. Borgsteede, and H. Van Loon.** 1989. Reliability and reproducibility of the larval paralysis test as an in vitro method for the detection of anthelmintic resistance of nematodes against levamisole and morantel tartrate. Vet. Parasitol. **30:**223–232.
61. **Geerts, S., J. Brandt, V. Kumar, and L. Biesemans.** 1987. Suspected resistance of *Ostertagia ostertagi* in cattle to levamisole. Vet. Parasitol. **23:**77–82.
62. **Geerts, S., G. C. Coles, and B. Gryseels.** 1997. Anthelmintic resistance in human helminths: learning from the problems with worm control in livestock. Parasitol. Today **13:**149–151.
63. **Gettinby, G., S. Hazelwood, and J. Armour.** 1990. Computer models applied to drug resistance in parasites, p. 213–219. *In* J. C. Boray, P. J. Martin, and R. T. Roush (ed.), Resistance of parasites to antiparasitic drugs. MSD Agvet, Rahway, N.J.
64. **Gill, J. H., C. A. Kerr, W. L. Shoop, and E. Lacey.** 1998. Evidence of multiple mechanisms of avermectin resistance in *Haemonchus contortus*—comparison of selection protocols. Int. J. Parasitol. **28:**783–789.
65. **Gill, J. H., and E. Lacey.** 1998. Avermectin/milbemycin resistance in trichostrongyloid nematodes. Int. J. Parasitol. **28:**863–877.
66. **Gill, J. H., J. M. Redwin, J. A. van Wyk, and E. Lacey.** 1995. Avermectin inhibition of larval development in *Haemonchus contortus*—effects of ivermectin resistance. Int. J. Parasitol. **25:**463–470.
67. **Grant, W. N.** 1994. Genetic variation in parasitic nematodes and its implications. Int. J. Parasitol. **24:**821–830.
68. **Grant, W. N., and L. J. Mascord.** 1996. Beta-tubulin gene polymorphism and benzimidazole resistance in *Trichostrongylus colubriformis*. Int. J. Parasitol. **26:**71–77.
69. **Gryseels, B.** 1996. Uncertainties in the epidemiology and control of schistosomiasis. Am. J. Trop. Med. Hyg. **55:**103–108.
70. **Gryseels, B., and S. J. de Vlas.** 1996. Worm burdens in schistosome infections. Parasitol. Today **12:**115–119.
71. **Gryseels, B., L. Nkulikyinka, and H. Coosemans.** 1987. Field trials of praziquantel and amniquine for the treatment of schistosomiasis mansoni in Burundi. Trans. R. Soc. Trop. Med. Hyg. **81:**641–644.
72. **Gryseels, B., F. F. Stelma, I. Talla, G. J. van Dam, K. Polman, S. Sow, M. Diaw, R. F. Sturrock, E. Doehring-Schwerdtfeger, R. Kardorff, C. Decam, M. Niang, and A. M. Deelder.** 1994. Epidemiology, immunology and chemotherapy of *Schistosoma mansoni* infections in a recently exposed community in Senegal. Trop. Geogr. Med. **46:**209–219.
73. **Guisse, F., K. Polman, F. F. Stelma, A. Mbaye, I. Talla, M. Niang, A. M. Deelder, and B. Gryseels.** 1997. Therapeutic evaluation of two different dose regimens of praziquantel in a recent *Schistosoma mansoni* focus in Northern Senegal. Am. J. Trop. Med. Hyg. **56:**511–514.
74. **Hall, A.** 1972. Quantitative variability of nematode eggs in faeces: a study among rural Kenyans. Trans. R. Soc. Trop. Med. Hyg. **75:**682–687.
75. **Hall, A., K. S. Anwar, and A. M. Tomkins.** 1992. Intensity of reinfection with *Ascaris lumbricoides* and its implications for parasite control. Lancet **339:**1253–57.
76. **Hayes, J. D., and C. R. Wolf.** 1990. Molecular mechanisms of drug resistance. Biochem. J. **272:**281–295.
77. **Hennessy, D. R.** 1994. The disposition of antiparasitic drugs in relation to the development of resistance by parasites of livestock. Acta Trop. **56:**125–141.
