REVIEW ARTICLE

NEMATODE CONTROL AND THE POSSIBLE DEVELOPMENT OF ANTHELMINTIC RESISTANCE

M.B. MOLENTO* & R.K. PRICHARD

Institute of Parasitology, McGill University, 21,111 Lakeshore Road, Sainte Anne-de-Bellevue, Quebec, Canada, H9X-3V9
* Corresponding author: marcelo@parasit.lan.mcgill.ca, FAX (514) 398-7857, Phone (514) 398 7730

SUMMARY: Although drug treatment is regarded as the most efficacious way of controlling parasitic infections, the reality is that drug resistance to all the anthelmintic groups is spreading all over the world. This paper focuses on the methods that are being used to control nematode parasite infections, and the implications of the development of anthelmintic resistance. It also introduces new approaches to worm control that might be helpful for veterinary medicine and animal science students, clinicians and researchers. The ideal approach to possibly avoid anthelmintic resistance is to use an integrated parasite management combining different strategies. Therefore, if an anti-parasitic program is going to be implemented to eliminate the loss caused by parasitic infections of animals and to control the development of drug resistance many factors have to be evaluated.

KEY WORDS: Anthelmintic resistance, nematode control.

INTRODUCTION

Nematode parasites such as *Haemonchus spp*, *Ostertagia spp.*, *Trichostrongylus spp.* or *Cooperia spp.* are responsible for immense decreases in animal performance reducing meat, milk, and wool production in sheep, goats, and cattle, and increasing mortality in young animals. The degree of damage is, in most circumstances, impossible to quantify for the livestock industries. Anthelmintics are regarded as the sole treatment for a heavily infected flock or herd and many times they are used without taking into consideration parasite specificity, formulation, routes of administration, or other crucial details. So, producers appeal to the constant use of chemicals as a prophylactic treatment for parasitic infections. Thus, as a consequence, selection of drug resistant organisms into a population may occur and this phenotype will be passed to future generations of parasites.

Soon after the introduction of the benzimidazoles in the mid 60's, drug failure was observed, and the discovery of the avermectin and milbemycin class of anthelmintics in the early 80's provided a new means of parasite control in domestic ruminant. Unfortunately, in some instances, parasites acquired resistance to ivermectin after only a few treatments. For a review of the mechanisms of action of anthelmintic drugs including levamisole and morantel see SHOOP *et alii*, (1995) and MARTIN (1997).

As we know, there is no single formula for worm control that can be applied worldwide. A combination of strategic and tactical treatments along with the evaluation of anthelmintic efficacy is required to enable producers to raise livestock under optimal production conditions (CRAIG, 1993). In regions such as Australia, South Africa, and southern Latin America, there is a critical need for such strategies that will render efficient parasite control. One has to keep in mind that drug failure is an individual farm problem, and has to be evaluated as to which strategy can be best used.

PARASITE BIOLOGY AND CONTROL STRATEGIES

Gastrointestinal nematode populations generally follow a

seasonal abundance on the pasture. Larvae available to grazing animals in the spring are either those that survived the winter, or dry season depending on the region or pasture and will only survive as long as their energy stores last. Or, they are the progeny of adult worms which have survived in animals over that period or are the progeny of hypobiotic larvae which have resumed development. The second population will be the offspring of the population acquired in the spring, or wet season. Susceptibility of the host population and environmental conditions will determine the level of available larvae. Because of the small temperature variability and humid climate the tropical and subtropical countries have proved to be suitable for the development and survival of parasitic larvae throughout the year.

In terms of the parasite population, each adult female parasite needs to replace herself in the next generation by at least one new offspring (effective reproduction ratio, R_e) or the population will decline (less than one). The R_e is always less than the basic reproduction ratio, R_o, which is defined as the average number of female parasites produced by one mated female in a completely susceptible host population, that is, when no other parasites are present. The difference between R_e and R_o is density dependent which is related to parasite distribution. The importance of this effect to chemotherapy is that density dependence governs the rate of recovery of the parasite population following chemotherapy. Where R_e will increase and the population will grow until the parasite density is again at its equilibrium (MEDLEY, 1994).

The primary information required by producers when having to deal with worm infection is which anthelmintic should be used, when, and at what cost. Facing this situation, the effectiveness of any anthelmintic has to be evaluated, and only those drugs which are known to be effective against the target parasites should be used. After deciding which drug to use, some factors have to be taken into consideration such as the route of administration and the animal's weight. A proper delivery system has to be followed if the commercial drug is to be used orally, sub-cutaneously, intra-abomasum, or intra-muscularly. In the case of oral treatments for ruminants, the importance of the oesophageal groove closure was reported to have altered the pharmacokinetics of oxfendazole in 42% of the animals showing a complete rumen bypass, decreasing drug efficacy and maybe selecting for drug resistance (PRICHARD & HENNESSY, 1981). An adequate dosage is imperative, because if some animals receive underdosed treatments, parasites which are heterozygous for resistance may well survive (COLES, 1988). Thus, inadequately drenching animals might precipitate selection for resistance in the field. CHARLES *et alii*, (1989) reported that therapeutic dosages of levamisole, albendazole, and parbendazole that are prescribed for sheep were not as effective (underdosed) for goats against nematode parasites. On the other hand, some drugs may have negative environmental effects or cause host toxicity when used above therapeutic doses.

Where the parasite survival mechanism during periods of adverse weather is larval hypobiosis, it is imperative that treatment with products, effective against hypobiotic larvae, is carried out during the period of arrested development. Treatment should be directed toward preventing pasture contamination associated with activation of arrested larvae, which may accompany periparturient relaxation of immunity. Treating livestock at such a time, so as to preclude the spring pasture rise of larvae would effectively lower pasture contamination (HERD *et alii*, 1985). Unless there is a concomitant increase in immunity or prolonged pasture rest, selection for resistance will start with the first treatment. GATONGI and colleagues (1998) have demonstrated that treatment before the long rains would reduce the adult worm and hypobiotic larval populations and therefore lead to low pasture contamination during the next wet season in Kenya.

In small ruminants, treatments may be determined on the basis of faecal egg count reduction tests (FECRT), as there is a direct relationship between faecal egg counts and population size of important nematodes (ROBERTS & SWAN, 1981). Although this relationship has been proved to be not always strong, a tactical treatment based on FECRT is more rational than treating every 3 or 4 weeks as practised by many producers (WALLER *et alii*, 1993), and as is commonly done by sheep producers in the southern state of Paraná, Brazil (M. MOLENTO, personal observations).

The effectiveness of alternating anthelmintic treatments is still not satisfactorily answered. Some have advocated the strategy of using a single drug until it is no longer effective, then changing it (LE JAMBRE *et alii*, 1978). Rapid rotation of anthelmintics has been largely discredited, as it selects for resistance to all of the drugs that are used in the rotation (BARNES & DOBSON, 1990). Slow rotation (once a year) of anthelmintics with different modes of action has been shown to be quite effective (PRICHARD *et alii*, 1980). At the end of the first year, the surviving worms may have developed only a low level of resistance to the utilized anthelmintic, and should have no resistance to the new class of anthelmintic introduced. In practice, the slow rotation of anthelmintics has proved to be most effective in reducing the rate of selection for anthelmintic resistance. However, to be effective it must be practised with two or three classes of anthelmintic that are fully effective. Once resistance is present in a class of anthelmintic, a slow rotation program will be compromised in some years.

