



Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance

J.A. VAN WYK

Department of Veterinary Tropical Diseases, Faculty of Veterinary Science, University of Pretoria Private Bag X04, Onderstepoort, 0110 South Africa

ABSTRACT

VAN WYK J.A. 2001. Refugia—overlooked as perhaps the most potent factor concerning the development of anthelmintic resistance. *Onderstepoort Journal of Veterinary Research*, 68:55–67

Anthelmintic resistance involving particularly the gastrointestinal nematodes of small ruminants is escalating globally, to the extent that in certain countries, such as South Africa, it has already reached alarming proportions, and is affecting practically all the anthelmintics.

In this paper it is argued that the high levels of resistance in nematodes of veterinary importance indicate that the drugs have been used incorrectly. It is suggested that the phenomenon of refugia plays a much more important role in the selection of anthelmintic resistance than other phenomena that are more frequently investigated and recommended for counteracting it, such as reduced drenching frequency and avoiding under-dosing. While refugia is commonly mentioned in passing in most papers on anthelmintic resistance, it is, almost without exception, not incorporated in the final control/management proposals.

On the strength of the conclusions arrived at in the present paper strategies such as the drench-and-move system in which all the animals in a flock are drenched before they are moved to pastures containing few or no worms in refugia, and the system of strategic drenching on safe pastures should be condemned and never recommended. If such strategies are indeed unavoidable, the farmer should be warned that the farming system would probably not be sustainable even in the short term, in view of the generally high levels of resistance already present in most of the important sheep-producing regions. Farmers should be educated to consider refugia above all else when designing worm management programmes.

Finally there seems to be too much complacency concerning the possibility that anthelmintic resistance may also escalate in cattle, eventually to reach the proportions that it has in sheep.

Keywords: Anthelmintic resistance, cattle, gastrointestinal nematodes, refugia, sheep

INTRODUCTION

In 1985, after anthelmintic resistance in sheep nematodes had started to escalate in South Africa, Van Wyk said: "... unless we use [the anthelmintics] to better advantage we may find ourselves in the situ-

ation where we no longer have effective remedies available for worm control." At the time, some helminthologists regarded the statement as alarmistic (Van Wyk 1990a). Nevertheless, the phenomenon has progressed globally to reach "... alarming proportions throughout the world and [to threaten] the future viability of continued small ruminant production in many countries" (Waller 1999).

In recent surveys in South Africa, in which a lenient method was used to analyze the results of faecal egg

¹ E-mail: janvw@moon.o.vi.ac.za

Accepted for publication 9 January 2001—Editor

count reduction tests in order to give the drugs the best chance of showing efficacy, it was determined that strains of *Haemonchus contortus* were resistant to at least one drug on 99% of 80 farms (Van Wyk, Stenson, Van der Merwe, Vorster & Viljoen 1999). On 8% of 26 farms in one district all four compounds (rafoxanide, albendazole, ivermectin and levamisole) representing the anthelmintic groups available for drenching against nematodes of small ruminants in the country, were less than 40% effective against strains of *Haemonchus* encountered (Van Wyk *et al.* 1999). In another study Van Wyk, Malan & Randles (1997b) recorded what was apparently the first case of resistance in a strain of *H. contortus* to drugs from five different action groups, namely the above four, plus trichlorphon. Reduction on the arithmetic mean worm burdens indicated < 79% susceptibility and the geometric mean < 91.7% to 8 of 9 compounds tested in a slaughtering trial at their recommended therapeutic dosage rates, namely albendazole 3.8 mg kg⁻¹, closantel 5 mg kg⁻¹, dinitrophenol 10 mg kg⁻¹, ivermectin 0.2, levamisole 7.5, morantel 14.5, nitroxy-nil 10, rafoxanide 7.5 and trichlorphon 33 mg kg⁻¹. Only closantel was highly effective.

The state of anthelmintic resistance in the veterinary field should be seriously considered in the light of evidence that resistance may also be developing in the helminths of humans (Anonymous 1998). Of particular importance is a move to treat entire human populations in an attempt to reduce the prevalence and severity of their helminth infections (Geerts & Gryseels 2000).

The global rate of development and extent of anthelmintic resistance in helminths of sheep and goats indicates that the numerous anthelmintics and strategies developed over the course of the previous century have been incorrectly applied.

How did this crisis situation come about?

Have anthelmintics been used incorrectly because farmers did not listen to the experts, or was the advice of the experts wrong, or is it, perhaps, a bit of each? Alternatively, was resistance inevitable? In 1990a Van Wyk asked whether "... even the experienced helminthologist had the answer for preventing ... resistance." Subsequent results of various surveys worldwide indicate that we do not have it.

Today I believe that much of the blame for the global escalation of anthelmintic resistance can be ascribed to the advice given by us, the helminthologists. For instance, in some worm control programmes, principles such as to drench and move to "safe" or "clean" pasture are prescribed (Van Wyk 1990b; 1990c). Furthermore, I contend that while many faults in the conventional advice given to the stockman have been recognized, we have failed to condemn such practices, or place sufficient emphasis on alternatives. Few, if any, have heeded the words of

Barger (1982): "While economists can advise ... farmers on the extent to which investments in worm control are profitable, there seem to be few clear indications from parasitologists of what the ultimate biological aims of control should be. Farmers are motivated to travel along the profitable road to worm control, and economists can determine how far they should travel, but the parasitologists seem unwilling to point out the most desirable route". On the other hand, the farmer has without doubt also helped to dig his own grave. For instance, on the farm on which one of the first field cases of resistance of *H. contortus* to ivermectin was recorded, the sheep had been drenched a mean of every 23 days over the preceding 5 months (J.A. van Wyk, personal observations 1986; Van Wyk & Malan 1988; Van Wyk, Malan, Gerber & Alves 1989), thus giving susceptible worms almost no chance to reproduce. In addition, rams and even whole flocks of sheep infected with highly resistant worms are sold at auctions, thus widely disseminating resistant worms (Van Wyk 1990a; Van Wyk, Van Schalkwyk, Bath, Gerber & Alves 1991).

Fortunately, progress is being made with various forms of integrated worm management using anthelmintics plus alternative methods of control, as reviewed by Waller (1997) and Van Wyk, Malan & Bath (1997a). Anthelmintics remain the cornerstone of worm control/management, but need to be supported by methods such as drenching only a proportion of the flock to reduce selection for resistance (Besier 1997a), or by breeding resistant/resilient animals (Morris & Bisset 1997). Hopefully practical problems concerning vaccines and management via nematophagous fungi will be solved so that these will become available for use in the field (Smith 1997; Waller 1997).

Fact and conjecture

In my opinion a common error of us parasitologists has been to elevate conjecture to fact, as a result of "... a tendency to seek general rules for action [that unfortunately results in] probabilistic statements [being transformed] into certainties" (Smith, Grenfell, Isham & Cornell 1999). Furthermore, it all too often happens that "facts", derived from unreplicated trials with laboratory-manipulated strains of parasites in relatively small numbers of animals, are used for far-reaching recommendations to the farmer on worm control. Sometimes this occurs because the technical difficulty and expense involved in field-testing of factors that can affect selection for resistance makes it impractical to replicate the work; in such cases the tentative nature of the results should be stressed.

Mathematical models

Some extrapolations and deductions from simulation studies using mathematical models also appear to

be elevated to the status of fact, without being backed by sufficient experimental evidence. One example is the statement that: "... highly effective anthelmintics, or combinations of anthelmintics, because they leave very few survivors, select less strongly for resistance than less effective drugs that leave many ..." (Barger 1996). In the first place, this statement apparently has its origin in genetic theory and computer simulation, without having been confirmed experimentally; if so, this fact should have been mentioned. Secondly, but equally important, it is unclear what is meant by "highly effective". If in accordance with the present recommendations of the World Association for the Advancement of Veterinary Parasitology (WAAVP) (Wood, Amaral, Bairden, Duncan, Kassai, Malone, Pankavich, Reinecke, Slocombe, Taylor & Vercruysse 1995), it means an efficacy of as low as 98%, a mean 100 of 5000 *H. contortus* will remain per sheep after treatment. If 50 of these worms are females, each adult sheep may void up to half a million eggs daily in its faeces (Gordon 1981), which probably gives the worms a good chance to become established on "clean" grazing. At this level of efficacy practically only homozygous resistant individuals are likely to survive the drench, compared to both homo- and heterozygous individuals after lower dosage rates have been administered, as graphically illustrated by Barger (1995a), and the homozygous worms will definitely be more difficult to kill.