78. **Hoekstra, R., F. H. M. Borgsteede, J. H. Boersema, and M. H. Roos.** 1997. Selection for high levamisole resistance in *Haemonchus contortus* monitored with an egg-hatch assay. Int. J. Parasitol. **27:**1395–1400.
79. **Hoekstra, R., A. Visser, L. J. Wiley, A. S. Weiss, N. C. Sangster, and M. H. Roos.** 1997. Characterization of an acethylcholine receptor gene of *Haemonchus contortus* in relation to levamisole resistance. Mol. Biochem. Parasitol. **84:**179–187.
80. **Hunt, K. R., and M. A. Taylor.** 1989. Use of the egg hatch assay on sheep faecal samples for the detection of benzimidazole resistant worms. Vet Rec. **125:**153–154.
81. **Ismail, M., A. Metwally, A. Farghaly, J. Bruce, L.-F. Tao, and J. L. Bennett.** 1996. Characterization of isolates of *Schistosoma mansoni* from Egyptian villagers that tolerate high doses of praziquantel. Am. J. Trop. Med. Hyg. **55:**214–218.

81a. **Ismail, A., S. Botros, A. Metwally, S. William, A. Farghally, L. F. Tao, T. A. Day, and J. L. Bennett.** 1999. Resistance to praziquantel: direct evidence from *Schistosoma mansoni* isolated from Egyptian villagers. Am. J. Trop. Med. Hyg. **60:**932–935.

82. **Jung, M. K., I. B. Wilder, and B. R. Oakley.** 1992. Amino acid alterations in the benA (β-tubulin) gene of *Aspergillus nidulans* that confer benomyl resistance. Cell Motil. Cytoskeleton **22:**170–174.
83. **Katz, N., A. Chaves, and J. Pellegrino.** 1972. A simple device for quantitative tool thick smear technique in schistosomiasis mansoni. Rev. Inst. Med. Trop. Sao Paulo **14:**397–400.
84. **Kerboeuf, D., P. Chambrier, Y. Le Vern, and J. Aycardi.** 1999. Flow cytometry analysis of drug transport mechanisms in *Haemonchus contortus* susceptible or resistant to anthelmintics. Parasitol. Res. **85:**118–123.
85. **Koenraadt, H., S. C. Sommerville, and A. L. Jones.** 1992. Characterization of mutations in the beta-tubulin gene of benomyl-resistant field strains of *Venturia inaequalis* and other pathogenic fungi. Mol. Plant Pathol. **82:**1348–1354.
86. **Komiya, Y., T. Ishizaki, Y. Ichikawa, H. Takayama, K. Tomabechi, S. Sato, and H. Kutsumi.** 1957. The clinical studies of those positive for *Ascaris* ova after successive administration of santonin preparations. The problem of santonin resistance of *Ascaris lumbricoides*. Kiseichugaku Zasshi **4:**319–326.
87. **Korte, R., B. Schmidt-Ehry, A. A. Kielman, and U. K. Brinkmann.** 1986. Cost and effectiveness of different approaches to schistosomiasis control in Africa. Trop. Med. Parasitol. **37:**149–152.
88. **Krepel, H. P., T. Haring, S. Baeta, and A. M. Polderman.** 1993. Treatment of mixed *Oesophagostomum* and hookworm infection: effect of albendazole, pyrantel pamoate, levamisole and thiabendazole. Trans. R. Soc. Trop. Med. Hyg. **87:**87–89.
89. **Kumar, V., and B. Gryseels.** 1994. Use of praziquantel against schistosomiasis: a review of the current status. Int. J. Antimicrob. Agents **4:**313–320.
90. **Kwa, M. S. G., M. N. Okoli, H. Schulz-Key, P. O. Okongkwo, and M. H. Roos.** 1998. Use of P-glycoprotein gene probes to investigate anthelmintic resistance in *Haemonchus contortus* and comparison with *Onchocerca volvulus*. Int. J. Parasitol. **28:**1235–1240.