The model suggested by SMITH (1990) is that of simultaneous use of two different anthelmintics. This strategy delays but does not prevent the development of resistance relative to, for example, drug rotation. If this strategy is contemplated, it is imperative that all of the anthelmintics used are effective at the start of the program. If worms already have a level of resistance

to one of the products, this resistance will be intensified, and selection will occur to the other product (SIVARAJ *et alii*, 1993). Continuous treatment with few different classes of anthelmintic has not received widespread acceptance as it effectively doubles the cost of the treatment, may cause toxicity to the host, and is disliked by drug registration authorities in most countries. Usually, helminth populations are overdispersed among hosts, so most individuals are lightly infected and a few hosts harbor many parasites (MEDLEY, 1994). Selective deworming of heavily infected individuals is a strategy that may prove to be of great value and which should not put very much selective pressure on all of the worms in a population (DUNCAN & LOVE, 1991).

If parasite management is practised in such a way that factors other than anthelmintics are contributed to reducing pasture contamination, then the selection pressure towards parasite resistance will be reduced considerably. The traditional approach of pasture hygiene for equines has been modernized. To some degree, the removal of faeces from pastures not only reduces the level of infective larvae, but it also increases the amount of grazable pasture (HERD, 1986). The practice of alternate grazing of species like ruminants/non-ruminants, or sheep/cattle may be useful in controlling nematodes. Grazing pastures with livestock, that have acquired resistance to parasites before allowing susceptible populations to graze, may also slow down the onset of anthelmintic resistance (EYSKER *et alii*, 1986). The treat-and-move principle, where animals are moved from contaminated to safe or clean pastures, may reduce the need for frequent anthelmintic treatment in susceptible host populations (WALLER *et alii*, 1989). On the other hand, when treated animals are moved to clean pastures, the only parasites available to contaminate the clean pastures will be those that have survived treatment, i.e., those that are resistant. Therefore, the practice of treat-and-move to clean pasture may act as a strong stimulus for resistance to develop. ECHEVARRIA *et alii*, (1993) reported that pastures that were reseeded after a crop of soya beans showed little immediate risk of nematode infection to sheep and cattle. Re-introducing a susceptible strain of a parasite would also decrease the frequency of a resistant allele in the population (VAN WYK & VAN SCHALKWYK, 1990).

Genetic selection of hosts for resistance against specific parasites may also be a useful alternative. Host-resistance against nematode parasites probably operates in two ways; through immunologic reactions, or by resilience to infection, which means a superior ability to compensate for parasite-induced damage. Genetic selection for immunologic enhancement will not only protect those individuals against the effects of worms but will lessen environmental exposure from and dependence on anthelmintics (WOOLASTON *et alii*, 1990). The major limiting factor is a method of selection of livestock resistant to parasites before exposing them to what could be fatal levels of infection (CRAIG, 1993). Genetic markers (haemoglobin, anaemia, MHC type, FEC) for resistance to gastrointestinal parasite infection in sheep have been developed (BEH & MADDOX, 1996). However, resistance to metazoan parasites is polygenic and modulated by environmental factors.A related approach was propose by ROOS (1997); which was to find molecular markers that are linked with resistance in sheep. In the future, these genes could be used to inject sheep embryos to make transgenic sheep that are resistant to nematodes. After the increasing awareness of the possible environmental hazards in using chemicals, and the desire to use anthelmintics minimally to reduce selection for drug resistance, biological control is getting more attention. Using agents such as nematode destroying fungi, viruses, bacteria, or protozoa to control nematodes, this technique has been defined as 'an ecological method designed by man to keep parasite populations at a non-harmful level using natural living antagonist' (GRÖNVOLD *et alii* 1996). Another very promising field is the development of specific or broad-spectrum vaccines against parasites (for review see EMERY, 1996). But the most probable time to commercial availability of a vaccine against *H. contortus* was judged to be 5 years, and in excess of 10 years for vaccines against other nematode species (BARGER, 1996). The only vaccines to be successfully developed against helminth parasites in veterinary medicine were irradiated larval vaccines against *Dictyocaulus viviparus* (URQUHART, 1985), *D. filaria* (SHARMA *et alii*, 1981) and *Ancylostomum caninum* (MILLER, 1978).

Regardless of whether chemical or non-chemical approaches are used, monitoring control programs is very important, given the host-parasite relationship. Because of this dynamic relationship, changes in the program have also to be continuously evaluated. MEDLEY (1994) suggests that one important measurement in determining successful control programs is the host morbidity reduction, which can be correlated with the number of heavily infected individuals.

Although the use of anthelmintic drugs to treat nematode infection is by far the simplest method used by farmers, especially in remote areas. The continuous use of these chemicals rapidly selected strains that are resistant to all the available drugs. Producers are looking for alternatives to maintain their productivity. As a consequence, researchers are testing different alternatives, as well as the combination of different management strategies, to be able to lessen the use of anthelmintic drugs; hoping that we can maintain control of parasites. In Europe, drug-free practices are being encouraged by market pressure where people are looking for a more ecological or nonchemical products.

ANTHELMINTIC RESISTANCE (AR)

Anthelmintic resistance has been selected when the drug,

which was previously effective against unselected parasites, is no longer effective at the same dose rate (PRICHARD, 1990). Early detection of emerging resistance is vital for the conservation of effective anthelmintics and for parasite control. Though, some information has to be supplied before suspecting the failure of an anthelmintic treatment, such as drug storage conditions, expiring dates, drenching techniques, and pasture conditions (DORNY & VERCRUYSSE, 1993).

Parasite selection can be originated on the farm after a few generations or when recently acquired animals are introduced into the flock without proper care for dissemination of eggs and larvae. In Brazil, a resistant *H. contortus* strain was introduced after a large number of sheep were purchased from the south of the country and shipped to the northeast (VIEIRA *et alii*, 1992). Resistance by various geographic strains of *Haemonchus*, *Ostertagia spp.*, and *Trichostrongylus* against all classes of anthelmintics in small ruminants has been established (VAN WYK & MALAN 1988, MALAN *et alii*, 1990; MILLER & BARRAS, 1994). Resistance was also demonstrated in horses (PICHE *et alii*, 1989), cattle (EAGLESON & BOWIE, 1986; JACKSON *et alii*, 1987), and in pigs (BJORG *et alii*, 1990). In addition, there is some potential of transmission to wild animals that could share contaminated pasture. This was confirmed by PRASLICKA *et alii* (1995) where they artificially infected mouflons (wild goats) with a benzimidazole-resistant strain of *H. contortus*. The recovered eggs were then used to infect naive lambs and examined by an egg hatch assay, where the strain maintained it's level of resistance.

Unless a drug treatment is 100% effective against an organism, there is the possibility that treatment is selecting for resistance (LE JAMBRE, 1990). Via a cell model, a single gene, rather than polygenes mutation, may be responsible for the development of resistance after continuous drug treatment (Fig. 1). This type of resistance will usually develop after the homozygous resistant gene is disseminated through the cell/parasite population.