Several workers have warned that mathematical models are subject to some wide-ranging assumptions concerning the dynamics of the infection process and its consequences, such as that each host is infected with the same number of worms, while in practice rank overdispersion is the rule rather than the exception (Barger 1985); that each larval genotype is ingested in simple proportion by each animal; and that in each infection there are sufficient male worms, so that every female is fertilized (Smith *et al.* 1999). Echevarria, Gettinby & Hazelwood (1993) add that the simulations do not allow for the crucial role of refugia in farming management practices, such as the drench-and-move strategies which constitute probably the most severe method of selection (see below). Lastly, Le Jambre, Dobson, Lenane & Barnes (1999) point out that post-treatment selection by anthelmintics with a persistent effect is not considered in simulations, while "... the resistant genotypes enjoy a considerable period of advantage." Echevarria *et al.* (1993), in agreement with Conder & Campbell (1995), conclude that: "The findings are based on genetic parameters, which remain theoretical estimates, ... as yet unsubstantiated by field or laboratory studies."

In another example it is concluded, with reference to a paper based on deductions from computer simulations by Barnes, Dobson & Barger (1995), that "The

principal cause of rapid selection for anthelmintic resistance remains treatment frequency" (Dobson, Le Jambre & Gill 1996). However, what should have been done was to compare drenching frequency with the effect of low levels of worms in refugia on selection for resistance, which, as discussed below, is much more likely to be the overriding factor in most instances.

Possible causes of selection for anthelmintic resistance

Factors such as under-dosing and the frequent use of anthelmintics have been blamed as being mainly responsible for the selection of anthelmintic resistance that has escalated to the extent that some strains have become practically uncontrollable (Waller 1985; Van Wyk *et al.* 1997b; 1999).

Refugia

The term "refugia" was coined to define the proportion of the parasite population that is not exposed to a particular given control measure, thus escaping selection for resistance. In the case of the gastrointestinal nematodes this comprises primarily the portion of a worm population that is free-living on pasture. After an "effective" drench the progeny of the worms that survive the treatment develop among the free-living worms in refugia. Thus the size of the population in refugia has a direct bearing on the degree of selection for resistance with a particular remedy as Martin, Le Jambre & Claxton (1981) convincingly showed two decades ago.

In the host's body the parasitic stages of some worm species, such as the histiotrophic larvae of the Cyathostominae in the intestinal mucosae of equids, also escape the effect of a drug. When used conventionally in a single dose, benzimidazoles do not kill these larvae, but recently Duncan, Bairden & Abbott (1998) reported that they can be killed by five daily treatments with fenbendazole at 7.5 mg kg⁻¹ per day. However, in my opinion, this drenching schedule should be used only when essential, as these histiotrophic larvae are in effect in refugia, and thus help to reduce selection for resistance by benefitting the susceptible portion of the worm population, by voiding "susceptible" worm eggs onto pasture when they mature.

As can be deduced from numerous papers (Martin *et al.* 1981; Michel 1985; Martin 1989; Swan, Gardner, Besier & Wroth 1994; Leathwick, Vlassoff & Barlow 1995; Anonymous 1998; Lloyd & Soulsby 1998; Barger 1999; Smith *et al.* 1999; Lloyd, Smith, Connan, Hatcher, Hedges, Humphrey & Jones 2000), refugia is generally regarded as an important factor in selecting for resistance through drenching with anthelmintics. Nevertheless, while it is often mentioned

in papers on worm control as a factor affecting selection for anthelmintic resistance, it is almost always overlooked when the recommendations for reducing selection for resistance are finally listed. A good example is a group of persons who recently reviewed the evidence on resistance in helminths affecting humans (Anonymous 1998). Because there is little pertinent information in the medical field, and apparently because the group were not directly involved in anthelmintic resistance in practice, they based their review principally on information and possible solutions gleaned from the veterinary literature. Thus their recommendations on policies for preventing resistance can be expected to reflect those of the experts in the field of veterinary parasitology. While they mention that selection is greater in sheep drenched on clean than on contaminated pasture, in other words that refugia plays a role, this is not listed under prevention, but only the following: to use the full therapeutic dosage rate; to screen worm populations for sensitivity; to alternate anthelmintic groups annually; to dose less frequently; to de-worm all new livestock, and to use narrow-spectrum drugs where appropriate. In contrast, as long ago as 1989 Martin stressed, when referring to Donald (1983), that selection for resistance depends largely on the “... success of the resistant progeny in contributing to subsequent generations.”

In the light of the results of Martin *et al.* (1981) and of the following words by the perspicacious Michel (1985) it is incomprehensible that refugia is still not accorded its rightful place in recommendations on worm control: “Selection pressures may be measured as the proportion of each generation that is the progeny of worms that have survived exposure to anthelmintic. ... If the treatment is given at a time when, or in circumstances in which all the worms on the farm are in the treated hosts, selection will be rigorous.” He goes so far as to class the recommendations to reduce selection for resistance by reducing drenching *per se* or by alternating anthelmintic groups, as “curious ideas”.

Under-dosing

That under-dosing, i.e. use of less than the therapeutic dosage level recommended by the manufacturers, will quickly select for resistance, is one of the most common assertions made concerning prevention of anthelmintic resistance (Besier & Hopkins 1988; Dorchies, Nicolas, Gevrey & Mage 1990; Prichard 1990; Dorchies 1991; Scott, Duncan, McKellar, Coop, Jackson & Mitchell 1991; Hazelby, Probert & Rowlands 1994; Hennessee 1994; Kieran 1994; Conder & Campbell 1995; Waller, Dash, Barger, Le Jambre & Plant 1995; Besier 1997b; Anonymous 1998; Chartier, Pors, Hubert, Rocheteau, Benoit & Bernard 1998; Geerts & Gryseels 2000).

However, experimental evidence is lacking.

Trials of Martin (1989) are frequently referred to as experimental confirmation that under-dosing is important for selecting for resistance, e.g.: “Martin (1989) has shown that low efficiency anthelmintic treatment can rapidly select for anthelmintic resistance in *Ostertagia* spp.” (Prichard 1990). But this is a misinterpretation. Martin (1989) actually said (my emphasis): “Selection for thiabendazole resistance was clearly demonstrated under field conditions *incorporating an anthelmintic treatment and move to worm-free pasture*. This increase resulted from the surviving sub-population selected from the upper extreme of the distribution of drug tolerances. The rapid development of resistance in this situation is, therefore, a direct result of using anthelmintics at a dose rate *less than 100 % efficient*.”

In the first place Martin (1989) concluded that low numbers of worms in refugia were of prime importance, as the sheep were moved to “worm-free” pastures after having been drenched. Secondly, in no way can it be deduced from his results or his statements that under-dosing in the conventional sense, i.e. drenching less than the therapeutic doses recommended by the manufacturers and regulating bodies, was the cause of the selection for resistance. It is probable, as suggested in theory by Barger (1995a) and also by Prichard (1990), that every compound that is less than 100 % effective will select for resistance and that the intensity of selection will increase in positive relationship to the percentage of control achieved with the anthelmintic treatment, practically until the 100 % level is reached. But despite a succession of very highly effective anthelmintics, there has not been a single one that has been truly 100 % effective. This is confirmed by the resistance that has been shown to practically every one of the modern nematocides (Shoop 1992; Shoop, Hines, Michael & Eary 1993; Leathwick 1995; Rolfe & Fitzgibbon 1996; Vermunt, West & Pomroy 1996; Watson, Hosking, Leathwick & McKee 1996; Sutherland, Leathwick, Brown & Miller 1997; Van Wyk *et al.* 1997b). In other words, with the possible exception of only one or two localized populations (Le Jambre 1981) no dosage regime using any of the available anthelmintics, whether alone or in combination, will not select for resistance. And the prime factor determining the degree of selection is probably the proportion of worms in refugia, and not the dosage rate, which is one of the secondary factors.