91. **Kwa, M. S. G., J. G. Veenstra, and M. H. Roos.** 1994. Benzimidazole resistance in *Haemonchus contortus* is correlated with a conserved mutation at amino acid 200 in β-tubulin isotype 1. Mol. Biochem. Parasitol. **63:**299–303.
92. **Kwa, M. S. G., J. G. Veenstra, M. Van Dijk, and M. H. Roos.** 1995. β-Tubulin genes from the parasitic nematode *Haemonchus contortus* modulate drug resistance in *Caenorhabditis elegans*. J. Mol. Biol. **246:**500–510.
93. **Lacey, E., J. M. Redwin, J. H. Gill, V. M. Demargheriti, and P. J. Waller.** 1990. A larval development assay for the simultaneous detection of broad spectrum anthelmintic resistance, p. 177–184. *In* J. C. Boray, P. J. Martin and R. T. Roush (ed.), Resistance of parasites to antiparasitic drugs. MSD Agvet, Rahway, N.J.
94. **Le Jambre, L. F.** 1976. Egg hatch as an in-vitro assay of thiabendazole resistance in nematodes. Vet. Parasitol. **2:**385–391.
95. **Maciel, S., A. M. Gimenez, C. Gaona, P. J. Waller, and J. W. Hansen.** 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: Paraguay. Vet. Parasitol. **62:**207–212.
96. **Martin, L. K., and P. C. Beaver.** 1968. Evaluation of Kato Thick Smear technique for quantitative diagnosis of helminth infections. Am. J. Trop. Med. Hyg. **17:**382–391.
97. **Martin, P. J., N. Anderson, and R. G. Jarrett.** 1989. Detecting benzimidazole resistance with faecal egg count reduction tests and *in vitro* assays. Aust. Vet. J. **66:**236–240.
98. **Martin, P. J., N. Anderson, R. G. Jarrett, T. H. Brown, and G. E. Ford.** 1982. Effects of a preventive and suppressive control scheme on the development of thiabendazole-resistance in *Ostertagia* spp. Aust. Vet. J. **58:**185–190.
99. **Martin, P. J., N. Anderson, T. Lwin, G. Nelson, and T. E. Morgan.** 1984. The association between frequency of thiabendazole treatment and the development of resistance in field isolates of *Ostertagia* spp. of sheep. Int. J. Parasitol. **14:**177–181.
100. **Reference deleted.**
101. **McGregor, A.** 1998. Call for renewed drive against schistosomiasis. Lancet **352:**1997.
102. **McKenna, P. B.** 1997. Use of arithmetic and geometric means in the calculation of anthelmintic efficacy. Vet. Rec. **141:**472–473.
103. **Metwally, A., J. L. Bennett, S. Botros, F. Ebeid, and G. El Attar.** 1995. Impact of drug dosage and brand on bio-availability and efficacy of praziquantel. Pharamacol. Res. **31:**53–59.
104. **Monteiro, A. M., S. W. Wanyangu, D. P. Kariuki, R. Bain, F. Jackson, and Q. A. McKellar.** 1998. Pharmaceutical quality of anthelmintics sold in Kenya. Vet. Rec. **142:**396–398.
105. **Pereira, C., P. G. Fallon, J. Cornette, A. Capron, M. J. Doenhoff, and R. J. Pierce.** 1998. Alterations in cytochrome-*c* oxidase expression between praziquantel-resistant and susceptible strains of *Schistosoma mansoni*. Parasitology **117:**63–73.
106. **Peters, P., M. El Alamy, K. Warren, and A. Mahmoud.** 1980. Quick Kato smear for field evaluation of *Schistosoma mansoni* eggs. Am. J. Trop. Med. Hyg. **29:**217–219.
107. **Pica-Mattoccia, L., L. C. Dias, R. Moroni, and D. Cioli.** 1993. *Schistosoma mansoni*: genetic complementation analysis shows that two independent hycanthone/oxamniquine-resistant starins are mutated in the same gene. Exp. Parasitol. **77:**445–449.
108. **Picquet, M., J. Vercruysse, D. J. Shaw, M. Diop, and A. Ly.** 1998. Efficacy of praziquantel against *Schistosoma mansoni* in northern Senegal. Trans. R. Soc. Trop. Med. Hyg. **92:**90–93.