The most widespread resistance occurs within the benzimidazoles and pro-benzimidazoles where alleles for resistance were apparently present before benzimidazoles were marketed (ROOS *et alii*, 1990). Significant allelic variation at two b-tubulin genes from cambendazole- and thiabendazole-resistant and susceptible strains of *H. contortus* were identified (BEECH *et alii*, 1994). These authors showed that drug treatment can cause a shift in the frequency of the alleles of the drug target, b-tubulin, present in a susceptible strain that can result in resistance. Levamisole and morantel have a similar mode of action, and resistance to the one anthelmintic is often associated with resistance to the other (PRICHARD, 1990). In this case, resistance appears to be associated with alterations in cholinergic receptors in resistant nematodes (SANGSTER *et alii*, 1991). Resistance to the macrocyclic lactones, avermectins and milbemycins, the most recently introduced group of compounds, has been reported under field conditions (VAN WYK & MALAN, 1988; ECHEVARRIA & TRINDADE, 1989; LEATHWICK, 1995). The phenomenon of resistance to the macrolactones appears to have a multiple mechanism. GILL *et alii*, (1998) reported that two strains of *H. contortus* that were selected under laboratory conditions had different responses when treated with ivermectin using *in vitro* assays compared to one selected-strain collected from the field. XU and colleagues (1998) reported that P-glycoprotein, a transport protein, over-expression in a resistant strains of *H. contortus* may be linked with the mechanism of resistance. It is believed that P-glycoprotein would decrease ivermectin's concentration in the parasite nerve cells by pumping ivermectin form the cell in an efflux mechanism (Fig. 2). A distinct mechanism was reported by BLACKHALL *et alii* (1998) where different alleles of the a-subunit gene of the glutamate-gated chloride channel were detected among susceptible and ivermectin- and moxidectin-resistant strains of *H. contortus*. The mechanism of ivermectin or moxidectin resistance in *H. contortus* is still to be determined in its totality. Thus, these data strongly suggest that the mechanism of resistance to the macrolactones could be a result of more than one process, separate or associated.

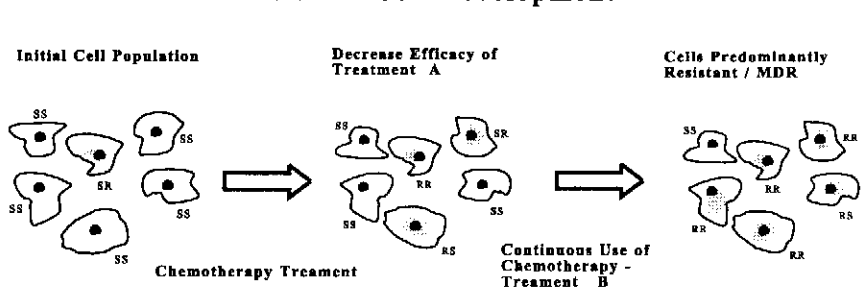

Fig. 1 - SS, Homozygous Susceptible, RR, Homozygous Resistant, and SR/RS, Heterozygous.

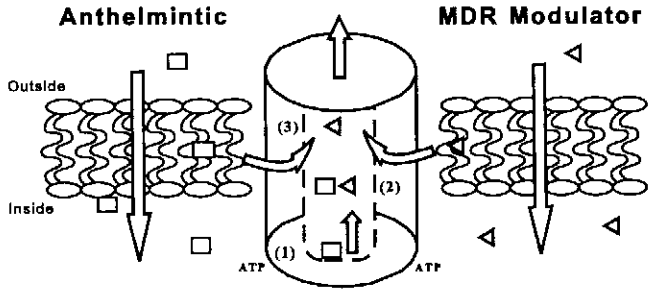

Fig. 2 - P-glycoprotein transport model. The ilustration demonstrates how the anthelmintic can be effluxed from the cell (1), and how the MDR modulator may compete for the anthelmintic binding site (2), or use a non-competitive approach (3). Adapted from Ford, (1995).

Side resistance occurs when a resistant population becomes resistant to other compounds which have a similar mode of action, whether or not the parasites have been exposed to the compound in question. Cross resistance, or as a more modern term, multidrug anthelmintic resistance (MAR), occurs when drugs from different chemical groups fail to eliminate those individuals from those populations to which resistance has already been manifest (PRICHARD *et alii*, 1980). Resistance to more than 2 anthelmintic groups is common and multiple anthelmintic resistance to gastrointestinal parasites was reported in New Zealand (VERMUNT *et alii*, 1995), India (YADAV *et alii*, 1995), Brazil (SOCCOL *et alii*, 1996), UK (COLES *et alii*, 1996), and in Kenya (MWAMACHI *et alii*, 1995, and WARUIRU *et alii*, 1997). Parasite reduction was determined based on farm diagnosis, where therapeutic dosages were applied on naturally infected animals or a drug-selected strain from the field was used to infect experimental animals.

DETECTION OF ANTHELMINTIC RESISTANCE

Anthelmintic resistance is generally not recognized until it becomes a problem, due to our inability to access subclinical resistance in the field (CONDER & CAMPBELL, 1995), and farmers are the ones who first point out that a certain drug is "failing to clean" the animals. The most effective method for determining the existence of an AR problem is that of post-mortem evaluation of treated and untreated animals. BEVERIDGE *et alii*, (1990) explained that this enables the investigator to determine unequivocally the species and stage of development of worms which are either susceptible or resistant to the tested compound. The use of laboratory animals rather than natural hosts have the advantage of being less costly, thus facilitating the use of a larger sample size. Although restricted in terms of parasite species and rate of parasite establishment, the use of laboratory animals as models for testing drug efficacy is being improved (CONDER *et alii*, 1991). MOLENTO *et alii*, (1999a E NÃO 98) have demonstrated reduced efficacy of ivermectin and moxidectin when used in jirds infected with a moxidectin-selected strain of *H. contortus*. The strain was shown to exhibit cross-resistance between the two drugs which are suspected to have a similar mode of action.

Several *in vitro* tests have been developed to determine if resistant populations of worms are present. Egg hatch assays, larval motility tests, larval development tests and tubulin binding assays have been developed and are simple and useful for different classes of anthelmintics based on their mode of action under stringent environmental conditions (LACEY & PRICHARD, 1986; FOLZ *et alii* 1987; TAYLOR, 1990; HUBERT & KERBOEUF, 1992; ROTHWELL & SANGSTER, 1993; D'ASSONVILLE *et alii*, 1996). A PCR technique was developed for the diagnostic of *Teladorsagia circumcincta* resistant to benzimidazoles (ELARD *et alii*, 1999). A very interesting, easy to teach, and useful method is the recently developed FAMACHA test (VAN WYK *et alii*, 1997a). Its objective is to identify the most resistant sheep, able to cope with *H. contortus* infection. The diagnostic is based on a five-picture chart of the ocular mucous membranes of sheep, and treatment is recommended only for those heavily parasitized, thus, selecting less for AR. This method is being validated in South Africa for its use in the field.

The application of methods from the field of molecular biology allows new understanding and improved possibilities for the diagnosis of resistance. In the near future, DNA-based techniques should be able to determine the level of resistance in a population to a given anthelmintic, and whether immediate measures should be taken to maintain parasite control and arrest the further selection for resistance.