Another consideration is what does the term, “therapeutic dosage rate”, entail? It is the rate decided on by the manufacturers, largely based on subjective economic considerations and “high efficacy” against dose-limiting parasites or parasitic stages (Conder & Campbell 1995). However, even the concept of “high efficacy” has changed from formerly being generally applied to anthelmintics with more than 80 % parasitocidal effect, to the present recommendations

of $\geq 98\%$ kill for “highly effective” and 80% for “effective”, as outlined in the present WAAVP guidelines aimed at international standardization of anthelmintic testing for ruminants. Wood *et al.* (1995) recommend that drugs with less than 80% efficacy should not be registered. Thus, for the sake of further discussion below, “under-dosing” is regarded as all levels of drenching less than the dosage rate required to kill 80% of a worm strain not previously exposed to the drug concerned.

Workers have suggested previously that under-dosing will cause both hetero- and homozygous resistant worms to survive drenching and that these will then contribute a disproportionately large component to the subsequent worm generation (Prichard 1990; Conder & Campbell 1995). Prichard (1990) further suggests that if dosage rates are high enough to kill the heterozygous resistant worms, resistance genes may be rendered effectively recessive. While Conder & Campbell (1995) concur with this statement, they add, referring to Roush & McKenzie (1987), that subtherapeutic doses make the resistance genes effectively dominant. In contrast, Smith *et al.* (1999) are of the opinion that it is not an all-or-nothing situation: “... as one reduces dosage levels, there is a range of dose levels where under-dosing promotes resistance and a range of dose levels where under-dosing impedes resistance.” As there is no great contrast in fitness between susceptible and resistant worms, it seems logical that it will be more deleterious if only homozygous and hence no heterozygous resistant or susceptible individuals survive drenching, these will then produce the entire ensuing generation of worms, as is likely to happen when the hosts are moved to worm-free pasture shortly after drenching at highly effective dosage rates. In the case of *H. contortus*, even a relatively small number of survivors can repopulate clean pastures, because of the exceptional egg-producing capacity of the females. Hence, if the reasoning above is correct, it is not under-dosing in the conventional sense, but rather killing all but the most resistant individuals, combined with low numbers of worms in refugia that is the essence of the problem. In contrast, Le Jambre (1978a) contends: “Even if complete mortality is not achieved, the population may be depleted enough to reduce the amount of genetic variability, and ... this will make response to selection less effective.” In the case of helminths this still seems to be conjecture, and the question is at what level the killing rate will be high enough to put the remaining worm population at a “disabling” disadvantage.

The results of Hoekstra, Borgsteede, Boersema & Roos (1997) support the suggestion of a disabling disadvantage, as a strain of *H. contortus* sequentially selected by dosing infected animals with levamisole until the effective dosage level had reached 30 mg kg^{-1} , was less viable than the initial population. On the other hand, most of the modern anthelmintics are

close to being completely effective when used for the first time against worm strains that had previously not been exposed to the drug, and yet resistance has developed to frightening levels (Waller 1999), with numerous instances where drenching had no apparent effect on reducing the numbers of worms in infected animals (Van Wyk *et al.* 1997b; 1999). Furthermore, reversion to susceptibility has been so slow as to be of little practical value (Scott, Robbins, Jackson, Jackson & Clarkson 1995; Borgsteede & Duyn 1989). Thus highly resistant strains seem well equipped to maintain themselves in the field, with the apparent single exception of a resistant strain of *Ostertagia* spp. which was eradicated by intensive drenching (Le Jambre 1981). Unfortunately, even moxidectin, one of the most effective anthelmintics yet against the most common gastrointestinal nematode species of small ruminants, has not escaped unscathed (Leathwick 1995; Rolfe & Fitzgibbon 1996; Vermunt *et al.* 1996; Watson *et al.* 1996; Sutherland *et al.* 1997; F.S. Malan, personal communication 2000). An important consideration is that in a trial such as that of Le Jambre *et al.* (1999), consisting of laboratory selection of an initially susceptible worm strain (Hoekstra *et al.* 1997), there are very few resistant individuals at the start. Therefore the resultant resistant population selected from this strain will have much less genetic variation than would be expected with selection from the same strain in flocks of sheep under field conditions, and their viability will not necessarily be similar.

There is a general perception that selection for anthelmintic resistance is more severe when goats are drenched as opposed to sheep (Watson *et al.* 1996) and this often seems to be regarded as support for the viewpoint that under-drenching is an important cause of enhanced selection for resistance. The reasoning behind this assumption is that, while goats require higher dosage rates than sheep for similar blood level profiles with associated higher minimal effective dosage levels, the drugs are mostly administered at the same rate for both host species (Conder & Campbell 1995). Thus goats are in effect usually under-drenched. The question is whether there are not perhaps other possible explanations than that of under-drenching for the common occurrence of resistance in worm strains infecting goats, for instance more intensive drenching of goats (Kettle, Vlassoff, Reid & Horton 1983; Jackson 1993), or factors related to the minimum effective dosage (MED) levels. It is theoretically possible that, because the MED level in goats is higher than in sheep (Conder & Campbell 1995), resistance will be detected more quickly at a common therapeutic dosage level, much like the difference between ivermectin and moxidectin, and to some extent between thiabendazole and the later benzimidazoles. A case in point is the marked resistance found in *Cooperia* spp. of cattle against moxidectin (Vermunt *et al.* 1996), for which *Cooperia* spp.

are a dose-limiting genus, thus having a higher MED rate than other genera, such as *Ostertagia* and *Haemonchus*. In the light of higher MED levels of moxidectin against *Ostertagia* spp., than against *H. contortus*, is it coincidence that the first cases of resistance recorded in the field against moxidectin involved *Ostertagia* spp. in New Zealand and Australia, where there is relatively little resistance in any of the worm species against ivermectin (Conder & Campbell 1995) and not *H. contortus* in South Africa, where ivermectin resistance is rife in this worm species (Van Wyk *et al.* 1999)?

Drenching frequency

As stated by Martin in 1989, "... previous reports on the development of resistance have implicated treatment frequency as the prime selection mechanism ...". Even now practically no list of recommendations is drawn up without reduction of drenching frequency being included (Van Wyk 1990b; Dorchie 1991; Hazelby *et al.* 1994; Conder & Campbell 1995; Scott *et al.* 1995; Anonymous 1998; Claxton, Zambrano, Ortiz, Delgado, Escurra & Clarkson 1998; Geerts & Gryseels 2000; Lloyd *et al.* 2000).

There is no doubt that, all else being equal, and given drenching frequencies approaching the prepatent period of a helminth, resistance will develop in relation to the frequency of drenching (Kelly, Webster, Griffin, Whitlock, Martin & Gunawan 1981; Martin, Anderson, Jarrett, Brown & Ford 1982; Barton 1983; Martin, Anderson, Lwin, Nelson & Morgan 1984). Questionnaire surveys of anthelmintic resistance, combined with testing for susceptibility do suggest a correlation between drenching intensity and anthelmintic resistance (Chartier *et al.* 1998). However, in practice it is probably subordinate particularly to refugia, and should not be viewed independently of other factors, such as the degree of control attributed to the anthelmintic: "... selection pressure does not depend on the frequency of treatment as such, but on the circumstances in which it is given" (Michel 1985). Simulation studies by Leathwick *et al.* (1995) with a mathematical model even suggest that (my emphasis) *on its own* without considering the effect of differences in proportions of a given worm population in refugia, drenching frequency may even be a poor indicator of selection pressure for resistance. Thus, while drenching frequency is certainly extremely important, it is probable that it is the combination of the proportion of a given worm strain in refugia primarily and the drenching frequency secondly, that determines the degree of selection for resistance.

Drenching aimed at taking maximum advantage of "low worm" pastures

In nature "low-worm" pastures result from, amongst other factors, seasonal cycling and nomadic move-

ments of animals where this is still possible. Starting as early as 1912 with the advent of non-herbal anthelmintics (Theiler 1912a; 1912b; 1912c), a variety of ways has been developed to preserve the *status quo* of such safe pastures for as long as possible during the course of each year's "worm season".

Drench-and-move

One of the most common methods used to reduce the rate of re-infection of the low worm pastures has been the "drench-and-move" strategy, which has been ascribed to Weybridge in the United Kingdom (Stromberg & Averbek 1999), but is practically as old as the first non-herbal anthelmintics developed for sheep. In 1912 Theiler published three pamphlets totalling 62 pages, in which he suggested that worms could be eradicated by drenching animals and then moving them to pasture that had been left ungrazed for a year (Theiler 1912a; 1912b; 1912c). In addition, almost every helminthologist that followed Veglia 1918 at the Onderstepoort Veterinary Institute strongly propounded the drench-and-move system (Van Wyk 1987; 1990b; 1990c). This method of control is also one of the methods most adopted by farmers, both in South Africa (Joubert, Van Wyk & De Wet 1996) and elsewhere (Besier 1997b). Van Wyk (1990c) goes so far as to recommend that, when drenched, animals should be kept overnight in a pen before being spelled to safe pasture, so that most worm ova in the animals at the time of drenching are voided in the pen, instead of on the safe pasture.