109. **Polderman, A. M., K. Mpamila, J. P. Manshande, and M. L. Bouwhuis-Hoogerwerf.** 1985. Methodology and interpretation of parasitological surveillance of intestinal schistosomiasis in Maniema, Kivu Province, Zaire. Ann. Soc. Belg. Med. Trop. **65:**243–249.
110. **Polderman, A. M., and A. C. Rijpstra.** 1993. Medische parasitologie. Bohn Stafleu Van Loghum, Houten, The Netherlands.
111. **Redman, C. A., A. Robertson, P. G. Fallon, J. Modha, J. R. Kusel, M. J. Doenhoff, and R. J. Martin.** 1996. Praziquantel: an urgent and exciting challenge. Parasitol. Today **12:**14–20.
112. **Reinemeyer, C. R., B. W. Rohrbach, V. M. Grant, and G. L. Radde.** 1992. A survey of ovine parasite control practices in Tennessee. Vet. Parasitol. **42:**111–122.
113. **Remme, J. H. F.** 1995. The African Programme for Onchocerciasis Control: preparing to launch. Parasitol. Today **11:**403–406.
114. **Renganathan, E., and D. Cioli.** 1998. An international initiative on praziquantel use. Parasitol. Today **14:**390–391.
115. **Renganathan, E., E. Ercole, M. Albonico, G. De Gregorio, K. S. Alawi, U. M. Kisumku, and L. Savioli.** 1995. Evolution of operational research studies and development of a national control strategy against intestinal helminths in Pemba Island, 1988–92. Bull. W. H. O. **73:**183–190.
116. **Reynoldson, J. A., J. M. Behnke, L. J. Pallant, M. G. Macnish, F. Gilbert, S. Giles, R. J. Spargo, and R. C. A. Thompson.** 1997. Failure of pyrantel in treatment of human hookworm infections (*Ancylostoma duodenale*) in the Kimberley region of North West Australia. Acta Trop. **68:**301–312.
117. **Rohrer, S. P., E. T. Birzin, C. H. Eary, J. M. Schaeffer, and W. L. Shoop.** 1994. Ivermectin binding sites in sensitive and resistant *Haemonchus contortus*. J. Parasitol. **80:**493–497.
118. **Roos, M. H.** 1997. The role of drugs in the control of parasitic nematode infections: must we do without? Parasitology **114:**S137–S144.
119. **Roos, M. H., and M. S. G. Kwa.** 1994. Genetics of anthelmintic resistance in parasitic nematodes: comparison of a theoretical model with laboratory and field studies, p. 141–152. *In* G. C. Coles, F. H. M. Borgsteede, and S. Geerts (ed.), Anthelmintic resistance in nematodes of farm animals—1994. European Commission, Brussels, Belgium.
120. **Roos, M. H., M. S. G. Kwa, and W. N. Grant.** 1995. New genetic and practical implications of selection for anthelmintic resistance in parasitic nematodes. Parasitol. Today. **11:**148–150.
121. **Ross, A. G. P., Y. S. Li, A. C. Sleigh, and D. P. McManus.** 1997. Schistosomiasis control in the People's Republic of China. Parasitol. Today **13:**152–155.

121a. **Sacko, M., D. De Clercq, J. M. Behnke, F. S. Gilbert, P. Dorny, and J. Vercruysse.** 1999. Comparison of the efficacy of mebendazole, albendazole and pyrantel in treatment of human hookworm infections in the Southern Region of Mali, West Africa. Trans. R. Soc. Trop. Med. Hyg. **93:**195–203.

122. **Sangster, N.** 1996. Pharmacology of anthelmintic resistance. Parasitology **113:**S201–S216.
123. **Sangster, N. C.** 1999. Anthelmintic resistance: past, present and future. Int. J. Parasitol. **29:**115–124.
124. **Sangster, N. C., S. C. Bannan, A. S. Weiss, S. C. Nulf, R. D. Klein, and T. G. Geary.** 1999. *Haemonchus contortus*: sequence heterogeneity of internucleotide binding domains from P-glycoproteins and an association with avermectin/mylbemycin resistance. Exp. Parasitol. **91:**250–257.