The World Association for the Advancement of the Veterinary Parasitology (WAAVP) is very much aware of the problem of AR in domestic and farm animals, and it has released guidelines looking at identification and control of this emergent danger (POWERS *et alii*, 1982; COLES *et alii*, 1992; WOOD *et alii*, 1995). With the objective of supplying accurate assistance to the community at large, Governments and universities have to work together to perform national or continental anthelmintic surveys. As we have seen in Australia, USA, Europe, and South America (EDWARDS *et alii*, 1986; REINEMEYER, *et alii*, 1992; DANGOLLA, *et alii*, 1996; WALLER *et alii* 1996), this requires well-explained questionaries and trained field technicians to put together all the relevant information.

MANAGING ANTHELMINTIC RESISTANCE

The selection for resistance is enhanced by the high biotic

potential of gastrointestinal nematodes. Their fecundity can enable small populations of resistant worms to become large populations in a short time, especially if the climate is favourable for the free-living stages (WEBB, 1980). Over the years, programs aimed at providing minimal drug exposure to parasites and grazing strategies have been devised. The programs have been based on the approach of treating livestock when most, if not all, of the worm population is in the host, not on the pasture (strategic treatment). If properly managed, strategic treatment will require fewer treatments, and the critical numbers of worms required to cause disease may not be reached (CRAIG, 1993). There is no doubt that careful use of anthelmintics will at least retard the development of AR. Strategic or tactical treatment puts less selection pressure on worm populations than the suppressive use of anthelmintics. Strategic treatment is based on epidemiology, while tactical treatment relies on environmental conditions, increased faecal egg counts or other criteria that predict parasitic infection.

Because resistance is a heritable characteristic, logic indicates that if the resistant population of worms is no longer exposed to that anthelmintic, within several generations it may revert to susceptibility and the anthelmintic will once again be effective (CRAIG, 1993), assuming that resistance is maladaptive in the absence of anthelmintic (SCOTT & ARMOUR, 1991). However, observations of benzimidazole-resistant worm populations indicate no reversion to susceptibility and furthermore, that benzimidazole resistance may be associated with increased pathogenicity (MAIR & CRIPPS, 1991).

As we have seen above, drug specificity, the incidence of treatment, and drug alternation, are important factors to be considered in a strategic parasite control. But firstly, the drug pharmacokinetic relationship should be understood. HENNESSY (1997), explains that the quantity of a drug that is removed from a compartment over a given time depends on the quantity of drug present. For any drug, this can be represented as a curve, where the maximum concentration of a drug in the compartment occurs when the quantity absorbed is equivalent to the quantity excreted. Symbolized as the drug peak, that will gradually decline as the quantity in the compartment decreases, displaying the characteristic "tail". Analysing the curve profile of ivermectin (MOLENTO *et alii,* 1999b), we can observe that worms are exposed to high drug concentrations for a short period of time, where >99% of the worms are killed, followed by 2 other phases where drug-killing in less efficacious (Fig. 3). The importance of the phase 2 and 3 is that not all the worm population has been removed, clearly leaving resistant alleles present in the remaining population within the host, exposing the worm population to sub-therapeutic doses of the drug. On phase 4, a few worms would be eliminated. The sum of the 4 phase times is the drug persistence period (DOBSON *et alii*, 1996). The ideal drug then, would be one that maintains a high level of killing for a long period with a short "tail".

Another approach is that of multiple or prolonged dose administration where a more or less constant level of anthelmintic is present over prolonged periods of time (PRICHARD *et alii*, 1978; HASS *et alii*, 1982). The desirable consequence of that is the presence of an extended period of exposure of worms to toxic levels of the anthelmintic followed by a short "tail". In order to survive these conditions, a worm would require a mutation which could provide a major change in some physiological function, a reduced susceptibility of the target protein, or a decrease in the rate of absorption (LE JAMBRE, 1990). This is the situation with the slow release boluses that are available on the market. They deliver a measured quantity of a drug into the rumen-reticulum, protecting the animal for approximately 80 to 180 days. Apart from its cost, this strategy is more feasible in the non-tropical countries where there is a much shorter grazing season and the animals need to be treated only once a year.

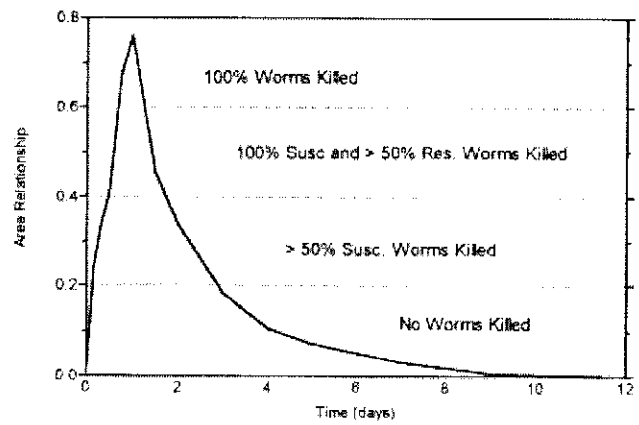

Fig. 3 - Area relationship profile of ivermectin elimination in sheep plasma over 12 days and its hypothetical efficacy against susceptible and resistance nematode isolates.

ENHANCING ANTHELMINTIC EFFICACY

The observation of the failure of all antiparasitic drugs in the field confirms that the situation is indeed very critical. A knowledge of host physiology can contribute to better pharmacological performance. SANCHEZ *et alii*, (1996) demonstrated that different feed-restriction programs can induce a great change in the plasma disposition kinetics of the anthelmintic albendazole and its metabolites. In these studies, calves received a restricted diet of 40% of the animal's maintenance for up to 35 days before treatment. The restricted group showed an enhancement in the AUC and plasma half-life for the metabolites compared to the *ad libitum* diet group. The same group of investigators, using a feed-restriction protocol

of a 24 h fasting period, reported similar results for adult sheep (LIFSCHITZ, *et alii*, 1997).

As is included in this paper, P-glycoprotein is believed to reduce drug accumulation inside the parasite in an ATP-dependent mechanism. In the free-living nematode, *Caenorhabditis elegans*, P-glycoprotein has the function of protection against environmental or chemical toxins. P-glycoprotein is also overexpressed in mammalian tumor cells and resistance can be reversed by the association of multidrug resistance (MDR) modulators to chemosensitizers. YUSA & TSURUO (1989) blocked P-glycoprotein activity by adding verapamil, a calcium channel blocker, to vincristine, an antitumor agent, enhancing its toxicity against resistant cancer cells. After the discovery that P-glycoprotein is overexpressed in ivermectin-resistant *H. contortus*, our group tested the possibility of the MDR reversing agent, verapamil, enhances the efficacy of moxidectin or ivermectin in this parasite in jirds. The drug combination was found to have a positive result, significantly increasing moxidectin and ivermectin's toxicity against selected strains (XU *et alii*, 1998). Evidences for a role for P-glycoproteins in anthelmintic resistance was produced by flow cytometry analysis of drug transport in *H. contortus*. The fluorescent probe, rhodamine 123, gave a significantly higher level of green fluorescence in resistant parasite eggs, when associated with verapamil, which would block rhodamine efflux by P-glycoprotein, as compared with susceptible eggs (KERBOEUF *et alii*, 1999). A comparative experiment using 4 different chemoenhancers was also performed utilizing a larval motility assay. Verapamil and CL 347,099, another non-toxic MDR reversing agent, significantly increased ivermectin and moxidectin's efficacy (MOLENTO & PRICHARD, 1998). Verapamil was also used *in vitro* in association with a benzimidazole, partially increasing the anthelmintic activity against an *H. contortus* benzimidazole-resistant strain (BEUGNET *et alii*, 1997). However, KOTZE, (1998) and KERBOEUF *et alii*, (1999), found that verapamil did not increase the efficacy of ivermectin or thiabendazole against ivermectin- or benzimidazole-resistant *H. contortus*, respectively, using an egg hatch assay.