Initially, after it had become common practice globally to spell to "safe" or "clean" pastures after drenching, no thought was apparently given to the possibility that this could strongly select for resistance (Parry 1975; Brunson 1976; Michel 1976; Morley & Donald 1980; Herd, Streitl, McClure & Parker 1983; Davidson 1987; Van Wyk 1987; 1990b; 1990c). It was even suggested that in the long term it could help delay selection for resistance (Morley & Donald 1980). Subsequently, as anthelmintic resistance began to escalate, the possibility that the drench-and-move system would select for resistance was considered, but some trials did not support this (Waller, Dobson, Lacey, Hennessy, Allerton & Prichard 1989). However, Martin (1989) convincingly demonstrated selection under such conditions. Even earlier Michel (1985) had warned that drenching during winter will select for resistance in a similar way as does drenching animals and moving them to worm-free, or low-worm pastures. Today the drench-and-move system is commonly listed as a practice that may select for resistance (Barger 1995a; 1999), although some still persist in recommending it, particularly for controlling worms in animal species other than sheep and goats (Amarante & Barbosa 1995; Williams, Loyacano, DeRosa, Gurie, Coombs & Skogerboe 1997; Stromberg & Averbek 1999). Most importantly the

system is seldom unequivocally condemned, e.g. the statement: "Obviously, the advantage of reducing exposure to re-infection and the risk of selecting for resistance must be considered in light of all other factors on a case-by-case basis before dose and move strategies are used." (Conder & Campbell 1995). This leaves the door for using this system wide open—no wonder that Besier (1997b) and Joubert *et al.* (1996) confirmed wide application thereof in the field.

In the case of an anthelmintic with persistent efficacy, doubt has recently been cast on the fore-going conclusions that the drench-and-move strategy should be avoided (Le Jambre *et al.* 1999). In trials on the selection for anthelmintic resistance in *H. contortus* by drenching with macrolactones, the results are interpreted as indicating that to drench animals with an anthelmintic with a persistent effect and then to move them to clean pasture is more sustainable than to leave the animals on the contaminated pasture after treatment. It is also emphasized in the paper concerned that to treat sheep with a macrolactone with persistent efficacy while grazing a contaminated paddock should be seen as an emergency procedure when there are no alternatives.

The recommendation by Le Jambre *et al.* (1999) to move sheep to clean pastures after a drench with moxidectin cannot be accepted without debate. The experiment was technically difficult and expensive and could hardly have been enlarged to give unequivocal answers. However, it suffered shortcomings that, in my opinion, make it necessary to delay any radical recommendations derived from it until the work can be replicated under more practice-related conditions. One of the strains of *H. contortus* used was unrepresentative, being derived from an unlisted number of inbred female worms selected for a certain morphological characteristic. In addition, considering the well-known large degree of variation between individual animals in susceptibility to worm infection (Barger 1985), only five sheep were used per group in each of 12/14 groups, and extremely low numbers of worms developed in the groups treated with moxidectin (geometric mean numbers of 2–8 worms in adult sheep, and 18–27 in young lambs). Furthermore, to test the development of worms after the drench, a trickle infection with undisclosed numbers of larvae and frequency of administration was given for a mere 5 weeks after the sheep had been treated with moxidectin. This is more or less the duration of the period of highly persistent efficacy of parenterally administered moxidectin against re-infection with *H. contortus*, while the *Haemonchus* season is much longer than this. In other words, during the 5 weeks of trickle challenge almost no susceptible worms could become established, and had the challenge lasted longer, the results could have been totally different. The authors apparently

expect that sheep with a measure of immunity to re-infection would not have been susceptible to trickle infection after the residual effect of the anthelmintic had worn off. If so, this is a generalization, as immunity is seldom solid in sheep, and individuals differ greatly in this respect. If sheep are moved to clean pasture after a moxidectin drench, all the *H. contortus* that develop subsequently will be the progeny of resistant worms. In contrast, I contend that, granted firstly a largely susceptible worm population, secondly that the drench is not given towards the end of the *Haemonchus* season, and in the light of the skew distribution, overdispersion of worm burdens in a population of sheep (Barger 1985), the few resistant worms picked up by the sheep during the effective "tail" of the moxidectin persistence as happened in the above trials, will be swamped by much larger numbers of susceptible worms that will establish thereafter. This possibility should be tested before farmers are advised to move sheep to clean pastures after a moxidectin drench.

In South Africa, a grazing management system developed by Kirkman & Moore (1995) for those regions of South Africa with a relatively high summer rainfall can play a crucial, positive role in worm control. However, if used with the drench-and-move strategy, it is also likely to select extremely severely for resistance. In the so-called "50–50" grazing management system, which has been shown to be excellent for sustainably maintaining and improving the quality of the pasture, all the animals are concentrated on one half of the pasture if only small ruminants are farmed, or on two-thirds for mixed cattle and sheep/goat farming, while the remainder rests for a full year (Kirkman & Moore 1995; Moore & Van Wyk 1997). When the rested pasture in these summer rainfall regions is utilized thereafter it is practically free from the principal gastrointestinal worm species. The danger is that, as this seems to be the "logical" thing to do for maximum potential worm control, the farmer will drench all the animals at the time they are spelled to the rested pasture. This topic, and possible solutions will be discussed elsewhere. In short, I will suggest that, amongst others, farmers should never drench an entire flock or herd of animals prophylactically when they are to be moved to "safe" pasture, and should strive for the minimum alternation of anthelmintics, unless FECR tests are done regularly to monitor the development of resistance. Furthermore, the viewpoint that phenomena such as underdosing are of crucial importance in selecting for anthelmintic resistance will be put in perspective, in relation to the overriding importance of refugia.

Strategic drenching

This has commonly been used for controlling worms with anthelmintics at times when there are few worms

in refugia on pasture. The drenching is timed to “reduce or prevent parasite build-up on pasture” (Stromberg & Averbek 1999). For instance, in an attempt to delay the seasonal build-up of worms on pasture, animals are drenched in winter, or at the beginning of spring in summer rainfall regions (Mönnig 1940; 1942; 1944; 1949; Reinecke 1983; Van Wyk 1987; 1990b; 1990c), or twice in the dry, hot summers of winter rainfall regions (Anderson 1972; 1973).

Numerous field trials have confirmed that strategic drenching can be highly effective for worm control, and computer simulation studies have predicted almost without exception that preventive strategic drenching is superior for worm control than most schedules of suppressive drenching that are applied at a time when the worm challenge on pasture is high (Michel 1969; 1976; Brunsdon 1980; Herd 1988; Abbott, Cobb & Glass 1995; Claxton *et al.* 1998; Lloyd *et al.* 2000). However, selection for anthelmintic resistance needs to be considered. Once again, the greater the success of the system, the greater the degree of selection is likely to be (Barger 1995a).

As in the case of the drench-and-move system, the possibility that strategic drenching could select for anthelmintic resistance by reducing the chances of susceptible worms to reproduce was initially overlooked. However, warnings have since been sounded (Echevarria, Gettinby & Hazelwood 1993; Besier 1997b; Barger 1999). On the other hand, it has been suggested that this approach may have prolonged “... the useful life of some anthelmintic groups...” by reducing drenching frequency (Sangster 1999). However, the consumer must be warned in no uncertain terms that to depend on strategic drenching for worm control in order to take advantage of low numbers of worms in refugia on pasture, will not be sustainable.