125. **Sangster, N. C., H. V. Whitlock, I. G. Russ, M. Gunawan, D. L. Griffin, and J. D. Kelly.** 1979. *Trichostrongylus colubriformis* and *Ostertagia circumcincta* resistant to levamisole, morantel tartrate and thiabendazole: occurrence of field isolates. Res. Vet. Sci. **27:**106–110.
126. **Shakoor, O., R. B. Taylor, and R. H. Behrens.** 1997. Assessment of the incidence of substandard drugs in developing countries. Trop. Med. Int. Health **2:**839–845.
127. **Shoop, W. L.** 1993. Ivermectin resistance. Parasitol. Today **9:**154–159.
128. **Smith, G.** 1990. Chemotherapy: future problems, p. 291–303. *In* G. A. Schad and K. S. Warren (ed.), Hookworm disease: current status and new directions—1990. Taylor & Francis, London, United Kingdom.
129. **Smith, G., B. T. Grenfell, V. Isham, and S. Cornell.** 1999. Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. Int. J. Parasitol. **29:**77–91.
130. **Stelma, M. M., S. Sall, B. Daff, S. Sow, M. Niang, and B. Gryseels.** 1997. Oxamniquine cures *Schistosoma mansoni* infection in a focus in which cure rates with praziquantel are unusually low. J. Infect. Dis. **176:**304–307.
131. **Stelma, F. F., I. Talla, K. Polman, M. Niang, R. F. Sturrock, A. M. Deelder, and B. Gryseels.** 1993. Epidemiology of *Schistosoma mansoni* infection in a recently exposed community in Northern Senegal. Am. J. Trop. Med. Hyg. **49:**701–706.
132. **Stelma, F., I. Talla, S. Sow, A. Kongs, M. Niang, K. Polman, A. M. Deelder, and B. Gryseels.** 1995. Efficacy and side effects of praziquantel in an epidemic focus of *Schistosoma mansoni*. Am. J. Trop. Med. Hyg. **63:**167–170.
133. **Tayler, E.** 1997. Antituberculosis drug resistance: practical solutions to

practical problems. J. Med. Microbiol. **46:**531–533.
134. **Taylor, M. A., and K. R. Hunt.** 1989. Anthelmintic drug resistance in the UK. Vet. Rec. **125:**143–147.
135. **Townson, S., S. K. Taghoto, J. Castro, A. Lujan, K. Awadzi, and V. P. K. Titanji.** 1994. Comparison of the sensitivity of different geographical races of *Onchocerca volvulus* microfilariae to ivermectin: studies *in vitro*. Trans. R. Soc. Trop. Med. Hyg. **88:**101–106.
136. **Van Etten, L., D. Engels, F. W. Krijger, L. Nkulikyinka, B. Gryseels, and A. M. Deelder.** 1996. Fluctuation of schistosome circulating antigen levels in urine of individuals with *Schistosoma mansoni* infection in Burundi. Am. J. Trop. Med. Hyg. **54:**348–351.
137. **Van Lieshout, L., F. F. Stelma, F. Guisse, S. T. M. Falcao Ferreira, K. Polman, G. J. van Dam, M. Diakhate, S. Sow, A. M. Deelder, and B. Gryseels.** 1999. The contribution of host-related factors to low cure rates of praziquantel for the treatment of *Schistosoma mansoni* in Senegal. Am J. Trop. Med. Hyg. **61:**760–765.
138. **van Wyk, J. A.** 1990. Occurrence and dissemination of anthelmintic resistance in South Africa and management of resistant worm strains, p. 103–114. *In* J. C. Boray, P. J. Martin, and R. T. Roush (ed.), Resistance of parasites to antiparasitic drugs. MSD Agvet, Rahway, N.J.
139. **van Wyk, J. A., F. S. Malan, H. M. Gerber, and R. M. R. Alves.** 1989. The problem of escalating resistance to *Haemonchus contortus* to the modern anthelmintics in South Africa. Onderstepoort J. Vet. Res. **56:**41–49.