Although very promising, this area of research is only at the beginning and there is a long way to be covered before a new control strategy based in drug association is introduced. One of the concerns is the fact that the use of the drug combination could have severe consequences by blocking P-glycoprotein in areas where its function is important to the host; e.g. brain, uterus, testicles. This was demonstrated by SCHINKEL *et alii*, (1994) using a P-glycoprotein knock-out mice where ivermectin was nearly 100-fold more neurotoxic, then to normal mice, after applying ivermectin to control a mite infestation. Verapamil was also showed to be toxic to jirds and sheep, in a dose-dependent manner, after being used subcutaneously (MOLENTO *et alii*, 1999a, MOLENTO *et alii*, 1999b). More work needs to be done to address the pharmacokinetic behaviour of the drug combination treatment and its consequences to the host and its efficacy against resistant parasites.

FUTURE DRUG DISCOVERY

The majority of antiparasite drugs were developed using the empirical methods of drug discovery, without knowledge of their mode of action. This has changed, and the imperative has been to define metabolic differences between parasite and host, identify biochemical targets for drugs and design specific inhibitors for these targets (CROFT, 1997). Because of that, the costs of product discovery and development in the pharmaceutical industry have escalated dramatically and this is refleted in the decrease in patent applications.

There are quite a number of techniques available to new chemical development. The data arising from sequencing of the entire genomes of some parasites provide a wealth of information on prospective drug targets (COOMBS & CROFT, 1997). Anthelmintics with different parasite target site are also to be expected in the future). The surveillance of natural substances, e.g., plant extracts and antibiotics, is also at an early stage, with only a small fraction of the microbial ecosystem having been searched (MARTIN *et alii*, 1997). Another essential adjunct to exploit the potential of this large chemical diversity is the use of robotically controlled assays of recombinant target enzymes (CROFT, 1997).

CONCLUSION

It is doubtful whether control programs, which have anthelmintic treatment as the main component, can avoid selecting for resistance. Furthermore, anthelmintic resistance will not disappear spontaneously, and it must inevitably increase if the traditional methods of worm control continue to be practised. It is also unrealistic to assume that the development and release of alternative, highly effective anthelmintic drugs will keep pace with resistance to existing drugs (WALLER, 1987). The development of new drugs is getting more and more expensive and it takes years of research and a great deal of effort and money to introduce a new candidate. The appearance of anthelmintic resistance in sheep nematodes to all broad-spectrum anthelmintics has emphasized the importance of alternative approaches to reduce the requirement for frequent anthelmintic treatments (ECHEVARRIA *et alii*, 1993; VAN WYK *et alii*, 1997b). Very importantly, understanding the biochemistry and the molecular genetics of different types of

anthelmintic resistance will lead to improvements in the methods for detection of resistance in parasites (PRICHARD, 1994).

Sometimes there are no veterinarians in a district. However, one can find the "Casas Veterinárias" with an enormous panacea of commercial drugs. Nevertheless, the careful use of anthelmintics, in easily understood, well presented, and properly serviced control programs, should at least help to delay selection for resistance and so extend the effective field life of these drugs (WALLER, 1987). Treatment frequency then, has to be reduced to only those treatments that are important to reduce morbidity, mortality and production losses. Otherwise, there will be an increase in selection for homozygous resistance alleles.

The control of domestic animal parasites is economically advantageous for livestock entrepreneurs considering the cost involved and the benefits for productivity and agro-business sales. Another no less-important consideration are subclinical infections, which are often disregarded and can cause significant losses in productivity. Using a cost-benefit computer model, MCLEOD (1995) estimated that sheep roundworm infection could cost over 222 million dollars / annum to the Australian grazing industry.

More and more we see the necessity for parasite control recommendations to be based on the concept of an integrated management system where all the variables are taken into account. The development of such strategic methods will depend on an assessment of each situation and transfer to producers with the objectives of improving farm benefits and controlling the development of anthelmintic resistance.

SUMÁRIO

Embora acredite-se que o uso de drogas anti-helmínticas seja o método mais eficaz no controle de infecções parasitárias, a resistência contra todos os grupos de drogas já é uma realidade mundial. Este trabalho visa detalhar os métodos que estão sendo utilizados no controle de parasitas nematodas e suas implicações no que se refere ao aparecimento da resistência às drogas anti-parasitárias. Novos métodos de controle parasitário são apresentados, visando auxiliar clínicos veterinários, zootecnistas, extensionistas, estudantes e pesquisadores. Para se evitar o aparecimento da resistência às drogas anti-helmínticas o ideal é que seja utilisado um manejo integrado de mais de uma estratégia de controle. Então, se o objetivo é montar um programa de controle antiparasitário para eliminar as perdas causadas por animais infectados e controlar a resistência às drogas, vários fatores devem ser avaliados.

PALAVRAS-CHAVE: resistência à anti-helmínticos, controle de nematóides.

REFERENCES

BARGER, I.A. (1996). Prospects for integration of novel parasite control options into grazing systems. International Journal for Parasitology, *26*:1001-1007.

BARNES, E.H. & DOBSON, R.J. (1990). Population dynamics of *Trichostrongylus colubriformis* in sheep: Computer model to simulate grazing systems and the evaluation of anthelmintic resistance. International Journal for Parasitology, *20*:823-831.

BEECH, R.N.; PRICHARD, R.K. & SCOTT, M.E. (1994). Genetic variability of the b-tubulin genes in benzimidazole-susceptible and -resistant strains of *Haemonchus contortus*. Genetics, *138*:103-110.

BEH, K., J. & Maddox, J., F. (1996). Prospects for development of genetic markers for resistance to gastrointestinal parasite infection in sheep. International Journal of Parasitology, *26*:879-896.

BEUGNET, F.; GAUTHEY, M. & KERBOEUF, D. (1997). Partial *in vitro* reversal of benzimidazole resistance by the free-living stages of Haemonchus contortus with verapamil. Veterinary Record, *141*:575-576.

BEVERIDGE, I.; ELLIS, N.J.S.; RILEY, M. J. & BROWN, T.H. (1990). Prevalence of resistance in sheep nematode populations to benzimidazole and levamisole anthelmintics in the high rainfall areas of South Australia. *Australian Veterinary Journal*, *67*:413-415.

BJORG, H.; ROEPSTORFF, A.; WALLER, P.J. & NANSEN, P. (1990). Resistance to levamisole and cross resistance between pyrantel and levamisole in *Oesophagostomum quadrispinulatum* and *Oesophagostomum dentatum* of pigs. *Veterinary Parasitology*, *37*:21-30.

BLACKHALL, W.J.; POULIOT, J-F.; PRICHARD, R.K. & BEECH, R.N. (1998). *Haemonchus contortus*: selection at a glutamate-gated chloride channel gene in ivermectin- and moxidectin-selected strains. *Experimental Parasitology*, *90*:42-48.