Alternation of anthelmintics

In common with reduced drenching, there is almost no discussion of methods for reducing selection for anthelmintic resistance that does not also emphasize alternation of anthelmintics (Le Jambre 1978a; Hall & Kelly 1979; Donald, Waller, Dobson & Axelsen 1980; Prichard, Hall, Kelly, Martin & Donald 1980; Dobson, Griffiths, Donald & Waller 1987; Van Wyk 1987; Waller, Dobson & Axelsen 1988; Waller *et al.* 1989; Dorchie 1991; Hazelby *et al.* 1994; Barger 1995a; 1995b; Conder & Campbell 1995; Scott *et al.* 1995; Waller *et al.* 1995; Anonymous 1998; Geerts & Gryseels 2000; Lloyd *et al.* 2000). Alternation is perhaps one of the recommendations most accepted in principle by farmers, even though they often apply it incorrectly (Kettle *et al.* 1983; Tritschler, Giordano & Coles 1986). Furthermore, it is widely accepted that annual rotation of anthelmintics will select less intensively for resistance than when they are al-

ternated within a given worm generation (Prichard *et al.* 1980; Dobson *et al.* 1987; Waller *et al.* 1988; 1989; Barger 1995b; Conder & Campbell 1995; Scott *et al.* 1995; Anonymous 1998; Lloyd & Soulsby 1998; Lloyd *et al.* 2000).

It is questionable, however, whether alternation, either “fast” or “slow”, has contributed, or can contribute substantially to reduction in selection for resistance, particularly if compared to the probable effect of refugia. The only experimental confirmation I could find was that of Donald *et al.* (1980) and Waller *et al.* (1988) that levamisole drenching may select against benzimidazole resistance; in other words, it could bring some relief for resistance to the benzimidazoles. On the other hand, Uhlinger & Kristula (1992) could find no effect from alternation on the rate of selection for resistance, and Smith (1990) indicated from simulation studies with a mathematical model, that a combination of drugs with different actions will be more effective for preventing escalation of resistance, than alternation of the same compounds. Once again experimental evidence is lacking and, in my opinion, alternation is likely at best to have only a marginal effect on reducing selection for resistance. According to Elard, Sauve & Humbert (1998) a strategy of alternation “... has no effect on resistant worms if their fitness is similar to that of susceptible worms.” Also, computer simulation studies have predicted that the endpoint of no remaining effective drugs will be reached at the same time, whether the drugs are used in alternation, or in series, until each one fails (Barnes *et al.* 1995).

For the following reasons it may under some circumstances be better not to alternate. Firstly, in a country like South Africa with a dearth of researchers and facilities for diagnosing resistance, farmers should be informed that progressive development of resistance will probably be easier to gauge without sophisticated testing if one group of anthelmintics after the other is used until no longer effective. It is hoped that in this way the present situation will be averted, where resistance is often very far developed before it is discovered (Van Wyk *et al.* 1997b; 1999). Secondly, if it becomes practical under commercial farming conditions to obtain reversion through dilution of resistant with susceptible worm strains (Van Wyk 1990a; Van Wyk & Van Schalkwyk 1990), the farmer should strongly be advised not to alternate. Instead, despite some practical problems associated with the variety of parasite species e.g. *Fasciola* spp. and *Oestrus ovis* affecting the animals, farmers will be advised to use each group in sequence for as long as possible, until efficacy is too low for managing the worms. In the case of *H. contortus* infection a system such as the FAMACHA[®] ovine clinical anaemia guide for identifying and treating only heavily infected animals (Bath, Malan & Van Wyk 1996; 1997; Van Wyk *et al.* 1997a), will help to indicate timeously

when the anthelmintic group in use is becoming ineffective.

The suggestion to continue using a group of anthelmintics until it fails entirely is contrary to the conventional recommendation that drenching with a compound should cease when resistance is detected (Martin, Anderson, Brown & Miller 1988; Prichard 1990; Conder & Campbell 1995; Isaza, Courtney & Kollias 1995; Geary, Sangster & Thompson 1999). Prichard (1990) implies that if the selection continues, "... resistance alleles re-associate with other fitness characters and the highly pressured resistant individuals survive better than more moderately resistant worms," and that "early detection of emerging resistance is vital for the conservation of effective anthelmintics and for parasite control" (Prichard 1990). But why should efficacy be conserved? Thus far cross-resistance between anthelmintic groups with different modes of action has apparently not occurred, and little useful reversion to susceptibility appears to take place after a group of compounds has been withdrawn from use for periods even as long as 10 years (Scott *et al.* 1995; Borgsteede & Duyn 1989). Hence, for all practical purposes, an anthelmintic compound or group appears to "die" in any case as resistance develops, and continued use when resistance has started to develop should not affect the efficacy of other groups. By not alternating, the farmer will know when resistance becomes critical, and can get help before the last effective anthelmintic group becomes practically useless. A drug can still be very useful for as long as efficacy remains above $\pm 50\%$ —in other words, long after diagnostic tests have shown definite resistance. The best practical demonstration of this is probably that for half a century mixtures of copper sulphate, arsenicals and nicotine helped to make sheep farming economically feasible in South Africa, while the last remaining anthelmintic containing a combination of such compounds in the country ("Gow's Remedy For Wire Worm and Tape Worm In Sheep, Goats, Lambs, Cattle and Calves", Registration GD65) was found to be barely 50% effective against adult *H. contortus* (R.K. Reinecke & J.A. van Wyk, unpublished observations 1968).

CONCLUSION

Refugia should be considered above all else when worm management in both domestic livestock and humans is planned. Furthermore, practices such as drenching all the animals in a flock or herd before they are moved to safe or worm-free pasture should be unequivocally condemned. Some animals should be left undrenched, to propagate susceptible worms.

The simple choice is either to conserve susceptible individuals in the worm population to give them the best possible advantage over resistant worms as

regards contributing to subsequent generations—therefore, farming with "good" worms, in a manner of speaking, or to accept that worm control with anthelmintics will not be sustainable. Consequently refugia is crucial, and farmers should be educated to consider this first when planning chemical worm control/management. The farmer must be brought to realize that in contrast to the approach of the past, it is optimum production that should be strived for in grazing animals, and not unsustainable maximum production that is almost completely dependent on effective anthelmintics.

Furthermore, the possibility that anthelmintic resistance may become as important in cattle as it is in small stock seems to be disregarded (e.g. Williams *et al.* 1997; Stromberg & Averbek 1999). This is a mistake, as Waller (1999) points out: "... an informed opinion is that the situation for anthelmintic resistance in cattle parasites is about a decade behind that for parasites of sheep and goats."

ACKNOWLEDGEMENTS

Proff. Roy Tustin, Ivan Horak and Gareth Bath, together with Drs Pierre Dorny and Francois Malan gave very helpful comments and encouragement and assisted with editing.

REFERENCES

- ABBOTT, K.A., COBB, R.M. & GLASS, H.M. 1995. Duration of the persistent activity of moxidectin against *Haemonchus contortus* in sheep. *Australian Veterinary Journal*, 72:408–410.
- AMARANTE, A.F.T. & BARBOSA, M.A. 1995. Seasonal variations in populations of infective larvae on pasture and nematode faecal egg output in sheep. *Veterinaria e Zootecnica*, 7:127–133. [From Central Agricultural Bureaux VETCD 1989–1999/08]
- ANDERSON, N. 1972. Trichostrongylid infections of sheep in a winter rainfall region. I. Epizootiological studies in the Western District of Victoria, 1966–1967. *Australian Journal of Agricultural Research*, 23:1113–1129.
- ANDERSON, N. 1973. Trichostrongylid infections of sheep in a winter rainfall region. II. Epizootiological studies in the Western District of Victoria, 1967–1968. *Australian Journal of Agricultural Research*, 24:599–611.
- ANONYMOUS. 1998. Report of the WHO Informal Consultation on monitoring of drug efficacy in the control of shistosomiasis and intestinal nematodes. (WHO/CDS/CPC/SIP/99.1) Geneva, 8–10 July 1998.
- BARGER, I.A. 1982. Helminth parasites and animal production, in *Biology and control of endoparasites*, edited by L.E.A. Symons, A.D. Donald & J.K. Dineen. New York: Academic Press: 133–155.
- BARGER, I.A. 1985. The statistical distribution of trichostrongylid nematodes in grazing lambs. *International Journal for Parasitology*, 15:645–649.
- BARGER, I.A. 1995a. Control strategies minimising the use of anthelmintics, in *Proceedings of 25th sheep and beef cattle seminar*, edited by G. Budge. Publication No. 165, Veterinary Continuing Education, Massey University, Palmerston North, New Zealand: 59–66.