140. **van Wyk, J. A., F. S. Malan, and J. L. Randles.** 1997. How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics. Vet. Parasitol. **70:**111–122.
141. **van Wyk, J. A., F. S. Malan, L. J. van Rensburg, P. T. Oberem, and M. J. Allan.** 1997. Quality control in generic anthelmintics: is it adequate? Vet. Parasitol. **72:**157–165.
142. **Varady, M., and J. Corba.** 1999. Comparison of six in vitro tests in determining benzimidazole and levamisole resistance in *Haemonchus contortus* and *Ostertagia circumcincta* of sheep. Vet Parasitol. **80:**239–249.
143. **Vargas, L., and J. Tovar.** 1957. Resistance of *Onchocerca volvulus* microfilariae to diethylcarbamazine. Bull. W. H. O. **16:**682–683.
144. **Visser, E. L., P. C. Van Schalkwyk, and S. M. Kotze.** 1987. Aanduidings van weerstand by lintwurms van kleinvee, p. 24–28. *In* J. Schröder (ed.), Worm resistance Workshop—1987.
145. **Waller, P. J.** 1985. Resistance to anthelmintics and the implications for animal production, p. 1–12. *In* N. Anderson and P. J. Waller (ed.), Resistance in nematodes to anthelmintic drugs—1985. CSIRO, Australia.
146. **Waller, P. J., K. M. Dash, A. Barger, L. F. Le Jambre, and J. Plant.** 1995. Anthelmintic resistance in nematode parasites of sheep: learning from the Australian experience. Vet. Rec. **136:**411–413.
147. **Waller, P. J., F. Echevarria, C. Eddi, S. Maciel, A. Nari, and J. W. Hansen.** 1996. The prevalence of anthelmintic resistance in nematode parasites of sheep in Southern Latin America: general overview. Vet. Parasitol. **62:**181–187.
148. **Warren, K. S.** 1990. An integrated system for the control of the major helminth parasites. Acta Leiden. **59:**433–442.
149. **Warren, K. S.** 1993. For the new millenium: control of helminth diseases throughout the world. Med. J. Aust. **159:**461–463.
150. **Wiley, L. J., D. R. Ferrara, N. C. Sangster, and A. S. Weiss.** 1997. The nicotinic acetylcholine α-subunit gene *tar-1* is located on the X chromosome but its coding sequence is not involved in levamisole resistance in an isolate of *Trichostrongylus colubriformis*. Mol. Biochem. Parasitol. **90:**415–422.
151. **Warren, K. S., D. A. P. Bundy, R. M. Anderson, A. R. Davis, D. A. Henderson, D. T. Jamison, N. Prescott, and A. Senft.** 1993. Helminth infection, p. 131–160. *In* D. T. Jamison, W. H. Mosley, A. R. Measham, and J. L. Bobadilla (eds), Disease control priorities in developing countries—1993. Oxford University Press, Oxford, United Kingdom.
152. **World Health Organization.** 1983. The role of chemotherapy in schistosomiasis control. WHO/Schisto/83.70. World Health Organization, Geneva, Switzerland.
153. **World Health Organization.** 1985. The control of schistosomiasis. Report of a WHO expert committee. W. H. O. Tech. Rep. Ser. **728:**1–113.
154. **World Health Organization.** 1995. WHO model prescribing information—drugs used in parasitic diseases, 2nd ed. World Health Organization, Geneva, Switzerland.
155. **World Health Organization.** 1992. The control of schistosomiasis: report of the Expert Committee. W. H. O. Tech. Rep. Ser. **830:**1–86.
156. **World Health Organization.** 1995. Onchocerciasis and its control. W. H. O. Tech. Rep. Ser. **852:**1–103.
157. **World Health Organization.** 1999. Report of the WHO Informal Consultation on monitoring drug efficacy in the control of schistosomiasis and intestinal nematodes, Geneva 8–10 July 1998, WHO/CDS/CPC/SIP/99.1. World Health Organization, Geneva, Switzerland.
158. **Xu, M., M. Molento, W. Blackhall, P. Ribeiro, R. Beech, and R. Prichard.** 1998. Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. Mol. Biochem. Parasitol. **91:**327–335.