COOMBS, G.H. & CROFT, S.L. (1997). Molecular basis of drug design and resistance. *Parasitology*, *114*:1-2.

CHARLES, T.P.; POMPEU, J. & MIRANDA, D.B. (1989). Efficacy of three broad-spectrum anthelmintics against gastrointestinal nematode infections of goats. *Veterinary Parasitology*, *34*:71-75.

COLES, G.C. (1988). Strategies for control of anthelmintic resistant nematodes in ruminants. *Journal of American Veterinary Medicine Association*, *192*:867-870.

COLES, G.C.; BAUER, C.; BORGSTEEDE, F.H.M.; GEERTS, S.; KLEI, T.R.; TAYLOR, M.A. & Waller, P.J. (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. *Veterinary Parasitology*, *44*:35-44.

COLES, G.C.; WARNER, A.K. & BEST, J.R. (1996). Triple resistant *Ostertagia* from angora goats. *The Veterinary Record, 21*:299-300.

CONDER, G.A.; JOHNSON, S.S.; GUIMOND, P.M.; COX, D.L. & LEE, B.L. (1991). Concurrent infections with the ruminant nematodes *Haemonchus contortus* and *Trichostrongylus colubriformis* in jirds, *Meriones unguiculatus*, and use of this model for anthelmintic studies. *Journal of Parasitology, 77*:621-623.

CONDER, G.A. & CAMPBELL, W.C. (1995). Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. *Advances in Parasitology, 35*:1-84.

CRAIG, T.M. (1993). Anthelmintic resistance. *Veterinary Parasitology, 46*:121-131.

CROFT, S.L. (1997). The current status of antiparasite chemotherapy. *Parasitology, 114*:3-15.

DANGOLLA, A.; BJORN, H.; WILLEBERG, P.; ROEPSTORFF, A. & NANSEN, P. (1996). A questionnaire investigation on factors of importance for the development of anthelmintic resistance of nematodes in sow herds in Denmark. *Veterinary Parasitology, 63*:257-271.

D'ASSONVILLE, J.A.; JANOVSKY, E. & VERSLEY, A. (1996). *In vitro* screening of *Haemonchus contortus* third stage larvae for ivermectin resistance. *Veterinary Parasitology, 61*:73-80.

DOBSON, R.J.; LE JAMBRE, L. & GILL, J.H. (1996). Management of anthelmintic resistance: Inheritance of resistance and selection with persistent drugs. *International Journal for Parasitology, 26*:993-1000.

DUNCAN, J.L. & LOVE, S. (1991). Preliminary observations on an alternative strategy for the control of horse strongyles. *Equine Veterinary Journal, 23*:226-228.

EAGLESON, J.S. & BOWIE, J.Y. (1986). Oxfendazole resistance in *Trichostrongylus axei* in cattle in Australia. *Veterinary Record, 119*:604.

ECHEVARRIA, F.A.M. & TRINDADE, G.N.P. (1989). Anthelmintic resistance by *Haemonchus contortus* to ivermectin in Brazil: A preliminary report. *Veterinary Record, 124*:147-148.

ECHEVARRIA, F.A.M.; ARMOUR, J.; DUNCAN, J.L. & PINHEIRO, A.C. (1993). Use of reseeded pastures as an aid in the control of gastrointestinal nematodes. *Veterinary Parasitology, 50*:151-155.

EDWARDS, J.R.; WROTH, R.; DE CHANEET, G.C.; BESIER, R.B.; KARLSSON, J.; MORCOMBE, P.W.; DALTON-MORGAN, G. & ROBERTS, D. (1986). Survey of anthelmintic resistance in Western Australia sheep flocks. 2. Relationship with sheep management and parasite control practices. *Australian Veterinary Journal, 63*:139-144.

ELARD, L.; CABARET, J. & HUMBERT, J.F. (1999). PCR diagnostic of benzimidazole-susceptibility or -resistance in natural populations of the small ruminant parasite, *Teladorsagia circumcincta*. *Veterinary Parasitology, 80*:231-237.

EMERY, D.L. (1996). Vaccination against worm parasites in animals. *Veterinary Parasitology, 64*:31-45.

EYSKER, M.; JANSEN, J. & MIRCK, M.H. (1986). Control of strongylosis in horses by alternate grazing of horses and sheep and some other aspects of the epidemiology of Strongylidae infections. *Veterinary Parasitology, 19*:103-115.

FOLZ, S.D.; PAX, R.A.; THOMAS, E.M.; BENNETT, J.L. & CONDER, G.A. (1987). Detecting *in vitro* anthelmintic effects with a micromotility meter. *Veterinary Parasitology, 24*:241-250.

FORD, J.M. (1995). Modulators of multidrug resistance-preclinical studies. *In*: Haematology/ Oncology clinics of North America. Fisher, G.A.; & Sikic, B.I. editors. W.B. Saunders.

GATONGI, P.M.; PRICHARD, R.K.; RANJAN, S.; GATHUMA, J.M.; MUNYUA, W.K.; CHERUIYOT, H. & SCOTT, M.E. (1998). Hypobiosis of *Haemonchus contortus* in natural infections of sheep and goats in a semi-arid area of Kenya. *Veterinary Parasitology, 77*:49-61.

GILL, J.H.; KERR, C.A.; SHOOP, W.L. & LACEY, E. (1998). Evidence of multiple mechanisms of avermectin resistance in *Haemonchus contortus* - comparison of selection protocols. *International Journal for Parasitology, 28*:783-789.

GRONVOLD, J.; HENRIKSEN, S.A.; LARSEN, M.; NANSEN, P. & WOLSTRUP, J. (1996). Aspects of biological control - with special reference to arthropods, protozoans and helminths of domestic animals. *Veterinary Parasitology, 64*:47-64.

GUTTERIDGE, W.E. (1997). Designer drugs: pipe-dreams or realities? *Parasitology, 114*:145-151.

HASS, D.K.; HOLLOWAY, F.L. & BROWN, L.J. (1982). Comparison of ruminant anthelmintics using multiple dose administration. *American Journal of Veterinary Medicine, 43*:534-537.

HENNESSY, D.R. (1997). Physiology, pharmacology and parasitology. *International Journal of Parasitology, 27*:145-152.

HERD, R.P. (1985). Epidemiological approach to the control of horse strongyles. *Equine Veterinary Journal, 17*:202-207.

HERD, R.P. (1986). Pasture hygiene: A nonchemical approach to equine endoparasite control. *Modern Veterinary Practice, 67*:36-38.

HUBERT, J. & KERBOEUF, D. (1992). A microlarval development assay for the detection of anthelmintic resistance in sheep nematodes. *Veterinary Record, 130*:442-446.

JACKSON, R.A.; TOWNSEND, K. G.; PYKE, C. & LANCE, D.M. (1987). Isolation of oxfendazole resistant *Cooperia oncophora* in cattle. *New Zealand Veterinary Journal*, *35*:187-189.

KERBOEUF, D.; CHAMBRIER, P.; LE VERN, I. & AYCARDI, J. (1999). Flow cytometry analysis of drug transport mechanisms in Haemonchus contortus susceptible or resistant to anthelmintics. *Parasitology Research*, *85*:118-123.