- BARGER, I.A. 1995b. Control of nematodes in the presence of anthelmintic resistance – Australian experience, in *Proceedings of 25th Sheep and Beef Cattle Seminar*, edited by G. Budge. Publication No. 165, Veterinary Continuing Education, Massey University, Palmerston North, New Zealand: 87–92.
- BARGER, I. 1996. Grazing management for the control of nematode parasites, in *Sustainable parasite control in small ruminants*, edited by L.F. Le Jambre & M.R. Knox. An international workshop sponsored by ACIAR and held in Bogor, Indonesia, 22–25 April 1996: 146–150.
- BARGER, I.A. 1999. The role of epidemiological knowledge and grazing management for helminth control in small ruminants. *International Journal for Parasitology*, 29:41–47.
- BARNES, E.H., DOBSON, R.J. & BARGER, I.A. 1995. Worm control and anthelmintic resistance: adventures with a model. *Parasitology Today*, 11:56–63.
- BARTON, N.J. 1983. Development of anthelmintic resistance in nematodes from sheep in Australia subjected to different treatment frequencies. *International Journal for Parasitology*, 13: 125–132.
- BATH, G.F., MALAN, F.S. & VAN WYK, J.A. 1996. The "FAMA-CHA" Ovine Anaemia Guide to assist with the control of haemonchosis, in *Proceedings of the 7th Annual Congress of the Livestock Health and Production Group of the South African Veterinary Association*, Port Elizabeth.
- BATH, G.F., MALAN, F.S. & VAN WYK, J.A. 1997. The use of clinical anaemia as an aid in the control of haemonchosis in sheep, in *Proceedings of the 4th International Congress for Sheep Veterinarians*, Armidale, NSW, Australia: 381–385.
- BESIER, R.B. 1997a. Ecological selection for anthelmintic resistance: re-evaluation of sheep worm control programs, in *Managing anthelmintic resistance in endoparasites. Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology*, edited by J.A. van Wyk & P.C. van Schalkwyk. Sun City, South Africa: 30–38.
- BESIER, R.B. 1997b. Sheep disease control plans, internal parasites: Wormkill, Wormwise, Drenchplan, Wormplan, Wormcheck, Wormbuster, in *Conference Proceedings. Fourth International Congress for Sheep Veterinarians*. Australian Sheep Veterinary Society, University of New England, Armidale, New South Wales, Australia: 74–80.
- BESIER, R.B. & HOPKINS, D.L. 1988. Anthelmintic dose selection by farmers. *Australian Veterinary Journal*, 65:193–194.
- BORGSTEEDE, F.H.M. & DUYN, S.P.J. 1989. Lack of reversion of a benzimidazole resistant strain of *Haemonchus contortus* after six years of levamisole usage. *Research in Veterinary Science*, 47:270–272.
- BRUNSDON, R.V. 1976. Responses to the anthelmintic treatment of lambs at weaning: the relative importance of various sources of contamination in trichostrongyle infections. *New Zealand Journal of Experimental Agriculture*, 4:275–279. [From Central Agricultural Bureaux VETCD 1973–1988].
- BRUNSDON, R.V. 1980. Principles of helminth control. *Veterinary Parasitology*, 6:185–215.
- CHARTIER, C., PORS, I., HUBERT, J., ROCHETEAU, D., BENOIT, C. & BERNARD, N. 1998. Prevalence of anthelmintic resistant nematodes in sheep and goats in western France. *Small Ruminant Research*, 29:33–41. [From Central Agricultural Bureaux VETCD 1989–1999/08].
- CLAXTON, J.R., ZAMBRANO, H., ORTIZ, P., DELGADO, E., ESCURRA, E. & CLARKSON, M.J. 1998. Strategic control of fasciolosis in the inter-Andean valley of Cajamarca, Peru. *The Veterinary Record*, 143:42–45.
- 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.
- DAVIDSON, S. 1987. Drenchplan: research-based worm control. *Rural Research*, 135:4–9. [From Central Agricultural Bureaux VETCD 1973–1988].
- DOBSON, R.J., GRIFFITHS, D.A., DONALD A.D. & WALLER, P.J. 1987. A genetic model describing the evolution of levamisole resistance in *Trichostrongylus colubriformis*, a nematode parasite of sheep. *IMA Journal of Mathematics Applied in Medicine & Biology*, 4:279–293.
- 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.
- DONALD, A.D. 1983. Anthelmintic resistance in parasites of sheep, in *Sheep production and preventive medicine, Refresher course for veterinarians*, cited by Martin. Proceedings No. 67, University of Sydney Post-Graduate Committee in Veterinary Science, Sydney: 493–507.
- DONALD, A.D., WALLER, P.J., DOBSON, R.J. & AXELSEN, A. 1980. The effect of selection with levamisole on benzimidazole resistance in *Ostertagia* spp. of sheep. *International Journal for Parasitology*, 10:381–389.
- DORCHIES, P. 1991. Les resistance aux anthelminthiques: position du probleme. [Resistance to anthelmintics: an outline of the problems]. *Revue de Medecine Veterinaire*, 142:615–621. [From Central Agricultural Bureaux VETCD 1989–1999/08].
- DORCHIES, P., NICOLAS, J., GEVREY, J. & MAGE, C. 1990. Vermifugation du mouton: prevenir les risques d'apparition des chimioresistances. [Anthelmintic treatment of sheep: preventing the risks of the appearance of drug resistance]. *Revue de Medecine Veterinaire*, 141:379–381. [From Central Agricultural Bureaux VETCD 1989–1999/08].
- DUNCAN, J.L., BAIRDEN, K. & ABBOTT, E.M. 1998. Elimination of mucosal cyathostome larvae by five daily treatments with fenbendazole. *The Veterinary Record*, 142:268–271.
- ECHEVARRIA, F.A.M., GETTINBY, G. & HAZELWOOD, S. 1993. Model predictions for anthelmintic resistance amongst *Haemonchus contortus* populations in southern Brazil. *Veterinary Parasitology*, 47:315–325.
- ELARD, L., SAUVE, C. & HUMBERT, J.F. 1998. Fitness of benzimidazole-resistant and -susceptible worms of *Teladorsagia circumcincta*, a nematode parasite of small ruminants. *Parasitology*, 117:571–578.
- GEARY, T.G., SANGSTER, N.C. & THOMPSON, D.P. 1999. Frontiers in anthelmintic pharmacology. *Veterinary Parasitology*, 84:275–295.
- GEERTS, S. & GRYSEELS, B. 2000. Drug resistance in human helminths: current situation and lessons from livestock. *Clinical Microbiology Reviews*, 13:207–222.
- GORDON, H.M.C.L. 1981. Epidemiology of helminthosis of sheep: diagnosis, in *Refresher course for veterinarians. Proceedings no. 58. Refresher course for sheep*. University of Sydney (The Post-Graduate Committee in Veterinary Science): 607–615.
- HALL, C.A. & KELLY, J.D. 1979. Anthelmintic resistance in nematodes, II. Treatment of resistant nematodes. *New South Wales Veterinary Proceedings*, 15:32–36. [From Central Agricultural Bureaux VETCD 1973–1988].
- HAZELBY, C.A., PROBERT, A.J. & ROWLANDS, D.A.P.-T. 1994. Anthelmintic resistance in nematodes causing parasitic gastroenteritis of sheep in the UK. *Journal of Veterinary Pharmacology and Therapeutics*, 17:245–252. [From Central Agricultural Bureaux VETCD 1989–1999/08].
- HENNESSEE, D.R. 1994. The disposition of antiparasitic drugs in relation to the development of resistance by parasites of livestock. *Acta Tropica*, 56:125–141.
- HERD, R.P. 1988. Control strategies for ostertagiasis. *Veterinary Parasitology*, 27:111–123.