KOTZE, A.C. (1998). Effects of macrocyclic lactones on ingestion in susceptible and resistant *Haemonchus contortus* larvae. *Journal of Parasitology*, *84*:631-635.

LACEY, E. & PRICHARD, R.K. (1986). Interaction of benzimidazoles (BZ) with tubulin from BZ-sensitive and BZ-resistant isolates of *Haemonchus contortus*. *Molecular and Biochemical Parasitology*, *19*:171-181.

LEATHWICK, D.M. (1995). A case of moxidectin failing to control ivermectin resistant *Ostertagia* species in goats. *Veterinary Record*, *136*:443-444.

LE JAMBRE, L.F.; SOUTHCOTT, W.H. & DASH, K.M. (1978). Development of simultaneous resistance in *Ostertagia circumcincta* to thiabendazole, morantel tartrate and levamisole. *International Journal for Parasitology*, *8*:443-447.

LE JAMBRE, L.F. (1990). Molecular biology and anthelmintic resistance in parasitic nematodes. Resistance of Parasites to Antiparasitic Drugs. Round Table Conference, TR9-2. Paris, France.

LIFSCHITZ, A.; VIRKEL, G.; MASTROMARINO, M. & LANUSSE, C., 1997. Enhanced plasma availability of the metabolites of albendazole in fasted adult sheep. *Veterinary Research Communications*, *21*:201-211.

MAJR, T.S. & CRIPPS, P.J. (1991). Benzimidazole resistance in equine strongyles: Association with clinical disease. *Veterinary Record*, *128*:613-614.

MALAN, F.S.; VAN WYK, J.A.; GERBER, H.M. & ALVES, R.M.R. (1990). First report of organophosphate resistance in a strain of *Haemonchus contortus* in the Republic of South Africa. *South Africa Journal of Science*, *86*:49-50.

MARTIN, R.J. (1997). Modes of action of anthelmintic drugs. *Veterinary Journal*, *154*:11-34.

MARTIN, R.J.; ROBERTSON, A.P. & BJORN, H. (1997). Target sites of anthelmintics. *Parasitoloygy*, *114*:111-124.

MCLEOD, R.S. (1995). Costs of major parasites to the Australian livestock industries. *International Journal for Parasitology*, *25*:1363-1367.

MEDLEY, G.F. (1994). Chemotherapy. *In*: Parasitic and Infectious Diseases. Scott, M. E., and Smith, G., (Editors). Academic Press, San Diego. 398 pp.

MILLER, T.A. (1978). Industrial development and field use of the canine hookworm vaccine. *Advances in Parasitology*, *16*:333-342.

MILLER, J.E. & BARRAS, S.R. (1994). Ivermectin resistant *Haemonchus contortus* in Louisiana lambs. *Veterinary Parasitology*, *55*:343-346.

MOLENTO, M.B. & PRICHARD, R.K. (1998). Comparative activity of MDR modulators against resistant *Haemonchus contortus*. 73rd Annual Meeting of the American Society of Parasitologists, Kona, Hawaii, USA. abstr. 56.

MOLENTO, M.B.; WANG, G.T. & PRICHARD, R.K. (1999a). Decrease ivermectin and moxidectin sensitivity in *Haemonchus contortus* selected with moxidectin over fourteen generations. *Veterinary Parasitology*, in press.

MOLENTO, M.B.; LIFSCHITZ, A.; SALLOVITZ, J.; LANUSSE, C. & PRICHARD, R. (1999b). Verapamil modifies the pharmacokinetics of ivermectin in sheep. 17th Conference of the World Association for the Advancement of Veterinary Parasitology. Copenhagen, Denmark.

MWAMACHI, D.M.; AUDHO, J.O.; THORPE, W. & BAKER, R.L. (1995). Evidence for multiple anthelmintic resistance in sheep and goats reared under the same management in coastal Kenya. *Veterinary Parasitology*, *60*:303-313.

PICHE, C.A.; KENNEDY, M.J. & Bauck, S. (1989). Benzimidazole resistance in horses in Western Canada. *Canadian Veterinary Journal*, *30*:173-174.

POWERS, K.G.; WOOD, I.B.; ECKERT, J.; GIBSON, T. & SMITH, H.J. (1982). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine and ovine). *Veterinary Parasitology*, 10: 265-284.

PRASLICKA, J.; VARADY, M.; CORBA, J. & MISKO, J. (1995). A model transmission of anthelmintic resistance by wild ruminants. *Helminthologia*, *32*:251-252.

PRICHARD, R.K.; HENNESSY, D.R. & STEEL, J.W. (1978). Prolonged administration: A new concept for increasing the spectrum and effectiveness of anthelmintics. *Veterinary Parasitology*, *4*:309-315.

PRICHARD, R.K. & HENNESSY D.R. (1981). Effect of oesophageal groove closure on the pharmacokinetic behaviour and efficacy of oxfendazole in sheep. *Research in Veterinary Science*, *30*:22-27.

PRICHARD, R.K.; HALL, C.A.; KELLY, J.D.; MARTIN, I.C. A. & DONALD, A.D. (1980). The problem of anthelmintic resistance in nematodes. *Australian Veterinary Journal*, *56*:239-251.

PRICHARD, R.K. (1990). Anthelmintic resistance in nematodes: Extent, recent understanding and future directions for control and research. *International Journal for Parasitology*, *20*:515-523.

PRICHARD, R.K. (1994). Anthelmintic resistance. *Veterinary Parasitology*, *54*:259-268.

REINEMEYER, C.R.; ROHRBACH, B.W.; GRANT, V.M. & RADDE, G., L. (1992). A survey of ovine parasite control practices in Tennessee. *Veterinary Parasitology*, *42*:111-122.

ROBERTS, J.L. & SWAN, R.A. (1981). Quantitative studies of ovine haemonchosis. I. Relationship between faecal egg counts and total worm counts. *Veterinary Parasitology*, *8*:165-171.

ROOS, M.H.; BOERSEMA, J.H.; BORGSTEEDE, F.H.M.; CORNELISSEN, J.; TAYLOR, M. & RUITENBERG, E.J. (1990). Molecular analysis of selection for benzimidazole resistance in the sheep parasite *Haemonchus contortus*. *Molecular and Biochemical Parasitology*, *43*:77-88.

ROSS, M.H. (1997). The role of drugs in the control of parasite nematode infections: must we do without? *Parasitology*, *114*:137-144.

ROTHWELL, J.T. & SANGSTER, N.C. (1993). An *in vitro* assay utilizing parasitic larval *Haemonchus contortus* to detect resistance to closantel and other anthelmintics. *International Journal for Parasitology*, *23*:573-578.

SANCHEZ, S.F.; ALVAREZ, L.I. & LANUSSE, C.E., 1996. Nutritional conditions affecs the disposition kinetics of albendazole in cattle. *Xenobiotica*, *26*:307-320.

SANGSTER, N.C.; DAVIS, C.W. & COLLINS, G.H. (1991). Effects of cholinergic drugs on longitudinal contraction in levamisole-susceptible and -resistant *Haemonchus contortus*. *International Journal for Parasitology*, *21*:689-695.

SCHINKEL, A.H.; SMIT, J.J.M.; VAN TELLINGEN, O.; BEIJNEN, J.H.; WAGENAAR, E.; VAN DEEMTER, L.; MOL, C.A.A.M.; VAN DER VALK, M.A.; ROBANUS-MAANDAG, E.C.; TE RIELE, H.P.J.; BERNS, A.J.M. & BORST, P. (1994). Disruption of the mouse *mdr-1a* P-glycoprotein gene leads to a deficiency in the blood-brain barrier and to increased sensitivity to drugs. *Cell*, *77*:491-502.

SCOTT, E.W.; BAXTER, P. & ARMOUR, J. (1991). Fecundity of anthelmintic resistant adult *Haemonchus contortus* after exposure to ivermectin or benzimidazoles *in vivo*. *Research in Veterinary Science*, *50*:247-249.

SHARMA, R.L.; BHAT, T.K. & DHAR, D.N. (1981). Control of sheep lungworm in India. *Parasitology Today*, *4*:33-36.

SHOOP, W.L.; MROZIK, H. & FISHER, M.H. (1995). Structure and activity of avermectins and milbemycins in animal health. *Veterinary Parasitology*, *59*:139-156.

SIVARAJ, S.; DORNY, P.; VERCRUYSSE, J. & PANDEY, V. S. (1993). Multiple drug resistance of *Haemonchus contortus* of sheep in Malaysia and the efficacy of moxidectin. 14th International Conference of the W.A.A.V.P., Cambridge, U.K., p 64.

SMITH, G. (1990). A mathematical model for the evaluation of anthelmintic resistance in a direct life cycle nematode parasite. *International Journal for Parasitology*, *20*:913-921.

SOCCOL, V.T.; SOTOMAIOR, C.; SOUZA, F.P.; CASTRO, E.A.; PESSÔA SILVA, M.C. & MILCZEWSKI. Occurence of resistance to anthelmintics in sheep in Paraná state, Brazil. *The Veterinary Record*, *139*:421-422.

TAYLOR, M.A. (1990). A larval development test for the detection of anthelmintic resistance in nematodes of sheep. *Research in Veterinary Science*, *49*:198-202.

TSURUO, T.; IIDA, H.; TSUKAGOSHI, S. & SAKURAI, Y. (1981). Overcoming of vincristine resistance in P388 leukemia *in vivo* and *in vitro* through enhanced cytotoxicity of vincristine and vinblastine by verapamil. *Cancer Research*, *41*:1967-1972.

URQUHART, G.M.; (1985). Field experience with the bovine lungworm vaccine. *Developmental Biology Stand.*, *62*:109-112.

VAN WYK, J.A. & MALAN, F.S. (1988). Resistance of field strains of *Haemonchus contortus* to ivermectin, closantel, rafoxanide and the benzimidazoles in South Africa. *Veterinary Record*, *123*:226-228.

VAN WYK, J.A.; MALAN, F.S. & BATH, G.F. (1997a). Rampant anthelmintic resistance in sheep in South Africa - What are the options? 16th International Conference of the W.A.A.V.P., Workshop on managing anthelmintic resistance in endoparasites. Sun City, South Africa, 51-63.

VAN WYK, J.A.; MALAN, F.S. & RANDLES, J.L. (1997b). How long before resistance makes it impossible to control some field strains of *Haemonchus contortus* in South Africa with any of the modern anthelmintics? *Veterinary Parasitology*, *70*:111-122.

VAN WYK, J., A. & VAN SCHALKWYK, P.C. (1990). A novel approach to the control of anthelmintic-resistance *Haemonchus contortus* in sheep. *Veterinary Parasitology*, *35*:61-69.

VERMUNT, J.J.; WEST, D.M. & POMROY, W.E. (1995). Multiple resistance to ivermectin and oxfendazole in *Cooperia* species of cattle in New Zealand. *Veterinary Record*, *137*:43-45.

VIEIRA, L.S.; BERNE, M.E.A.; CAVALCANTE, A.C.R. & COSTA, C.A.F. (1992). *Haemonchus contortus* resistance to ivermectin and netobimin in Brazilian sheep. *Veterinary Parasitology*, *45*: 111-116.

WALLER, P.J. (1987). Anthelmintic resistance and the future for roundworm control. *Veterinary Parasitology*, *25*:177-191.

WALLER, P.J.; DONALD, A.D.; DOBSON, R.J.; LACEY, E.; HENNESSY, D.R.; ALLERTON, G.R. & PRICHARD, R.K. (1989). Changes in anthelmintic resistance status of *Haemonchus contortus* exposed to different anthelmintic selection pressures in grazing sheep. *International Journal for Parasitology*, *19*:99-110.

WALLER, P.J.; ECHEVARRIA, F.A.M.; EDDI, C.; MACIEL, S. & NARI, A. (1993). Anthelmintic resistance in Southern Latin America: a potential time bomb? 14th International Conference of the W.A.A.V.P., Cambridge, U.K., p 68.

WALLER, P.J.; ECHEVARRIA, F.; EDDI C.; MACIEL, S.; NARI, A. & HANSEN, J., W. (1996). The prevalence of anthelmintic resistance in nematode parasites of sheep in southern Latin America: General overview. *Veterinary Parasitology*, *62*:181-187.

WARUIRU, R.M.; NGOTHO, J.W. & MUKIRI, J.G. (1997). Multiple anthelmintic resistance in *Haemonchus contortus* on a sheep farm in Kenya. *Veterinary Research Communications*, *21*:483-491.

WEBB, R.F. (1980). Epidemiological factors contributing to a high incidence of anthelmintic resistance in field populations in *Haemonchus contortus*. In: Geering, W. A., Roe, R. T., and Chapman, L. A. (editors), Proceedings 2nd International Symposium on Veterinary Epidemiology and Economics, Canberra, Australia Government Publishing, Canberra, 220-224.

WOOD, I.B.; AMARAL, N.K.; BAIRDEN, K.; DUNCAN, J.L.; KASSAI, T.; MALONE Jr., J.B.; PANKAVICH, J.A.; REINECKE, R.K.; SLOCOMBE, O.; TAYLOR, S.M. & VERCRUYSSE, J. (1995). World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) Second edition of guidelines for evaluating the efficacy of anthelmintic in ruminants (bovine, ovine, caprine). *Veterinary Parasitology*, *58*:181-213.

WOOLASTON, R.R.; BARGER, I.A. & PIPER, L.R. (1990). Response to helminth infection of sheep selected for resistance to *Haemonchus contortus*. *International Journal for Parasitology*, *20*:1015-1018.

XU, M.; MOLENTO, M.; BLACKHALL, W.; RIBEIRO, P.; BEECH, R. & PRICHARD, R. (1998). Ivermectin resistance in nematodes may be caused by alteration of P-glycoprotein homolog. *Molecular and Biochemical Parasitology*, *91*:327-335.

YADAV, C.L.; KUMAR, R.; UPPAL, R.P. & VERMA, S.P. (1995). Multiple anthelmintic resistance in *Haemonchus contortus* on a sheep farm in India. *Veterinary Parasitology*, *60*:355-360.

YUSA, K. & TSURO, T. (1989). Reversal mechanism of multidrug resistance by verapamil: Direct binding of verapamil to P-glycoprotein on specific sites and transport of verapamil outward across the plasma membrane of K562/ADM cells. *Cancer Research*, *49*:5002-5006.

(Received 27 January 1999, Accepted 10 May 1999)