- HERD, R.P., STREITEL, R.H., MCCLURE, K.E. & PARKER, C.F. 1983. Control of periparturient rise in worm egg counts of lambing ewes. *Journal of the American Veterinary Medical Association*, 182:375–379.
- HOEKSTRA, R., BORGSTEEDE, F.H.M., BOERSEMA, J.H. & ROOS, J.H. 1997. Selection of high levamisole resistance in *Haemonchus contortus* monitored with an egg-hatch assay. *International Journal for Parasitology*, 27:1395–1400.
- ISAZA, R., COURTNEY, C.H. & KOLLIAS, G.V. 1995. The prevalence of benzimidazole-resistant trichostrongyloid nematodes in antelope collections in Florida. *Journal of Zoo and Wildlife Medicine*, 26:260–264. [From Central Agricultural Bureaux VETCD 1989–1999/08].
- JACKSON, F. 1993. Anthelmintic resistance—the state of play. *British Veterinary Journal*, 149:123–138.
- JOUBERT, J.P.J., VAN WYK, J.A. & DE WET, J.A.L. 1996. Vraelys van Direktooraat Diergesondheid en Onderstepoort oor inwendige parasietbeheer by skape, in *Proceedings of the 7th Annual Congress of the Livestock Health & Production Group of the South African Veterinary Association*. Port Elizabeth: 120–133a.
- KELLY, J.D., WEBSTER, J.H., GRIFFIN, D.L., WHITLOCK, H.V., MARTIN, I.C.A. & GUNAWAN, M. 1981. Resistance to benzimidazole anthelmintics in equine strongyles. *Australian Veterinary Journal*, 57:163–171.
- KETTLE, P.R., VLASSOFF, A., REID, T.C. & HORTON, C.T. 1983. A survey of nematode control measures used by milking goat farmers and of anthelmintic resistance on their farms. *New Zealand Veterinary Journal*, 31:139–143.
- KIERAN, P.J. 1994. Moxidectin against ivermectin-resistant nematodes—a global view. *Australian Veterinary Journal*, 71:18–20.
- KIRKMAN, K.P. & MOORE, A. 1995. Veld management strategies for livestock farmers in the sourveld regions of South Africa. *Bulletin of the Grassland Society of South Africa*, 6:25–28.
- LEATHWICK, D.M. 1995. A case of moxidectin failing to control ivermectin resistant *Ostertagia* species in goats. *Veterinary Record*, 136:443–444.
- LEATHWICK, D.M., VLASSOFF, A. & BARLOW, N.D. 1995. A model for nematodiasis in New Zealand lambs: the effect of drenching regime and grazing management on the development of anthelmintic resistance. *International Journal for Parasitology*, 25:1479–1490.
- LE JAMBRE, L.F. 1978a. Anthelmintic resistance in gastrointestinal nematodes of sheep, in *The epidemiology and control of gastrointestinal parasites of sheep in Australia*, edited by A.D. Donald, W.H. Southcott & J.K. Dineen. Commonwealth Scientific and Industrial Research Organization, Australia [Shiels Printing, Mt Waverley]: 109–120.
- LE JAMBRE, L.F. 1981. Eradication of levamisole-resistant *Ostertagia*. *Australian Veterinary Journal*, 57:99–100.
- LE JAMBRE, L.F., DOBSON, R.J., LENANE, I.J. & BARNES, E.H. 1999. Selection for anthelmintic resistance by macrocyclic lactones in *Haemonchus contortus*. *International Journal for Parasitology*, 29:1101–1111.
- LLOYD, S. & SOULSBY, LORD 1998. Is anthelmintic resistance inevitable: back to basics? *Equine Veterinary Journal*, 30:280–283.
- LLOYD, S., SMITH, J., CONNAN, R.M., HATCHER, M.A., HEDGES, T.R., HUMPHREY, D.J. & JONES, A.C. 2000. Parasite control methods used by horse owners: factors predisposing to the development of anthelmintic resistance in nematodes. *The Veterinary Record*, 146:487–492.
- MARTIN, P.J. 1989. Selection for thiabendazole resistance in *Ostertagia* spp. by low efficiency anthelmintic treatment. *International Journal for Parasitology*, 19:317–325.
- MARTIN, P.J., ANDERSON, N., JARRETT, R.G., BROWN, T.H. & FORD, G.E. 1982. Effects of a preventive and suppressive control scheme on the development of thiabendazole-resistance in *Ostertagia* spp. *Australian Veterinary Journal*, 58:185–190.
- MARTIN, P.J., ANDERSON, N., LWIN, T., NELSON, G. & MORGAN, T.E. 1984. The association between frequency of thiabendazole treatment and the development of resistance in field isolates of *Ostertagia* spp. of sheep. *International Journal for Parasitology*, 14:177–181.
- MARTIN, P.J., ANDERSON, N., BROWN, T.H. & MILLER, D.W. 1988. Changes in resistance of *Ostertagia* spp. to thiabendazole following natural selection or treatment with levamisole. *International Journal for Parasitology*, 18:333–340.
- MARTIN, P.J., LE JAMBRE, L.F. & CLAXTON, J.H. 1981. The impact of refugia on the development of thiabendazole resistance in *Haemonchus contortus*. *International Journal for Parasitology*, 11:35–41.
- MICHEL, J.F. 1969. The epidemiology and control of some nematode infections of grazing animals, edited by B. Dawes. *Advances in Parasitology*, 7:211–282.
- MICHEL, J.F. 1976. The epidemiology and control of some nematode infections in grazing animals, edited by B. Dawes. *Advances in Parasitology*, 14:355–397.
- MICHEL, J.F. 1985. Strategies for the use of anthelmintics in livestock and their implications for the development of drug resistance. *Parasitology*, 90:621–628.
- MÖNNIG, H.O. 1940. Worms in sheep. Different types and their control. *Farming in South Africa*, August 1940: 315–322.
- MÖNNIG, H.O. 1942. Control of worms in sheep. *Farming in South Africa*, July 1942: 455–458.
- MÖNNIG, H.O. 1944. Wurms by skape, bokke en beeste. Die verskillende soorte en die bestryding daarvan. *Boerdery in Suid-Afrika*. November en Desember 1944 (Herdruk 83): 1–20.
- MÖNNIG, H.O. 1949. Bestryding van inwendige parasiete by skape. Die geskikste tyd vir behandeling. *Die Wolboer*, April 1949: 30–31.
- MOORE, A. & VAN WYK, J.A. 1997. Weidingsbestuur en haaruurbeheer by skape. *Goue Vag/Golden Fleece*, June 1997: 6–7.
- MORLEY, F.H.W. & DONALD, A.D. 1980. Farm management and systems of helminth control. *Veterinary Parasitology*, 6:105–134.
- MORRIS, C.A. & BISSET, S.A. 1997. Breeding sheep which require minimal anthelmintic treatment: a review of the genetics of resistance and resilience of sheep to nematode parasites, in *Managing anthelmintic resistance in endoparasites. Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology*, edited by J.A. van Wyk & P.C. van Schalkwyk. Sun City, South Africa: 21–28.
- PARRY, J. 1975. Clean-graze routine beats worm problem. *Big Farm Management*, 1975 (October): 69–70 [From Central Agricultural Bureaux VETCD 1973–1988].
- 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., 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.
- REINECKE, R.K. 1983. *Veterinary helminthology*. Durban: Butterworths: 1–392.
- ROLFE, P.F. & FITZGIBBON, C. 1996. Resistance to macrocyclic lactones in intestinal parasites of sheep: implications for the

- persistent effect of moxidectin, in *Sheep Sessions. Second Pan Pacific Veterinary Conference, Christchurch, New Zealand*. Publication—Veterinary Continuing Education, Massey University 1996, 170:191–194 [From Central Agricultural Bureau VETCD 1989–1999/08].
- ROUSH, R.T. & MCKENZIE, J.A. 1987. Ecological genetics of insecticide and acaricide resistance. *Annual Review of Entomology*, 32:361–380.
- SANGSTER, N.C. 1999. Anthelmintic resistance: past, present and future. *International Journal for Parasitology*, 29:115–124.
- SCOTT, E.W., DUNCAN, J.L., MCKELLAR, Q.A., COOP, R.L., JACKSON, F. & MITCHELL, G.B.B. 1991. Benzimidazole resistance in sheep nematodes. *The Veterinary Record*, 128: 618–619.
- SCOTT, E.W., ROBBINS, H., JACKSON, F., JACKSON, E. & CLARKSON, M. 1995. Limiting the spread of benzimidazole-resistant nematodes: a farm study. *Proceedings of the Sheep Veterinary Society*, 1993–1994, 18:181–182. [From Central Agricultural Bureau VETCD 1989–1999/08].
- SHOOP, W.L. 1992. Resistance to avermectins and milbemycins. *The Veterinary Record*, 130:563.
- SHOOP, W.L., HINES, H.W., MICHAEL, B.F. & EARY, C.H. 1993. Mutual resistance to avermectins and milbemycins: oral activity of ivermectin and moxidectin against ivermectin-resistant and susceptible nematodes. *The Veterinary Record*, 133: 445–447.
- SMITH, G. 1990. A mathematical model for the evolution of anthelmintic resistance in a direct life cycle nematode parasite. *International Journal for Parasitology*, 20:913–921.
- SMITH, G., GRENFELL, B.T., ISHAM, V. & CORNELL, S. 1999. Anthelmintic resistance revisited: under-dosing, chemoprophylactic strategies, and mating probabilities. *International Journal for Parasitology*, 29:77–91.
- SMITH, W.D. 1997. Prospects for vaccines against gastrointestinal helminth parasites of ruminants, in *Managing anthelmintic resistance in endoparasites. Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology*, edited by J.A. van Wyk & P.C. van Schalkwyk. Sun City, South Africa: 6–12.
- STROMBERG, B.E. & AVERBECK, G.A. 1999. The role of parasite epidemiology in the management of grazing cattle. *International Journal for Parasitology*, 29:33–39.
- SUTHERLAND, I.A., LEATHWICK, D.M., BROWN, A.E. & MILLER, C.M. 1997. Prophylactic efficacy of persistent anthelmintics against challenge with drug-resistant and susceptible *Ostertagia circumcincta*. *The Veterinary Record*, 141:120–123.
- SWAN, N., GARDNER, J.J., BESIEN, R.B. & WROTH, R. 1994. A field case of ivermectin resistance in *Ostertagia* of sheep. *Australian Veterinary Journal*, 71:302–303.
- THEILER, A. 1912a. Wire-worms in sheep and their treatment. *South African Agricultural Journal* (Bulletin no. 63).
- THEILER, A. 1912b. Experiments to determine the safe dose of white arsenic, Cooper's Dip and bluestone for sheep. *South African Agricultural Journal*, March 1912 (Bulletin no.17).
- THEILER, A. 1912c. Voorlopig rapport omtrent het ingeven van Cooper's Dip en blaauwsteen aan schapen op een zuurveld-plaats. *Landbouw Journaal van die Unie van Zuid-Afrika*, Augustus 1912 (Bulletin no. 48).
- TRITSCHLER, J.P. II., GIORDANO, D.J. & COLES, G.C. 1986. Use of anthelmintics by New England sheep producers. *Journal of the American Veterinary Medical Association*, 189:1309–1313.
- UHLINGER, C. & KRISTULA, M. 1992. Effects of alternation of drug classes on the development of oxibendazole resistance in a herd of horses. *Journal of the American Veterinary Medical Association*, 201:51–55.
- VAN WYK, J.A. 1985. The epidemiology and control of gastrointestinal nematode infestation of sheep and cattle in South Africa. 1. The historic role of Onderstepoort and a short discussion of present research priorities. *Onderstepoort Journal of Veterinary Research*, 49:257–262.
- VAN WYK, J.A. 1987. 'n Oorsig oor metodes wat vir die beheer van maagderm rondewurms van skape en beeste aangewend kan word, in *Worm resistance workshop*, edited by J. Schröder. Onderstepoort: 89–112.
- VAN WYK, J.A. 1990a. Occurrence and dissemination of anthelmintic resistance in South Africa, and management of resistant worm strains, in *Resistance of parasites to antiparasitic drugs. Round table conference held at the 7th International Congress of Parasitology*, edited by J.C. Boray, P.J. Martin & R.T. Roush. Paris: 103–113.
- VAN WYK, J.A. 1990b. A review of chemical methods available for the control of gastrointestinal nematodes of sheep and cattle. *Journal of the South African Veterinary Association*, 61: 136–140.
- VAN WYK, J.A. 1990c. Integrated worm control as a strategy in the control of gastrointestinal nematodes of sheep and cattle. *Journal of the South African Veterinary Association*, 61:141–145.
- 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. *The 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?, in *Managing anthelmintic resistance in endoparasites. Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology*, edited by J.A. van Wyk & P.C. van Schalkwyk. Sun City, South Africa: 51–63.
- VAN WYK, J.A., MALAN, F.S., GERBER, H.M. & ALVES, R.M.R. 1989. The problem of escalating resistance of *Haemonchus contortus* to the modern anthelmintics in South Africa. *Onderstepoort Journal of Veterinary Research*, 56:41–49.
- VAN WYK, J.A., MALAN, F.S. & RANGLES, 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 anthelmintics? *Veterinary Parasitology*, 70:111–122.
- VAN WYK, J.A., STENSON, M.O.S., VAN DER MERWE, J.S., VORSTER, R.J. & VILJOEN, P.G. 1999. Anthelmintic resistance in South Africa: surveys indicate an extremely serious situation in sheep and goat farming. *Onderstepoort Journal of Veterinary Research*, 66:273–284.
- VAN WYK, J.A. & VAN SCHALKWYK, P.C. 1990. A novel approach to the control of anthelmintic resistance in sheep. *Veterinary Parasitology*, 35:61–69.
- VAN WYK, J.A., VAN SCHALKWYK, P.C., BATH, G.F., GERBER, H.M. & ALVES, R.M.R. 1991. Die gevaar van wye verspreiding van weerstandbiedendheid teen wurmmiddels deur veldram-prestasietoetsentra/The threat of wide dissemination of anthelmintic resistance by veld ram performance testing units. *Journal of the South African Veterinary Association*, 62:171–175.
- VEGLIA, F. 1918. Chemotherapy of haemonchosis in sheep. *The Fifth and Sixth Reports of the Director of Veterinary Research, Union of South Africa*: 377–482.
- VERMUNT, J.J., WEST, D.M. & POMROY, W.E. 1996. Inefficacy of moxidectin and doramectin against ivermectin-resistant *Cooperia* spp. of cattle in New Zealand. *New Zealand Veterinary Journal*, 44:188–193.
- WALLER, P.J. 1985. Resistance to anthelmintics and their implications for animal production, in *Resistance in nematodes to anthelmintic drugs*, edited by N. Anderson & P.J. Waller.

- CSIRO Division of Animal Health, Australian Wool Corporation (Technical Publication 1985: 1–11).
- WALLER, P.J. 1997. Biological control of helminths, in *Managing anthelmintic resistance in endoparasites. Workshop held at the 16th International Conference of the World Association for the Advancement of Veterinary Parasitology*, edited by J.A. van Wyk & P.C. van Schalkwyk. Sun City: South Africa: 14–19.
- WALLER, P.J. 1999. International approaches to the concept of integrated control of nematode parasites of livestock. *International Journal for Parasitology*, 29:155–164.
- WALLER, P.J., DASH, K.M., BARGER, I.A., LE JAMBRE, L.F. & PLANT, J. 1995. Anthelmintic resistance in nematode parasites of sheep: learning from the Australian experience. *The Veterinary Record*, 136:411–413.
- WALLER, P.J., DOBSON, R.J. & AXELSEN, A. 1988. Anthelmintic resistance in the field: changes in resistance status of parasitic populations in response to anthelmintic treatment. *Australian Veterinary Journal*, 65:376–379.
- WALLER, P.J., DOBSON, R.J., LACEY, E., HENNESSEE, D.R., ALLERTON, G.R. & PRICHARD, R.K. 1989. Changes in anthelmintic resistance status of *Haemonchus contortus* and *Trichostrongylus colubriformis* exposed to different anthelmintic selection pressures in grazing sheep. *International Journal for Parasitology*, 19:99–110.
- WATSON, T.G., HOSKING, B.C., LEATHWICK, D.M. & MCKEE, P.F. 1996. Ivermectin-moxidectin side resistance by *Ostertagia* species isolated from goats and passaged to sheep. *The Veterinary Record*, 138:472–473.
- WILLIAMS, J.C., LOYACANO, A.F., DEROSA, A., GURIE, J., COOMBS, D.F. & SKOGERBOE, T.L. 1997. A comparison of the efficacy of two treatments of doramectin injectable, ivermectin injectable and ivermectin pour-on against naturally acquired gastrointestinal nematode infections of cattle during a winter-spring grazing season. *Veterinary Parasitology*, 72: 69–77.
- WOOD, I.B., AMARAL, N.K., BAIRDEN, K., DUNCAN J.L., KASSAI, T., MALONE, J.B. Jr, PANKAVICH, J.A., REINECKE, R.K., SLOCOMBE, O., TAYLOR, S.M. & VERCRUYSE, J. 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.) 2nd edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). *Veterinary Parasitology*, 58:181–213.